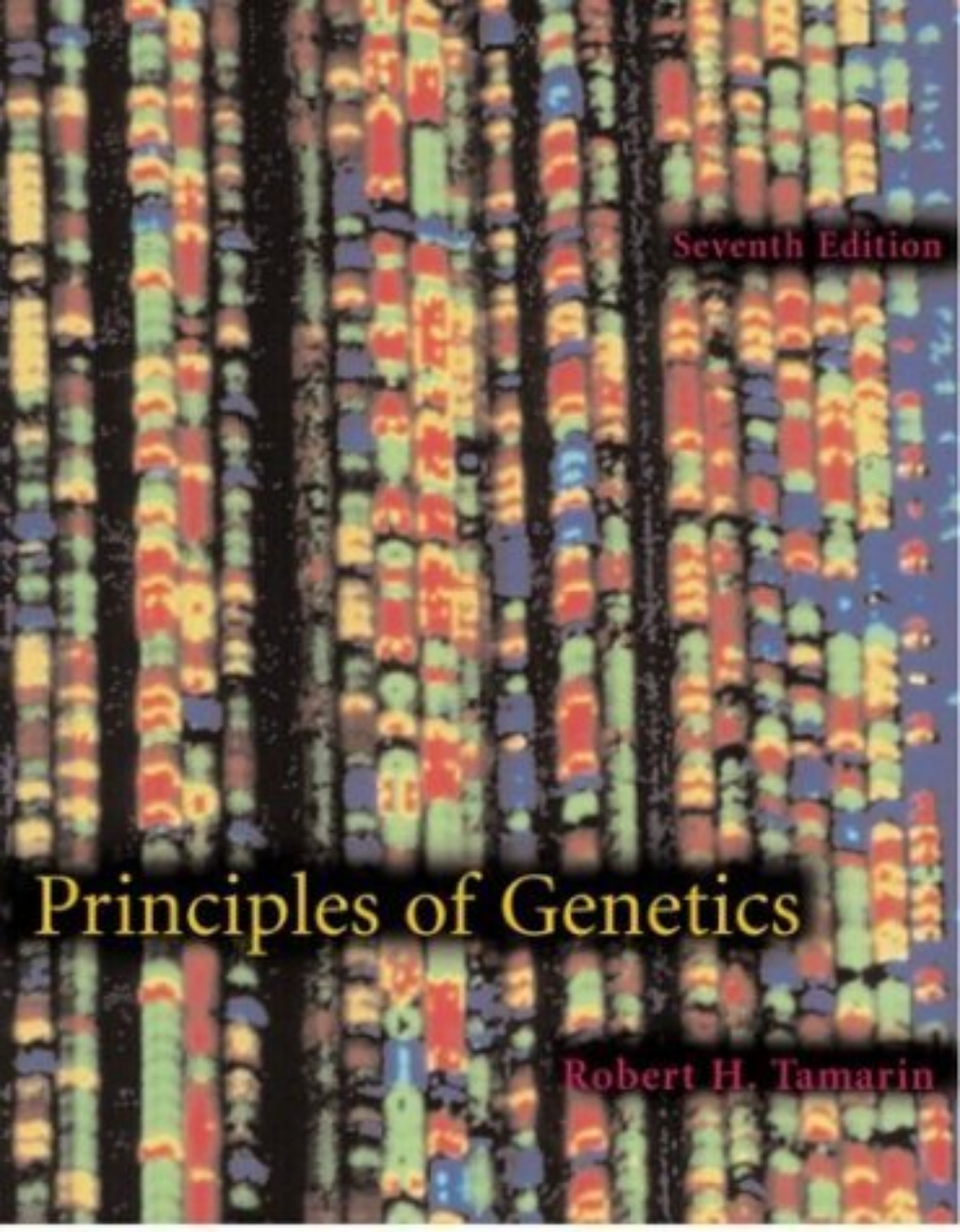

Seventh Edition

Principles of Genetics

Robert H. Tamarin

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PREFACE

The twentieth century began with the rediscovery of Mendel's rules of inheritance and ended with the complete sequence of the human genome, one of the most monumental scientific accomplishments of all time. What lies in the future? What will the twenty-first century, the century of genomics, bring? Will geneticists a hundred years from now speak of a complete cure for cancer, heart disease, and mental illness? Will we have a cure for autoimmune diseases such as diabetes and arthritis? Will aging be slowed or even prevented? Will we have a complete understanding of the process of development and a concurrent elimination of birth defects and developmental problems? Will genetics put an end to world hunger? How will we live, and what will be the quality of our lives? The students who now are taking genetics will learn the answers to these questions as time progresses. Some students will contribute to the answers.

The science of genetics includes the rules of inheritance in cells, individuals, and populations and the molecular mechanisms by which genes control the growth, development, and appearance of an organism. No area of biology can truly be appreciated or understood without an understanding of genetics because genes not only control cellular processes, they also determine the course of evolution. Genetic concepts provide the framework for the study of modern biology.

This text provides a **balanced treatment** of the major areas of genetics in order to prepare the student for upper-level courses and to help share in the excitement of research. Most readers of this text will have taken a general biology course and will have had some background in cell biology and organic chemistry. For an understanding of the concepts in this text, however, the motivated student will need to have completed only an introductory biology course and have had some chemistry and algebra in high school.

Genetics is commonly divided into three areas: classical, molecular, and population, although molecular advancements have blurred these distinctions. Many genetics teachers feel that a historical approach provides a sound introduction to the field and that a thorough grounding in Mendelian genetics is necessary for an understanding of molecular and population genetics—an approach this text follows. Other teachers, however, may prefer to begin with molecular genetics. For this reason, the chapters have been grouped as **units that allow for flexibility** in their use. A comprehensive glossary and index will help maintain continuity if the instructor chooses to change the order of the chapters from the original.

An understanding of genetics is crucial to advancements in medicine, agriculture, and many industries. Genetic controversies—such as the pros and cons of the Human Genome Project, the potential ethical and medical risks of recombinant DNA and cloning of mammals, and human behavioral genetic issues such as the degree of inheritance of homosexuality, alcoholism, and intelligence—have captured the interest of the general public. Throughout this text, we examine the **implications for human health and welfare** of the research conducted in universities and research laboratories around the world; boxed material in the text gives insight into genetic techniques, controversies, and breakthroughs.

Because genetics is the first analytical biology course for many students, some may have difficulty with its quantitative aspects. There is no substitute for work with pad and pencil. This text provides a larger number of problems to help the student learn and retain the material. All problems within the body of the text and a selection at the end of the chapters should be worked through as they are encountered. After the student has worked out the problems, he or she can refer to the answer section in Appendix A. We provide solved problems at the end of each chapter to help.

In this text, we stress **critical thinking,** an approach that emphasizes understanding over memorization, experimental proof over the pronouncements of authorities, problem solving over passive reading, and active participation in lectures. The latter is best accomplished if the student reads the appropriate text chapter before coming to lecture rather than after. That way the student can use the lecture to gain insight into difficult material rather than spending the lecture hectically transcribing the lecturer's comments onto the notebook page.

For those students who wish to pursue particular topics, a **reference section** in the back of the text provides chapter-by-chapter listings of review articles and articles in the original literature. Although some of these articles might be difficult for the beginner to follow, each is a landmark paper, a comprehensive summary, or a paper with some valuable aspect. Some papers may contain an insightful photograph or diagram. Some magazines and journals are especially recommended for the student to look at periodically, including *Scientific American,*

Science, and *Nature,* because they contain nontechnical summaries as well as material at the cutting edge of genetics. Some articles are included to help the instructor find supplementary materials related to the concepts in this book. Photographs of selected geneticists also are included. Perhaps the glimpse of a face from time to time will help add a human touch to this science.

The World Wide Web also can provide a valuable resource. The textbook has its own website: **www.mhhe.com/tamarin7**. In addition, the student can find much material of a supplemental nature by "surfing" the web. Begin with a search engine such as: www.yahoo.com, or www.google.com and type in a key word. Follow the links from there. Remember that the material on the web is "as is"; it includes a lot of misinformation. Usually, content from academic, industrial, and organizational sources is relatively reliable; however, caveat emptor—buyer beware. Often in surfing for scientific key words, the student will end up at a scientific journal or book that does not have free access. Check with the university librarian to see if access might be offered to that journal or book. The amount of information that is accurate and free is enormous. Be sure to budget the amount of time spent on the Internet.

NEW TO THIS EDITION

Since the last edition of this text, many exciting discoveries have been made in genetics. All chapters have been updated to reflect those discoveries. In particular:

- The chapter on Recombinant DNA Technology has been revised to be a chapter on Genomics, Biotechnology, and Recombinant DNA (sixth edition chapter 12 has become chapter 13 in this edition). The chapter includes new material on the completion of the Human Genome Project, bioinformatics, proteomics, and the latest techniques in creating cDNA and knockout mice.
- The chapter on Control of Transcription in Eukaryotes (sixth edition chapter 15 has become chapter 16 in this edition) has been completely reorganized and rewritten to emphasize signal transduction, specific transcription factors, methylation, and chromatin remodeling in control of gene expression; as in the last edition, there are specific sections on *Drosophila* and plant development, cancer, and immunogenetics.
- For better continuity, the chapter on Mutation, Recombination, and DNA Repair has been moved to follow the chapters on Transcription and Translation (sixth edition chapter 16 has become chapter 12 in this edition).
- The material in chapter 3 on Genetic Control of the Cell Cycle has been upgraded to a chapter section on the Cell Cycle.
- Molecular material throughout the book has been completely updated to include such subjects as numerous DNA repair polymerases and their functioning; base-flipping; TRAP control of attenuation; and chromatosomes.

LEARNING AIDS FOR THE STUDENT

To help the student learn genetics, as well as enjoy the material, we have made every effort to provide pedagogical aids. These aids are designed to help organize the material and make it understandable to students.

- **Study Objectives** Each chapter begins with a set of clearly defined, page-referenced objectives. These objectives preview the chapter and highlight the most important concepts.
- **Study Outline** The chapter topics are provided in an outline list. These headings consist of words or phrases that clearly define what the various sections of the chapter contain.
- **Boldface Terms** Throughout the chapter, all new terms are presented in boldface, indicating that each is defined in the glossary at the end of the book.
- **Boxed Material** In most chapters, short topics have been set aside in boxed readings, outside the main body of the chapter. These boxes fall into four categories: **Historical Perspectives, Experimental Methods, Biomedical Applications,** and **Ethics and Genetics.** The boxed material is designed to supplement each chapter with entertaining, interesting, and relevant topics.
- **Full Color Art and Graphics** Many genetic concepts are made much clearer with full-color illustrations and the latest in molecular computer models to help the student visualize and interpret difficult concepts. We've added thirty new photographs and over a hundred new and modified line drawings to this edition.
- **Summary** Each chapter summary recaps the study objectives at the beginning of the chapter. Thus, the student can determine if he or she has gained an understanding of the material presented in the study objectives and reinforce them with the summary.
- **Solved Problems** From two to four problems are worked out at the end of each chapter to give the student practice in solving and understanding basic problems related to the material.
- **Exercises and Problems** At the end of the chapter are numerous problems to test the student's

understanding of the material. These problems are grouped according to the sections of the chapter. Answers to the odd-numbered problems are presented in Appendix A, with the even-numbered problems answered only in the Student Study Guide so that the student and instructor can be certain that the student is gaining an understanding of the material.

- **Critical Thinking Questions** Two critical thinking questions at the end of each chapter are designed to help the student develop an ability to evaluate and solve problems. The answer to the first critical thinking question can be found in Appendix A, and the answer to the second question is in the Student Study Guide.

ANCILLARY MATERIALS

For the Instructor

- *Website.* Visit us at **www.mhhe.com/tamarin7.** Here instructors will find jpeg files of the line drawings and tables suitable for downloading into PowerPoint, quizzes for study support, and links to genetic sites. In addition, instructors will also find a link to our hugely successful **PageOut: The Course Website Development Center,** where instructors can create a professional-looking, customized course website. It's incredibly easy to use, and you need not know html coding.
- *Visual Resource Library (VRL).* This Windows- and Macintosh-compatible CD-ROM has all the line drawings and tables from the text suitable for PowerPoint presentations. (ISBN 0072334266)
- *Instructor's Manual with Test Item File.* Available on the website, the Instructor's Manual contains outlines, key words, summaries, instructional hints, and supplemental aids. The Test Item File contains 35 to 50 objective questions with answers for each chapter. (ISBN 0072334215)
- *Test Item File on MicroTest III Classroom Testing Software* is an easy-to-use CD-ROM test generator also offered free upon request to adopters of this text. The software requires no programming experience and is compatible with Windows or Macintosh systems. (ISBN 0072334231).

For the Student

- *Website.* Visit us at **www.mhhe.com/tamarin7.** Here the student will find quizzes for study support, web exercises and resources, and links to genetic sites.
- *Genetics: From Genes to Genomes CD-ROM,* by Ann E. Reynolds, University of Washington. Packaged free with every text, this CD-ROM covers the most challenging concepts in the course and makes them more understandable through the presentation of full-color, narrated animations and interactive exercises. The text indicates related topics on the CD with the following icon:

- *Student Study Guide.* This study guide features key concepts, problem-solving hints, practice problems, terms, study questions, and answers to even-numbered questions in the text. (ISBN 0072334207)
- *Laboratory Manual of Genetics 4/e,* by A. M. Winchester and P. J. Wejksnora, University of Wisconsin–Milwaukee. This manual for the genetics laboratory features classical and molecular biology exercises that give students the opportunity to apply the scientific method to "real"—not simulated—lab investigations. (ISBN 0697122875)
- *Case Workbook in Human Genetics, 2/e,* by Ricki Lewis, SUNY–Albany. The Workbook includes thought-provoking case studies in human genetics, with many examples gleaned from the author's experiences as a practicing genetic counselor. (ISBN 0072325305) Also included is the Answer Key. (ISBN 0072439009)

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Lowell, Massachusetts

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1

INTRODUCTION

STUDY OBJECTIVES

1. To examine a brief overview of the modern history of genetics 3
2. To gain an overview of the topics included in this book—the syllabus of genetics 4
3. To analyze the scientific method 5
4. To look at why certain organisms and techniques have been used preferentially in genetics research 7

STUDY OUTLINE

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Chameleon, *Cameleo pardalis.*

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Genetics is the study of inheritance in all of its manifestations, from the distribution of human traits in a family pedigree to the biochemistry of the genetic material in our chromosomes—deoxyribonucleic acid, or DNA. It is our purpose in this book to introduce and describe the processes and patterns of inheritance. In this chapter, we present a broad outline of the topics to be covered as well as a summary of some of the more important historical advancements leading to our current understanding of genetics.

A BRIEF OVERVIEW OF THE MODERN HISTORY OF GENETICS

For a generation of students born at a time when incredible technological advances are commonplace, it is valuable to see how far we have come in understanding the mechanisms of genetic processes by taking a very brief, encapsulated look at the modern history of genetics. Although we could discuss prehistoric concepts of animal and plant breeding and ideas going back to the ancient Greeks, we will restrict our brief look to events beginning with the discovery of cells and microscopes. For our purposes, we divide this recent history into four periods: before 1860, 1860–1900, 1900–1944, and 1944 to the present.

Before 1860

Before 1860, the most notable discoveries paving the way for our current understanding of genetics were the development of light microscopy, the elucidation of the cell theory, and the publication in 1859 of Charles Darwin's *The Origin of Species.* In 1665, Robert Hooke coined the term *cell* in his studies of cork. Hooke saw, in fact, empty cells observed at a magnification of about thirty power. Between 1674 and 1683, Anton van Leeuwenhoek discovered living organisms (protozoa and bacteria) in rainwater. Leeuwenhoek was a master lens maker and produced magnifications of several hundred power from single lenses (fig. 1.1). More than a hundred years passed before compound microscopes could equal Leeuwenhoek's magnifications. In 1833, Robert Brown (the discoverer of Brownian motion) discovered the nuclei of cells, and between 1835 and 1839, Hugo von Mohl described mitosis in nuclei. This era ended in 1858, when Rudolf Virchow summed up the concept of the cell theory with his Latin aphorism *omnis cellula e cellula:* all cells come from preexisting cells. Thus, by 1858, biologists had an understanding of the continuity of cells and knew of the cell's nucleus.

1860–1900

The period from 1860 to 1900 encompasses the publication of Gregor Mendel's work with pea plants in 1866 to the rediscovery of his work in 1900. It includes the discoveries of chromosomes and their behavior—insights that shed new light on Mendel's research.

From 1879 to 1885, with the aid of new staining techniques, W. Flemming described the chromosomes—first noticed by C. von Nägeli in 1842—including the way they split during division, and the separation of sister chromatids and their movement to opposite poles of the dividing cell during mitosis. In 1888, W. Waldeyer first used the term *chromosome.* In 1875, O. Hertwig described the fusion of sperm and egg to form the zygote. In the 1880s, Theodor Boveri, as well as K. Rabl and E. van Breden, hypothesized that chromosomes are individual structures with continuity from one generation to the next despite their "disappearance" between cell divisions. In 1885, August Weismann stated that inheritance is based exclusively in the nucleus. In 1887, he predicted the occurrence of a reductional division, which we now call meiosis. By 1890, O. Hertwig and T. Boveri had described the process of meiosis in detail.

1900–1944

From 1900 to 1944, modern genetics flourished with the development of the chromosomal theory, which showed

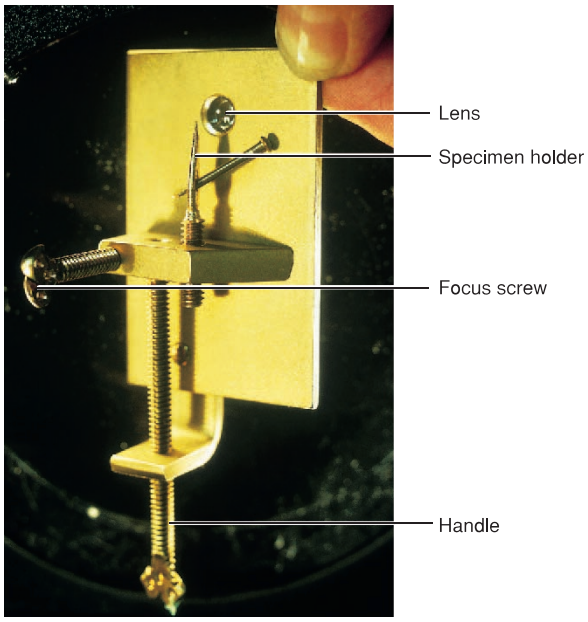

Figure 1.1 One of Anton van Leeuwenhoek's microscopes, ca. 1680. This single-lensed microscope magnifies up to 200x. (© Kathy Talaro/Visuals Unlimited, Inc.)

that chromosomes are linear arrays of genes. In addition, the foundations of modern evolutionary and molecular genetics were derived.

In 1900, three biologists working independently—Hugo de Vries, Carl Correns, and Erich von Tschermak—rediscovered Mendel's landmark work on the rules of inheritance, published in 1866, thus beginning our era of modern genetics. In 1903, Walter Sutton hypothesized that the behavior of chromosomes during meiosis explained Mendel's rules of inheritance, thus leading to the discovery that genes are located on chromosomes. In 1913, Alfred Sturtevant created the first genetic map, using the fruit fly. He showed that genes existed in a linear order on chromosomes. In 1927, L. Stadler and H. J. Muller showed that genes can be mutated artificially by X rays.

Between 1930 and 1932, R. A. Fisher, S. Wright, and J. B. S. Haldane developed the algebraic foundations for our understanding of the process of evolution. In 1943, S. Luria and M. Delbrück demonstrated that bacteria have normal genetic systems and thus could serve as models for studying genetic processes.

1944–Present

The period from 1944 to the present is the era of molecular genetics, beginning with the demonstration that DNA is the genetic material and culminating with our current explosion of knowledge due to recombinant DNA technology.

In 1944, O. Avery and colleagues showed conclusively that deoxyribonucleic acid—DNA—was the genetic material. James Watson and Francis Crick worked out the structure of DNA in 1953. Between 1968 and 1973, W. Arber, H. Smith, and D. Nathans, along with their colleagues, discovered and described restriction endonucleases, the enzymes that opened up our ability to manipulate DNA through recombinant DNA technology. In 1972, Paul Berg was the first to create a recombinant DNA molecule.

Since 1972, geneticists have cloned numerous genes. Scientists now have the capability to create transgenic organisms, organisms with functioning foreign genes. For example, we now have farm animals that produce pharmaceuticals in their milk that are harvested easily and inexpensively for human use. In 1997, the first mammal was cloned, a sheep named Dolly. The sequence of the entire human genome was determined in 2000; we will spend the next century mining its information in the newly created field of genomics, the study of the complete genetic complement of an organism. Although no inherited disease has yet been cured by genetic intervention, we are on the verge of success in numerous diseases, including cancer.

The material here is much too brief to convey any of the detail or excitement surrounding the discoveries of modern genetics. Throughout this book, we will expand on the discoveries made since Darwin first published his book on evolutionary theory in 1859 and since Mendel was rediscovered in 1900.

THE THREE GENERAL AREAS OF GENETICS

Historically, geneticists have worked in three different areas, each with its own particular problems, terminology, tools, and organisms. These areas are classical genetics, molecular genetics, and evolutionary genetics. In *classical genetics,* we are concerned with the chromosomal theory of inheritance; that is, the concept that genes are

Table 1.1 The Three Major Areas of Genetics—Classical, Molecular, and Evolutionary—and the Topics They Cover

Classical Genetics	Molecular Genetics	Evolutionary Genetics
Mendel's principles	Structure of DNA	Quantitative genetics
Meiosis and mitosis	Chemistry of DNA	Hardy-Weinberg equilibrium
Sex determination	Transcription	Assumptions of equilibrium
Sex linkage	Translation	Evolution
Chromosomal mapping	DNA cloning and genomics	Speciation
Cytogenetics (chromosomal changes)	Control of gene expression	
	DNA mutation and repair	
	Extrachromosomal inheritance	

located in a linear fashion on chromosomes and that the relative positions of genes can be determined by their frequency in offspring. *Molecular genetics* is the study of the genetic material: its structure, replication, and expression, as well as the information revolution emanating from the discoveries of recombinant DNA techniques (genetic engineering, including the Human Genome Project). *Evolutionary genetics* is the study of the mechanisms of evolutionary change, or changes in gene frequencies in populations. Darwin's concept of evolution by natural selection finds a firm genetic footing in this area of the study of inheritance (table 1.1).

Today these areas are less clearly defined because of advances made in molecular genetics. Information coming from the study of molecular genetics allows us to understand better the structure and functioning of chromosomes on the one hand and the mechanism of natural selection on the other. In this book we hope to bring together this information from a historical perspective. From Mendel's work in discovering the rules of inheritance (chapter 2) to genetic engineering (chapter 13) to molecular evolution (chapter 21), we hope to present a balanced view of the various topics that make up genetics.

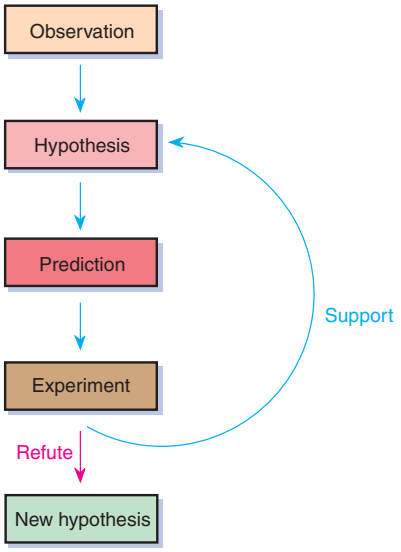

Figure 1.2 A schematic of the scientific method. An observation leads the researcher to propose a hypothesis, and then to make predictions from the hypothesis and to test these predictions by experiment. The results of the experiment either support or refute the hypothesis. If the experiment refutes the hypothesis, a new hypothesis must be developed. If the experiment supports the hypothesis, the researcher or others design further experiments to try to disprove it.

HOW DO WE KNOW?

Genetics is an empirical science, which means that our information comes from observations of the natural world. The *scientific method* is a tool for understanding these observations (fig. 1.2). At its heart is the experiment, which tests a guess, called a hypothesis, about how something works. In a good experiment, only two types of outcomes are possible: outcomes that support the hypothesis and outcomes that refute it. Scientists say these outcomes provide *strong inference.*

For example, you might have the idea that organisms can inherit acquired characteristics, an idea put forth by Jean-Baptiste Lamarck (1744–1829), a French biologist. Lamarck used the example of short-necked giraffes evolving into the long-necked giraffes we know of today. He suggested that giraffes that reached higher into trees to get at edible leaves developed longer necks. They passed on these longer necks to their offspring (in small increments in each generation), leading to today's long-necked giraffes. An alternative view, *evolution by natural selection,* was put forward in 1859 by Charles Darwin. According to the Darwinian view, giraffes normally varied in neck length, and these variations were inherited. Giraffes with slightly longer necks would be at an advantage in reaching edible leaves in trees. Therefore, over time, the longer-necked giraffes would survive and reproduce better than the shorter-necked ones. Thus, longer necks would come to predominate. Any genetic *mutations* (changes) that introduced greater neck length would be favored.

To test Lamarck's hypothesis, you might begin by designing an experiment. You could do the experiment on giraffes to test Lamarck's hypothesis directly; however, giraffes are difficult to acquire, maintain, and breed. Remember, though, that you are testing a general hypothesis about the inheritance of acquired characteristics rather than a specific hypothesis about giraffes. Thus, if you are clever enough, you can test the hypothesis with almost any organism. You would certainly choose one that is easy to maintain and manipulate experimentally. Later, you can verify the generality of any particular conclusions with tests on other organisms.

You might decide to use lab mice, which are relatively inexpensive to obtain and keep and have a relatively short generation time of about six weeks, compared with the giraffe's gestation period of over a year. Instead of looking at neck length, you might simply cut off the tip of the tail of each mouse (in a painless manner), using shortened tails as the acquired characteristic. You could then

BOX 1.1

Ethics and Genetics

The Lysenko Affair

As the pictures of geneticists throughout this book indicate, science is a very human activity; people living within societies explore scientific ideas and combine their knowledge. The society in which a scientist lives can affect not only how that scientist perceives the world, but also what that scientist can do in his or her scholarly activities. For example, the United States and other countries decided that mapping the entire human genome would be valuable (see chapter 13). Thus, granting agencies have directed money in this direction. Since much of scientific research is expensive, scientists often can only study areas for which funding is available. Thus, many scientists are working on the Human Genome Project. That is a positive example of society directing research. Examples also exist in which a societal decision has had negative consequences for both the scientific establishment and the society itself. An example is the Lysenko affair in the former Soviet Union during Stalin's and Krushchev's reigns.

Trofim Denisovich Lysenko was a biologist in the former Soviet Union researching the effects of temperature on plant development. At the same time, the preeminent Soviet geneticist was Nikolai Vavilov. Vavilov was interested in improving Soviet crop yields by growing and mating many varieties and selecting the best to be the breeding stock of the next generation. This is the standard way of improving a plant crop or livestock breed (see chapter 18, "Quantitative Inheritance"). The method conforms to genetic principles and therefore is successful. However, it is a slow process that only gradually improves yields.

Lysenko suggested that crop yields could be improved quickly by the inheritance of acquired characteristics (see chapter 21, "Evolution and Speciation"). Although doomed to fail because they denied the true and correct mechanisms of inheritance, Lysenko's ideas were greeted with much enthusiasm by the political elite. The enthusiasm was due not only to the fact that Lysenko promised immediate improvements in crop yields, but also to the fact that Lysenkoism was politically favored. That is, Lysenkoism fit in very well with communism; it promised that nature could be manipulated easily and immediately. If people could manipulate nature so easily, then communism could easily convert people to its doctrines.

Not only did Stalin favor Lysenkoism, but Lysenko himself was favored politically over Vavilov because Lysenko came from peasant stock, whereas Vavilov was from a wealthy family. (Remember that communism

mate these short-tailed mice to see if their offspring have shorter tails. If they do not, you could conclude that a shortened tail, an acquired characteristic, is not inherited. If, however, the next generation of mice have tails shorter than those of their parents, you could conclude that acquired characteristics can be inherited.

One point to note is that every good experiment has a *control,* a part of the experiment that ensures that some unknown variable, often specific to a particular time and place, is not causing the observed changes. For example, in your experiment, the particular food the mice ate may have had an effect on their growth, resulting in offspring with shorter tails. To control for this, you could handle a second group of mice in the exact same way that the experimental mice are handled, except you would not cut off their tails. Any reduction in the lengths of the tails of the offspring of the control mice would indicate an artifact of the experiment rather than the inheritance of acquired characteristics.

The point of doing this experiment (with the control group), as trivial as it might seem, is to determine the answer to a question using data based on what happens in nature. If you design your experiment correctly and carry it out without error, you can be confident about your results. If your results are negative, as ours would be here, then you would reject your hypothesis. Testing hypotheses and rejecting those that are refuted is the essence of the scientific method.

In fact, most of us live our lives according to the scientific method without really thinking about it. For example, we know better than to step out into traffic without looking because we are aware, from experience (observation, experimentation), of the validity of the laws of physics. Although from time to time anti-intellectual movements spread through society, few people actually give up relying on their empirical knowledge of the world to survive (box 1.1).

Nothing in this book is inconsistent with the scientific method. Every fact has been gained by experiment or observation in the real world. If you do not accept something said herein, you can go back to the *original literature,* the published descriptions of original experi-

was a revolution of the working class over the wealthy aristocracy.) Supported by Stalin, and then Krushchev, Lysenko gained inordinate power in his country. All visible genetic research in the former Soviet Union was forced to conform to Lysenko's Lamarckian views. People who disagreed with him were forced out of power; Vavilov was arrested in 1940 and died in prison in 1943. It was not until Nikita Krushchev lost power in 1964 that Lysenkoism fell out of favor. Within months, Lysenko's failed pseudoscience was repudiated and Soviet genetics got back on track.

For thirty years, Soviet geneticists were forced into fruitless endeavors, forced out of genetics altogether, or punished for their heterodox views. Superb scientists died in prison while crop improvement programs failed, all because the Soviet dictators favored Lysenkoism. The message of this affair is clear: Politicians can support research that agrees with their political agenda and punish scientists doing research that disagrees with this agenda, but politicians cannot change the truth of the laws of nature. Science, to be effective, must be done in a climate of open inquiry and free expression of ideas. The scientific method cannot be subverted by political bullies.

Trofim Denisovich Lysenko (1898–1976) shows branched wheat to collective farmers in the former Soviet Union. (© SOVFOTO.)

ments in scientific journals (as cited at the end of the book) and read about the work yourself. If you still don't believe a conclusion, you can repeat the work in question either to verify or challenge it. This is in keeping with the nature of the scientific method.

As mentioned, the results of experimental studies are usually published in scientific journals. Examples of journals that many geneticists read include *Genetics, Proceedings of the National Academy of Sciences, Science, Nature, Evolution, Cell, American Journal of Human Genetics, Journal of Molecular Biology,* and hundreds more. The reported research usually undergoes a process called *peer review* in which other scientists review an article before it is published to ensure its accuracy and its relevance. Scientific articles usually include a detailed justification for the work, an outline of the methods that allows other scientists to repeat the work, the results, a discussion of the significance of the results, and citations of prior work relevant to the present study.

At the end of this book, we cite journal articles describing research that has contributed to each chapter. (In chapter 2, we reprint part of Gregor Mendel's work, and in chapter 9, we reprint a research article by J. Watson and F. Crick in its entirety.) We also cite secondary sources, that is, journals and books that publish syntheses of the literature rather than original contributions. These include *Scientific American, Annual Review of Biochemistry, Annual Review of Genetics, American Scientist,* and others. You are encouraged to look at all of these sources in your efforts both to improve your grasp of genetics and to understand how science progresses.

WHY FRUIT FLIES AND COLON BACTERIA?

As you read this book, you will see that certain organisms are used repeatedly in genetic experiments. If the goal of science is to uncover generalities about the living world, why do geneticists persist in using the same few organisms

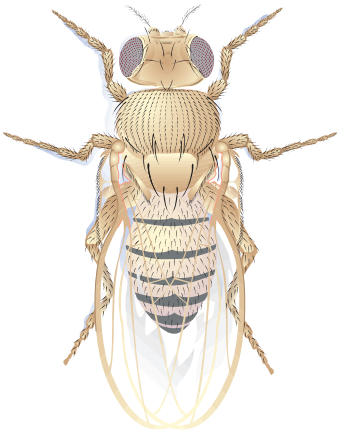

Figure 1.3 Adult female fruit fly, *Drosophila melanogaster.* Mutations of eye color, bristle type and number, and wing characteristics are easily visible when they occur.

Figure 1.4 Scanning electron micrograph of *Escherichia coli* bacteria. These rod-shaped bacilli are magnified 18,000x. (© K. G. Murti/Visuals Unlimited, Inc.)

in their work? The answer is probably obvious: the organisms used for any particular type of study have certain attributes that make them desirable *model organisms* for that research.

In the early stages of genetic research, at the turn of the century, no one had yet developed techniques to do genetic work with microorganisms or mammalian cells. At that time, the organism of preference was the fruit fly, *Drosophila melanogaster,* which developmental biologists had used (fig. 1.3). It has a relatively short generation time of about two weeks, survives and breeds well in the lab, has very large chromosomes in some of its cells, and has many aspects of its *phenotype* (appearance) genetically controlled. For example, it is easy to see the external results of mutations of genes that control eye color, bristle number and type, and wing characteristics such as shape or vein pattern in the fruit fly.

At the middle of this century, when geneticists developed techniques for genetic work on bacteria, the common colon bacterium, *Escherichia coli,* became a favorite organism of genetic researchers (fig. 1.4). Because it had a generation time of only twenty minutes and only a small amount of genetic material, many research groups used it in their experiments. Still later, bacterial viruses, called *bacteriophages,* became very popular in genetics labs. The viruses are constructed of only a few types of protein molecules and a very small amount of genetic material. Some can replicate a hundredfold in an hour. Our point is not to list the major organisms geneticists use, but to suggest why they use some so commonly. Comparative studies are usually done to determine which generalities discovered in the elite genetic organisms are really scientifically universal.

TECHNIQUES OF STUDY

Each area of genetics has its own particular techniques of study. Often the development of a new technique, or an improvement in a technique, has opened up major new avenues of research. As our technology has improved over the years, geneticists and other scientists have been able to explore at lower and lower levels of biological organization. Gregor Mendel, the father of genetics, did simple breeding studies of plants in a garden at his monastery in Austria in the middle of the nineteenth century. Today, with modern biochemical and biophysical techniques, it has become routine to determine the sequence of *nucleotides* (molecular subunits of DNA and RNA) that make up any particular gene. In fact, one of the most ambitious projects ever carried out in genetics is the mapping of the human genome, all 3.3 billion nucleotides that make up our genes. Only recently was the technology available to complete a project of this magnitude.

CLASSICAL, MOLECULAR, AND EVOLUTIONARY GENETICS

In the next three sections, we briefly outline the general subject areas covered in the book: classical, molecular, and evolutionary genetics.

Classical Genetics

Gregor Mendel discovered the basic rules of transmission genetics in 1866 by doing carefully controlled breeding experiments with the garden pea plant, *Pisum sativum.* He found that traits, such as pod color, were controlled by genetic elements that we now call *genes* (fig. 1.5). Alternative forms of a gene are called *alleles.* Mendel also discovered that adult organisms have two copies of each gene (*diploid* state); gametes receive just one of these copies (*haploid* state). In other words, one of the two parental copies segregates into any given gamete. Upon fertilization, the zygote gets one copy from each gamete, reconstituting the diploid number (fig. 1.6). When Mendel looked at the inheritance of several

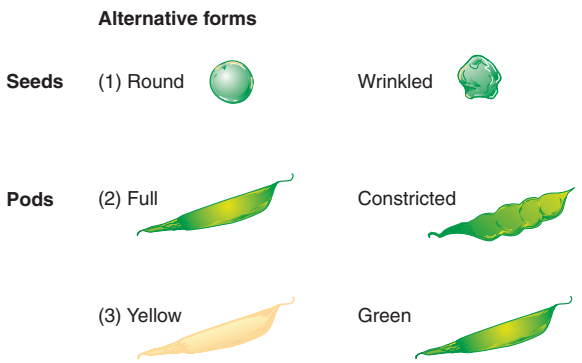

Figure 1.5 Mendel worked with garden pea plants. He observed seven traits of the plant—each with two discrete forms—that affected attributes of the seed, the pod, and the stem. For example, all plants had either round or wrinkled seeds, full or constricted pods, or yellow or green pods.

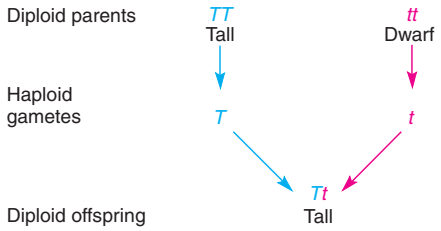

Figure 1.6 Mendel crossed tall and dwarf pea plants, demonstrating the rule of segregation. A diploid individual with two copies of the gene for tallness (*T*) per cell forms gametes that all have the *T* allele. Similarly, an individual that has two copies of the gene for shortness (*t*) forms gametes that all have the *t* allele. Cross-fertilization produces zygotes that have both the *T* and *t* alleles. When both forms are present (*Tt*), the plant is tall, indicating that the *T* allele is *dominant* to the *recessive t* allele.

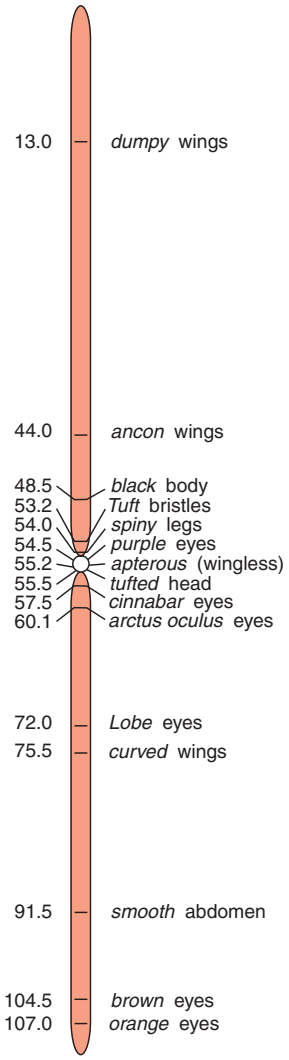

Figure 1.7 Genes are located in linear order on chromosomes, as seen in this diagram of chromosome 2 of *Drosophila melanogaster,* the common fruit fly. The centromere is a constriction in the chromosome. The numbers are map units.

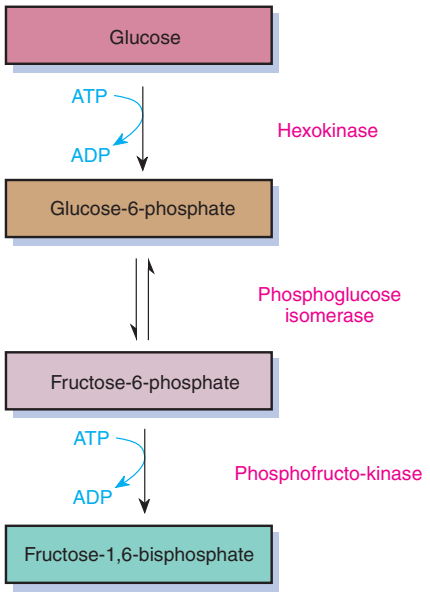

Figure 1.8 Biochemical pathways are the sequential changes that occur in compounds as cellular reactions modify them. In this case, we show the first few steps in the glycolytic pathway that converts glucose to energy. The pathway begins when glucose + ATP is converted to glucose-6-phosphate + ADP with the aid of the enzyme hexokinase. The enzymes are the products of genes.

traits at the same time, he found that they were inherited independently of each other. His work has been distilled into two rules, referred to as *segregation* and *independent assortment.* Scientists did not accept Mendel's work until they developed an understanding of the segregation of chromosomes during the latter half of the nineteenth century. At that time, in the year 1900, the science of genetics was born.

During much of the early part of this century, geneticists discovered many genes by looking for changed organisms, called *mutants.* Crosses were made to determine the genetic control of mutant traits. From this research evolved chromosomal mapping, the ability to locate the relative positions of genes on chromosomes by crossing certain organisms. The proportion of recombinant offspring, those with new combinations of parental alleles, gives a measure of the physical separation between genes on the same chromosomes in distances called *map units.* From this work arose the chromosomal theory of inheritance: Genes are located at fixed positions on chromosomes in a linear order (fig. 1.7, p. 9). This "beads on a string" model of gene arrangement was not modified to any great extent until the middle of this century, after Watson and Crick worked out the structure of DNA.

In general, genes function by controlling the synthesis of proteins called *enzymes* that act as biological catalysts in biochemical pathways (fig. 1.8). G. Beadle and E. Tatum suggested that one gene controls the formation of one enzyme. Although we now know that many proteins are made up of subunits—the products of several genes—and that some genes code for proteins that are not enzymes and other genes do not code for proteins, the *one-gene-one-enzyme* rule of thumb serves as a general guideline to gene action.

Molecular Genetics

With the exception of some viruses, the genetic material of all cellular organisms is double-stranded DNA, a double helical molecule shaped like a twisted ladder. The backbones of the helices are repeating units of sugars (deoxyribose) and phosphate groups. The rungs of the

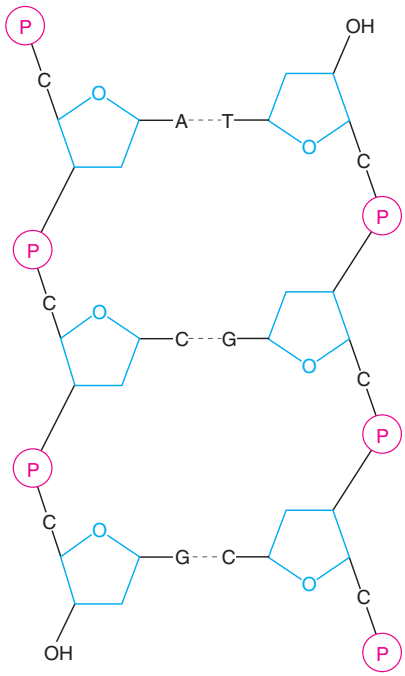

Figure 1.9 A look at a DNA double helix, showing the sugar-phosphate units that form the molecule's "backbone" and the base pairs that make up the "rungs." We abbreviate a phosphate group as a "P" within a circle; the pentagonal ring containing an oxygen atom is the sugar deoxyribose. Bases are either adenine, thymine, cytosine, or guanine (A, T, C, G).

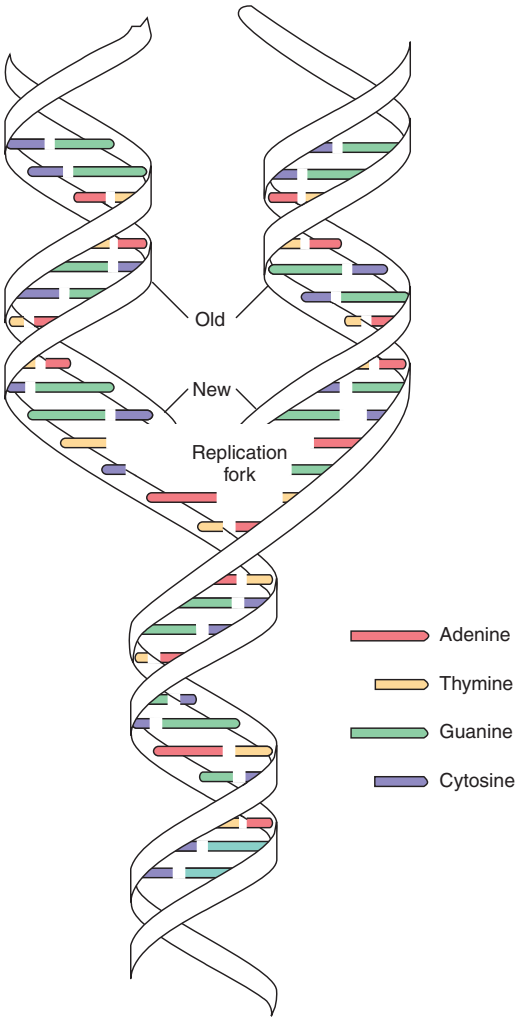

Figure 1.10 The DNA double helix unwinds during replication, and each half then acts as a template for a new double helix. Because of the rules of complementarity, each new double helix is identical to the original, and the two new double helices are identical to each other. Thus, an AT base pair in the original DNA double helix replicates into two AT base pairs, one in each of the daughter double helices.

ladder are base pairs, with one base extending from each backbone (fig. 1.9). Only four bases normally occur in DNA: adenine, thymine, guanine, and cytosine, abbreviated A, T, G, and C, respectively. There is no restriction on the order of bases on one strand. However, a relationship called *complementarity* exists between bases forming a rung. If one base of the pair is adenine, the other must be thymine; if one base is guanine, the other

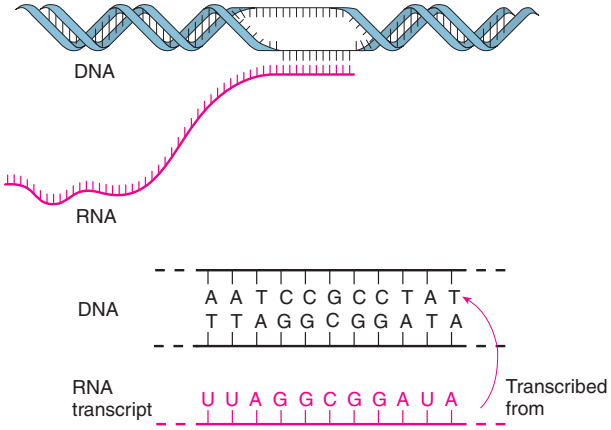

Figure 1.11 Transcription is the process that synthesizes RNA from a DNA template. Synthesis proceeds with the aid of the enzyme RNA polymerase. The DNA double helix partially unwinds during this process, allowing the base sequence of one strand to serve as a template for RNA synthesis. Synthesis follows the rules of DNA-RNA complementarity: A, T, G, and C of DNA pair with U, A, C, and G, respectively, in RNA. The resulting RNA base sequence is identical to the sequence that would form if the DNA were replicating instead, with the exception that RNA replaces thymine (T) with uracil (U).

must be cytosine. James Watson and Francis Crick deduced this structure in 1953, ushering in the era of molecular genetics.

The complementary nature of the base pairs of DNA made the mode of replication obvious to Watson and Crick: The double helix would "unzip," and each strand would act as a template for a new strand, resulting in two double helices exactly like the first (fig. 1.10). Mutation, a change in one of the bases, could result from either an error in base pairing during replication or some damage to the DNA that was not repaired by the time of the next replication cycle.

Information is encoded in DNA in the sequence of bases on one strand of the double helix. During gene expression, that information is *transcribed* into RNA, the other form of nucleic acid, which actually takes part in protein synthesis. RNA differs from DNA in several respects: it has the sugar ribose in place of deoxyribose; it has the base uracil (U) in place of thymine (T); and it usually occurs in a single-stranded form. RNA is transcribed from DNA by the enzyme *RNA polymerase,* using DNA-RNA rules of complementarity: A, T, G, and C in DNA pair with U, A, C, and G, respectively, in RNA (fig. 1.11). The DNA information that is transcribed into RNA codes for the amino acid sequence of proteins. Three nucleotide bases form a *codon* that specifies one of the twenty

Table 1.2 The Genetic Code Dictionary of RNA

Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid	Codon	Amino Acid
UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
UUA	Leu	UCA	Ser	UAA	STOP	UGA	STOP
UUG	Leu	UCG	Ser	UAG	STOP	UGG	Trp
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
AUG	Met (START)	ACG	Thr	AAG	Lys	AGG	Arg
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Note: A codon, specifying one amino acid, is three bases long (read in RNA bases in which U replaced the T of DNA). There are sixty-four different codons, specifying twenty naturally occurring amino acids (abbreviated by three letters: e.g., Phe is phenylalanine—see fig. 11.1 for the names and structures of the amino acids). Also present is *stop* (UAA, UAG, UGA) and *start* (AUG) information.

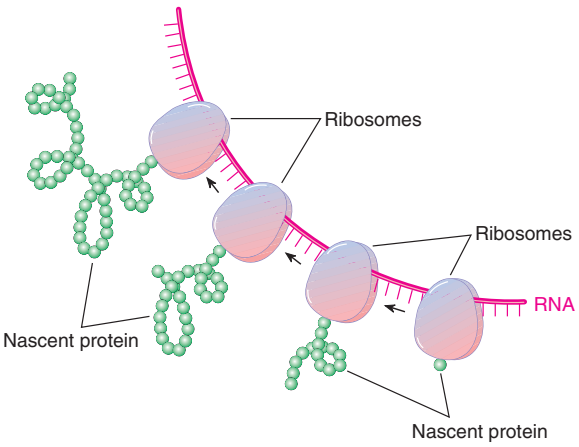

Figure 1.12 In prokaryotes, RNA translation begins shortly after RNA synthesis. A ribosome attaches to the RNA and begins reading the RNA codons. As the ribosome moves along the RNA, amino acids attach to the growing protein. When the process is finished, the completed protein is released from the ribosome, and the ribosome detaches from the RNA. As the first ribosome moves along, a second ribosome can attach at the beginning of the RNA, and so on, so that an RNA strand may have many ribosomes attached at one time.

naturally occurring amino acids used in protein synthesis. The sequence of bases making up the codons are referred to as the genetic code (table 1.2).

The process of *translation,* the decoding of nucleotide sequences into amino acid sequences, takes place at the ribosome, a structure found in all cells that is made up of RNA and proteins (fig. 1.12). As the RNA moves along the ribosome one codon at a time, one amino acid attaches to the growing protein for each codon.

The major control mechanisms of gene expression usually act at the transcriptional level. For transcription to take place, the RNA polymerase enzyme must be able to pass along the DNA; if this movement is prevented, transcription stops. Various proteins can bind to the DNA, thus preventing the RNA polymerase from continuing, providing a mechanism to control transcription. One particular mechanism, known as the *operon model,* provides the basis for a wide range of control mechanisms in prokaryotes and viruses. Eukaryotes generally contain no operons; although we know quite a bit about some control systems for eukaryotic gene expression, the general rules are not as simple.

In recent years, there has been an explosion of information resulting from *recombinant DNA techniques.* This revolution began with the discovery of *restriction endonucleases,* enzymes that cut DNA at specific se-

quences. Many of these enzymes leave single-stranded ends on the cut DNA. If a restriction enzyme acts on both a *plasmid,* a small, circular extrachromosomal unit found in some bacteria, and another piece of DNA (called foreign DNA), the two will be left with identical single-stranded free ends. If the cut plasmid and cut foreign DNA are mixed together, the free ends can re-form double helices, and the plasmid can take in a single piece of foreign DNA (fig. 1.13). Final repair processes create a completely closed circle of DNA. The hybrid plasmid is then reinserted into the bacterium. When the bacterium grows, it replicates the plasmid DNA, producing many copies of the foreign DNA. From that point, the foreign DNA can be isolated and sequenced, allowing researchers to determine the exact order of bases making up the foreign DNA. (In 2000, scientists announced the complete sequencing of the human genome.) That sequence can tell us much about how a gene works. In addition, the foreign genes can function within the bacterium, resulting in bacteria expressing the foreign genes and producing their protein products. Thus we have, for example, *E. coli* bacteria that produce human growth hormone.

This technology has tremendous implications in medicine, agriculture, and industry. It has provided the opportunity to locate and study disease-causing genes, such as the genes for cystic fibrosis and muscular dystrophy, as well as suggesting potential treatments. Crop plants and farm animals are being modified for better productivity by improving growth and disease resistance. Industries that apply the concepts of genetic engineering are flourishing.

One area of great interest to geneticists is cancer research. We have discovered that a single gene that has lost its normal control mechanisms (an *oncogene*) can cause changes that lead to cancer. These oncogenes exist normally in noncancerous cells, where they are called proto-oncogenes, and are also carried by viruses, where they are called viral oncogenes. Cancer-causing viruses are especially interesting because most of them are of the RNA type. AIDS is caused by one of these RNA viruses, which attacks one of the cells in the immune system. Cancer can also occur when genes that normally prevent cancer, genes called anti-oncogenes, lose function. Discovering the mechanism by which our immune system can produce millions of different protective proteins (*antibodies*) has been another success of modern molecular genetics.

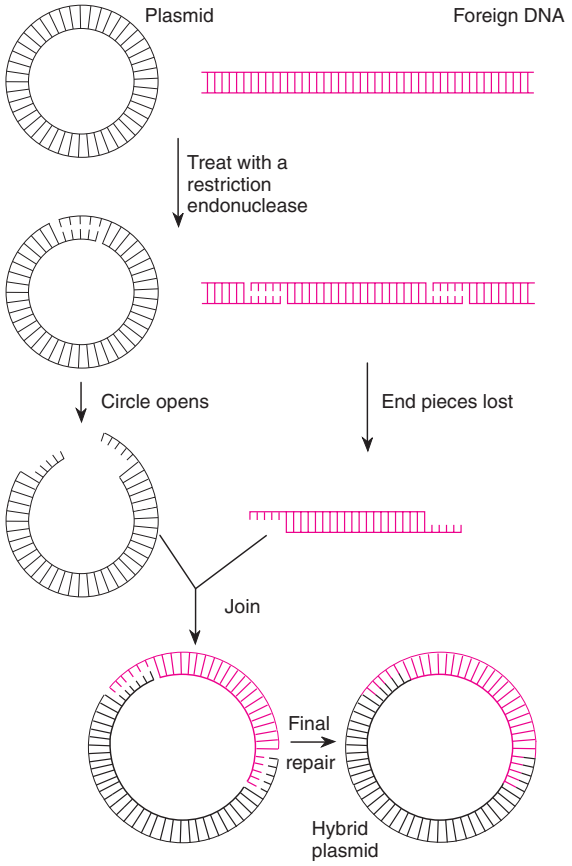

Figure 1.13 Hybrid DNA molecules can be constructed from a plasmid and a piece of foreign DNA. The ends are made compatible by cutting both DNAs with the same restriction endonuclease, leaving complementary ends. These ends will re-form double helices to form intact hybrid plasmids when the two types of DNA mix. A repair enzyme, DNA ligase, finishes patching the hybrid DNA within the plasmid. The hybrid plasmid is then reinjected into a bacterium, to be grown into billions of copies that will later be available for isolation and sequencing, or the hybrid plasmid can express the foreign DNA from within the host bacterium.

Evolutionary Genetics

From a genetic standpoint, evolution is the change in allelic frequencies in a population over time. Charles Darwin described evolution as the result of natural selection. In the 1920s and 1930s, geneticists, primarily Sewall Wright, R. A. Fisher, and J. B. S. Haldane, provided algebraic models to describe evolutionary processes. The marriage of Darwinian theory and population genetics has been termed *neo-Darwinism.*

In 1908, G. H. Hardy and W. Weinberg discovered that a simple genetic equilibrium occurs in a population if the population is large, has random mating, and has negligible effects of mutation, migration, and natural selection. This equilibrium gives population geneticists a baseline for comparing populations to see if any evolutionary

processes are occurring. We can formulate a statement to describe the equilibrium condition: If the assumptions are met, the population will not experience changes in allelic frequencies, and these allelic frequencies will accurately predict the frequencies of *genotypes* (allelic combinations in individuals, e.g., *AA, Aa,* or *aa*) in the population.

Recently, several areas of evolutionary genetics have become controversial. Electrophoresis (a method for separating proteins and other molecules) and subsequent DNA sequencing have revealed that much more *polymorphism* (variation) exists within natural populations than older mathematical models could account for. One of the more interesting explanations for this variability is that it is neutral. That is, natural selection, the guiding force of evolution, does not act differentially on many, if not most, of the genetic differences found so commonly in nature. At first, this theory was quite controversial, attracting few followers. Now it seems to be the view the majority accept to explain the abundance of molecular variation found in natural populations.

Another controversial theory concerns the rate of evolutionary change. It is suggested that most evolutionary change is not gradual, as the fossil record seems to indicate, but occurs in short, rapid bursts, followed by long periods of very little change. This theory is called *punctuated equilibrium.*

A final area of evolutionary biology that has generated much controversy is the theory of *sociobiology.* Sociobiologists suggest that social behavior is under genetic control and is acted upon by natural selection, as is any morphological or physiological trait. This idea is controversial mainly as it applies to human beings; it calls altruism into question and suggests that to some extent we are genetically programmed to act in certain ways. People have criticized the theory because they feel it justifies racism and sexism.

SUMMARY

The purpose of this chapter has been to provide a brief history of genetics and a brief overview of the following twenty chapters. We hope it serves to introduce the material and to provide a basis for early synthesis of some of the material that, of necessity, is presented in the discrete units called chapters. This chapter also differs from all the others because it lacks some of the end materials that should be of value to you as you proceed: solved problems, and exercises and problems. These features are presented chapter by chapter throughout the remainder of the book. At the end of the book, we provide answers to exercises and problems and a glossary of all **boldface** words throughout the book.

Suggested Readings for chapter 1 are on page B-1.

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2

MENDEL'S PRINCIPLES

STUDY OBJECTIVES

1. To understand that genes are discrete units that control the appearance of an organism 17
2. To understand Mendel's rules of inheritance: segregation and independent assortment 18
3. To understand that dominance is a function of the interaction of alleles; similarly, epistasis is a function of the interaction of nonallelic genes 22
4. To define how genes generally control the production of enzymes and thus the fate of biochemical pathways 37

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The garden pea plant, *Pisum sativum.*

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Genetics is concerned with the transmission, expression, and evolution of genes, the molecules that control the function, development, and ultimate appearance of individuals. In this section of the book, we will look at the rules of transmission that govern genes and affect their passage from one generation to the next. Gregor Johann Mendel discovered these rules of inheritance; we derive and expand upon his rules in this chapter (fig. 2.1).

In 1900, three botanists, Carl Correns of Germany, Erich von Tschermak of Austria, and Hugo de Vries of Holland, defined the rules governing the transmission of traits from parent to offspring. Some historical controversy exists as to whether these botanists actually rediscovered Mendel's rules by their own research or whether their research led them to Mendel's original paper. In any case, all three made important contributions to the early stages of genetics. The rules had been published previously, in 1866, by an obscure Austrian monk, Gregor Johann Mendel. Although his work was widely available after 1866, the scientific community was not ready to appreciate Mendel's great contribution until the turn of the century. There are at least four reasons for this lapse of thirty-four years.

Figure 2.1 Gregor Johann Mendel (1822–84). (Reproduced by permission of the Moravski Museum, Mendelianum.)

First, before Mendel's experiments, biologists were primarily concerned with explaining the transmission of characteristics that could be measured on a continuous scale, such as height, cranium size, and longevity. They were looking for rules of inheritance that would explain such **continuous variations,** especially after Darwin put forth his theory of evolution in 1859 (see chapter 21). Mendel, however, suggested that inherited characteristics were discrete and constant **(discontinuous):** peas, for example, were either yellow or green. Thus, evolutionists were looking for small changes in traits with continuous variation, whereas Mendel presented them with rules for discontinuous variation. His principles did not seem to apply to the type of variation that biologists thought prevailed. Second, there was no physical element identified with Mendel's inherited entities. One could not say, upon reading Mendel's work, that a certain subunit of the cell followed Mendel's rules. Third, Mendel worked with large numbers of offspring and converted these numbers to ratios. Biologists, practitioners of a very descriptive science at the time, were not well trained in mathematical tools. And last, Mendel was not well known and did not persevere in his attempts to convince the academic community that his findings were important.

Between 1866 and 1900, two major changes took place in biological science. First, by the turn of the century, not only had scientists discovered chromosomes, but they also had learned to understand chromosomal movement during cell division. Second, biologists were better prepared to handle mathematics by the turn of the century than they were during Mendel's time.

MENDEL'S EXPERIMENTS

Gregor Mendel was an Austrian monk (of Brünn, Austria, which is now Brno, Czech Republic). In his experiments, he tried to **crossbreed** plants that had discrete, nonoverlapping characteristics and then to observe the distribution of these characteristics over the next several generations. Mendel worked with the common garden pea plant, *Pisum sativum.* He chose the pea plant for at least three reasons: (1) The garden pea was easy to cultivate and had a relatively short life cycle. (2) The plant had discontinuous characteristics such as flower color and pea texture. (3) In part because of its anatomy, pollination of the plant was easy to control. Foreign pollen could be kept out, and **cross-fertilization** could be accomplished artificially.

Figure 2.2 shows a cross section of the pea flower that indicates the keel, in which the male and female parts develop. Normally, **self-fertilization** occurs when pollen falls onto the stigma before the bud opens. Mendel cross-fertilized the plants by opening the keel of

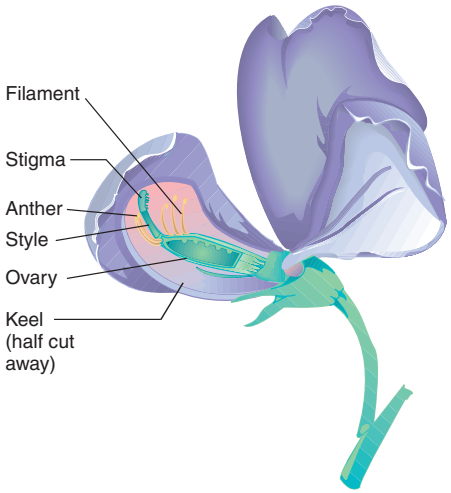


Figure 2.2 Anatomy of the garden pea plant flower. The female part, the pistil, is composed of the stigma, its supporting style, and the ovary. The male part, the stamen, is composed of the pollen-producing anther and its supporting filament.

a flower before the anthers matured and placing pollen from another plant on the stigma. In the more than ten thousand plants Mendel examined, only a few were fertilized other than the way he had intended (either self- or cross-pollinated).

Mendel used plants obtained from suppliers and grew them for two years to ascertain that they were homogeneous, or true-breeding, for the particular characteristic under study. He chose for study the seven characteristics shown in figure 2.3. Take as an example the characteristic of plant height. Although height is often continuously distributed, Mendel used plants that displayed only two alternatives: tall or dwarf. He made the crosses shown in figure 2.4. In the parental, or P_1, generation, dwarf plants pollinated tall plants, and, in a **reciprocal cross,** tall plants pollinated dwarf plants, to determine whether the results were independent of the parents' sex. As we will see later on, some traits follow inheritance patterns related to the sex of the parent carrying the traits. In those cases, reciprocal crosses give different results; with Mendel's tall and dwarf pea plants, the results were the same.

Offspring of the cross of P_1 individuals are referred to as the first **filial generation,** or F_1. Mendel also referred to them as **hybrids** because they were the offspring of unlike parents (tall and dwarf). We will specifically refer to the offspring of tall and dwarf peas as **monohybrids** because they are hybrid for only one characteristic (height). Since all the F_1 offspring plants were tall, Mendel referred to tallness as the **dominant** trait. The alternative, dwarfness, he referred to as **recessive.** Different forms of a gene that exist within a population are termed **alleles.** The terms *dominant* and *recessive* are used to describe both the relationship between the alleles and the traits they control. Thus, we say that both the allele for tallness and the trait, tall, are dominant. Dominance applies to the appearance of the trait when both a dominant and a recessive allele are present. It does not imply that the dominant trait is better, is more abundant, or will increase over time in a population.

When the F_1 offspring of figure 2.4 were self-fertilized to produce the F_2 generation, both tall and dwarf offspring occurred; the dwarf characteristic reappeared. Among the F_2 offspring, Mendel observed 787 tall and 277 dwarf plants for a ratio of 2.84:1. It is an indication of Mendel's insight that he recognized in these numbers an approximation to a 3:1 ratio, a ratio that suggested to him the mechanism of inheritance at work in pea plant height.

SEGREGATION

Rule of Segregation

Mendel assumed that each plant contained two determinants (which we now call **genes**) for the characteristic of height. For example, a hybrid F_1 pea plant possesses the dominant allele for tallness and the recessive allele for dwarfness for the gene that determines plant height. A pair of alleles for dwarfness is required to develop the recessive phenotype. Only one of these alleles is passed into a single **gamete,** and the union of two gametes to form a zygote restores the double complement of alleles. The fact that the recessive trait reappears in the F_2 generation shows that the allele controlling it was hidden in the F_1 individual and passed on unaffected. This explanation of the passage of discrete trait determinants, or genes, comprises Mendel's first principle, the **rule of segregation.** The rule of segregation can be summarized as follows: A gamete receives only one allele from the pair of alleles an organism possesses; fertilization (the union of two gametes) reestablishes the double number. We can visualize this process by redrawing figure 2.4 using letters to denote the alleles. Mendel used capital letters to denote alleles that control dominant traits and lowercase letters for alleles that control recessive traits. Following this notation, *T* refers to the allele controlling tallness and *t* refers to the allele controlling shortness (dwarf stature). From figure 2.5, we can see that Mendel's rule of segregation explains the homogeneity of the F_1 generation (all tall) and the 3:1 ratio of tall-to-dwarf offspring in the F_2 generation.

Let us define some terms. The **genotype** of an organism is the gene combination it possesses. In figure 2.5,

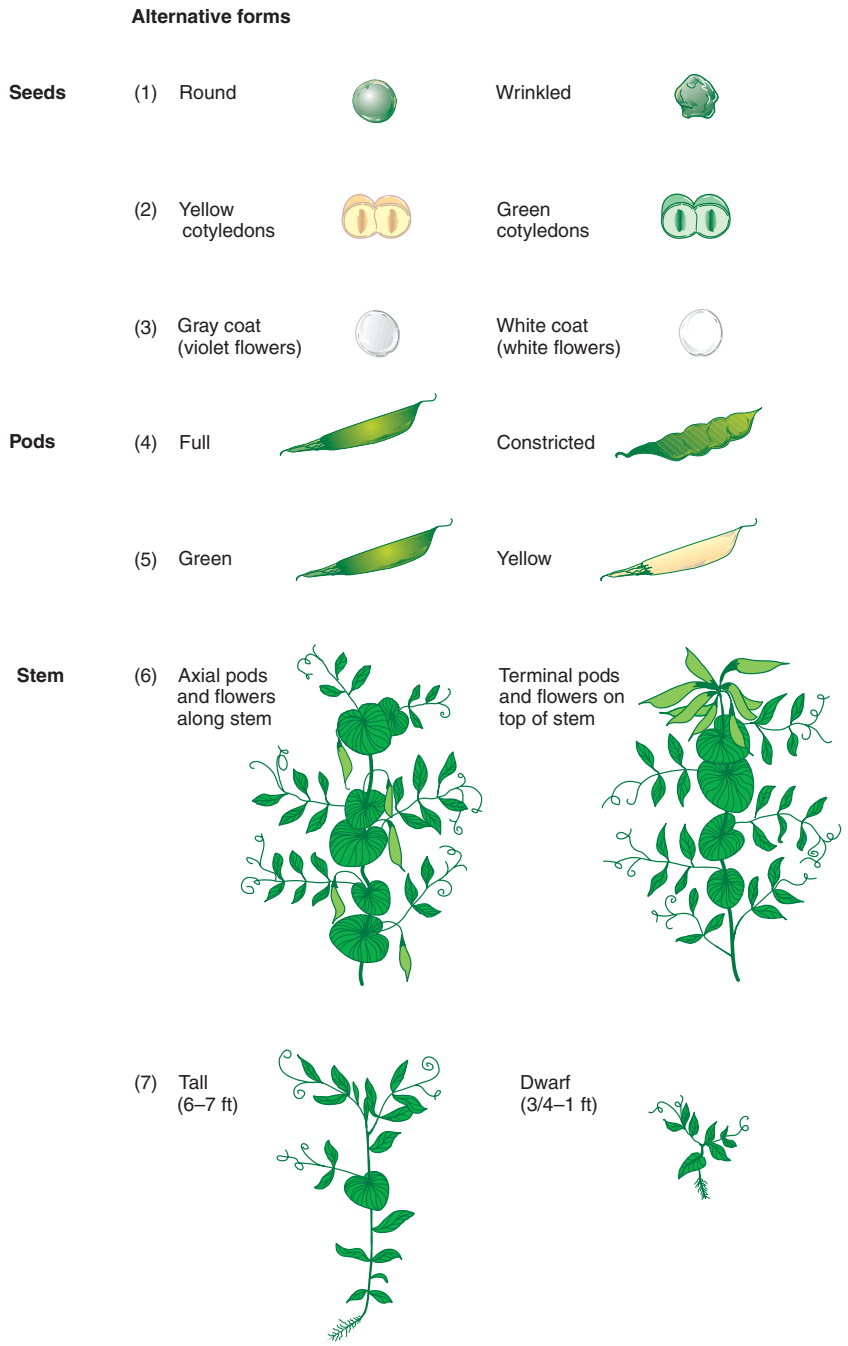


Figure 2.3 Seven characteristics that Mendel observed in peas. Traits in the left column are dominant.

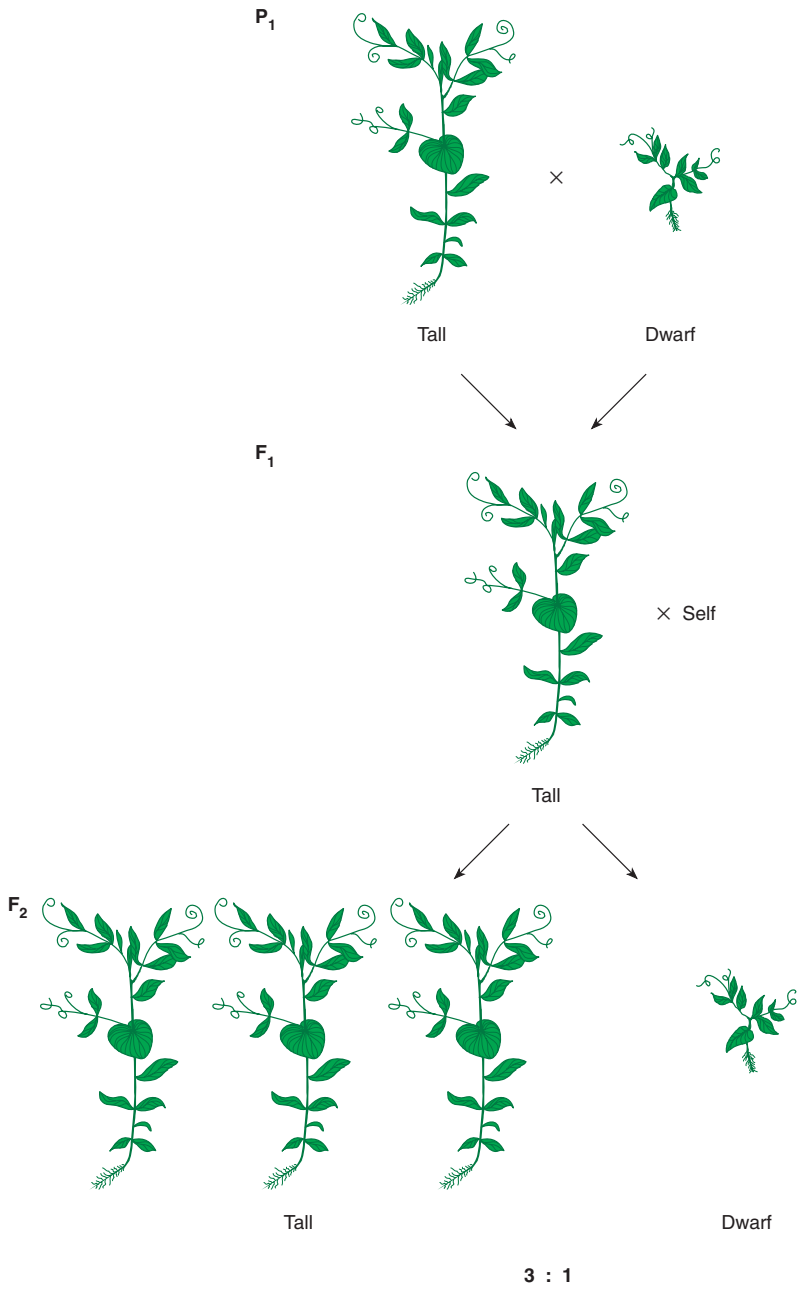


Figure 2.4 First two offspring generations from the cross of tall plants with dwarf plants.

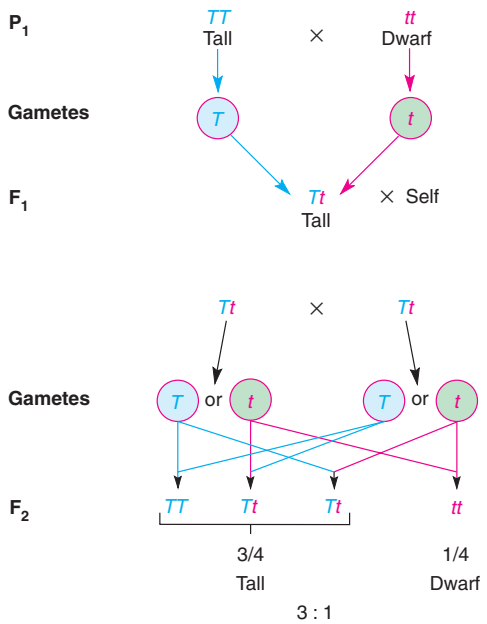


Figure 2.5 Assigning genotypes to the cross in figure 2.4.

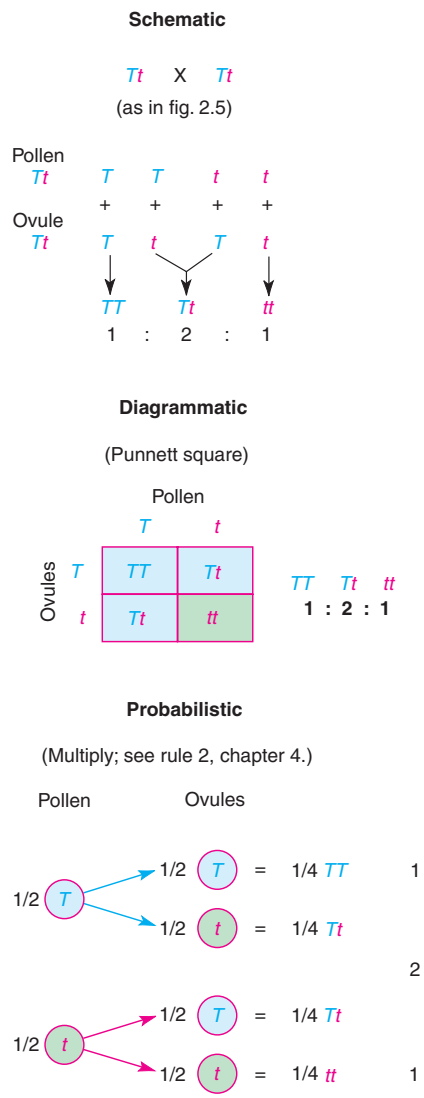


Figure 2.6 Methods of determining F_2 genotypic combinations in a self-fertilized monohybrid. The Punnett square diagram is named after the geneticist Reginald C. Punnett.

the genotype of the parental tall plant is *TT;* that of the F_1 tall plant is *Tt.* **Phenotype** refers to the observable attributes of an organism. Plants with either of the two genotypes *TT* or *Tt* are phenotypically tall. Genotypes come in two general classes: **homozygotes,** in which both alleles are the same, as in *TT* or *tt,* and **heterozygotes,** in which the two alleles are different, as in *Tt.* William Bateson coined these last two terms in 1901. Danish botanist Wilhelm Johannsen first used the word *gene* in 1909.

If we look at figure 2.5, we can see that the *TT* homozygote can produce only one type of gamete, the *T*-bearing kind, and the *tt* homozygote can similarly produce only *t*-bearing gametes. Thus, the F_1 individuals are uniformly heterozygous *Tt,* and each F_1 individual can produce two kinds of gametes in equal frequencies, *T*- or *t*-bearing. In the F_2 generation, these two types of gametes randomly pair during fertilization. Figure 2.6 shows three ways of picturing this process.

Testing the Rule of Segregation

We can see from figure 2.6 that the F_2 generation has a phenotypic ratio of 3:1, the classic Mendelian ratio. However, we also see a genotypic ratio of 1:2:1 for dominant homozygote:heterozygote:recessive homozygote. Demonstrating this genotypic ratio provides a good test of Mendel's rule of segregation.

The simplest way to test the hypothesis is by **progeny testing,** that is, by self-fertilizing F_2 individuals to produce an F_3 generation, which Mendel did (fig. 2.7). Treating the rule of segregation as a hypothesis, it is possible to predict the frequencies of the phenotypic classes that would result. The dwarf F_2 plants should be recessive homozygotes, and so, when **selfed** (self-fertilized), they should produce only *t*-bearing gametes and only dwarf offspring in the F_3 generation. The tall F_2 plants, however, should be a heterogeneous group, one-third of which should be homozygous *TT* and two-thirds heterozygous *Tt*. The tall homozygotes, when selfed, should produce only tall F_3 offspring (genotypically *TT*). However, the F_2 heterozygotes, when selfed, should produce

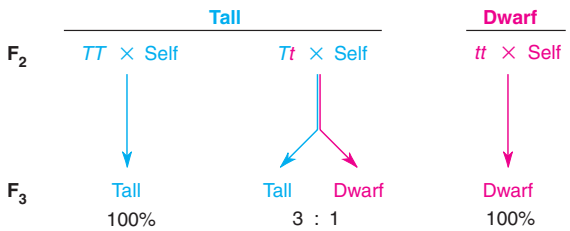


Figure 2.7 Mendel self-fertilized F_2 tall and dwarf plants. He found that all the dwarf plants produced only dwarf progeny. Among the tall plants, 72% produced both tall and dwarf progeny in a 3:1 ratio.

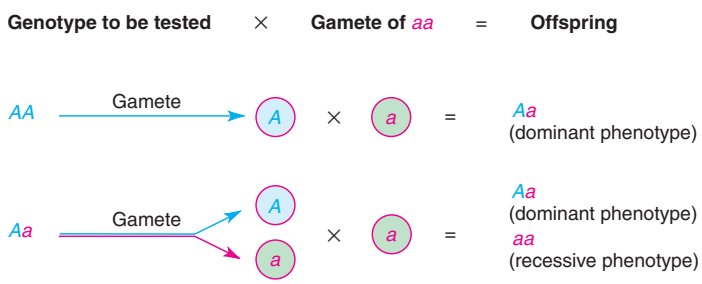


Figure 2.8 Testcross. In a testcross, the phenotype of an offspring is determined by the allele the offspring inherits from the parent with the genotype being tested.

tall and dwarf offspring in a ratio identical to that the selfed F_1 plants produced: three tall to one dwarf offspring. Mendel found that all the dwarf (homozygous) F_2 plants bred true as predicted. Among the tall, 28% (28/100) bred true (produced only tall offspring) and 72% (72/100) produced both tall and dwarf offspring. Since the prediction was one-third (33.3%) and two-thirds (66.7%), respectively, Mendel's observed values were very close to those predicted. We thus conclude that Mendel's progeny-testing experiment confirmed his hypothesis of segregation. In fact, a statistical test—developed in chapter 4—would also the support this conclusion.

Another way to test the segregation rule is to use the extremely useful method of the **testcross,** that is, a cross of any organism with a recessive homozygote. (Another type of cross, a **backcross,** is the cross of a progeny with an individual that has a parental genotype. Hence, a testcross can often be a backcross.) Since the gametes of the recessive homozygote contain only recessive alleles, the alleles that the gametes of the other parent carry will determine the phenotypes of the offspring. If a gamete from the organism being tested contains a recessive allele, the resulting F_1 organism will have a recessive phenotype; if it contains a dominant allele, the F_1 organism will have a dominant phenotype. Thus, in a testcross, the genotypes of the gametes from the organism being tested determine the phenotypes of the offspring (fig. 2.8). A testcross of the tall F_2 plants in figure 2.5 would produce the results shown in figure 2.9. These results further confirm Mendel's rule of segregation.

Tall (two classes)

TT × *tt* = all *Tt*

Tt × *tt* = *Tt* : *tt*

1 : 1

Figure 2.9 Testcrossing the dominant phenotype of the F_2 generation from figure 2.5.

DOMINANCE IS NOT UNIVERSAL

If dominance were universal, the heterozygote would always have the same phenotype as the dominant homozygote, and we would always see the 3:1 ratio when heterozygotes are crossed. If, however, the heterozygote were distinctly different from both homozygotes, we

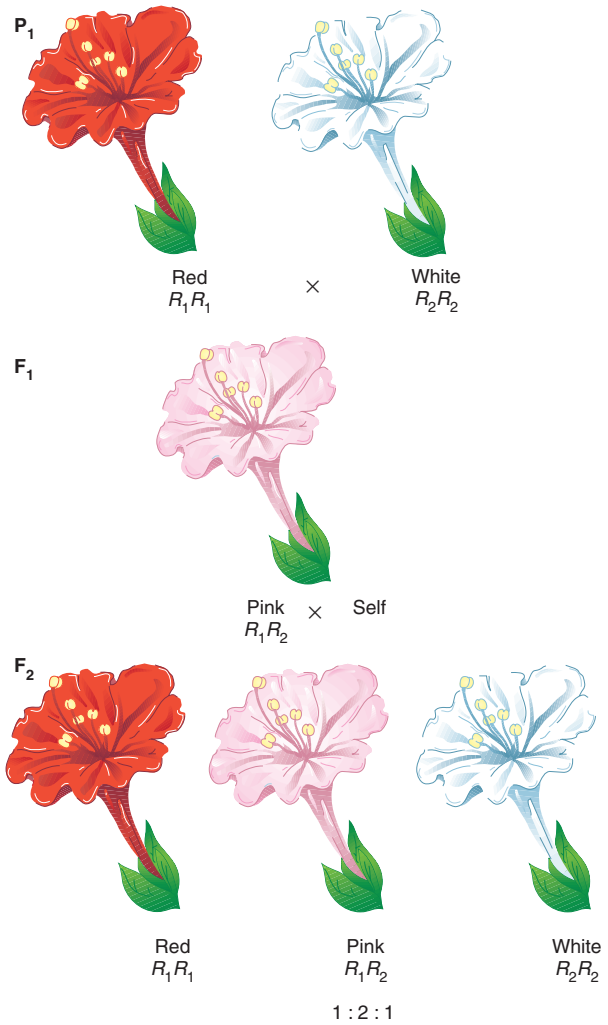


Figure 2.10 Flower color inheritance in the four-o'clock plant: an example of partial, or incomplete, dominance.

would see a 1:2:1 ratio of phenotypes when heterozygotes are crossed. In **partial dominance (or incomplete dominance),** the phenotype of the heterozygote falls between those of the two homozygotes. An example occurs in flower petal color in some plants.

Using four-o'clock plants (*Mirabilis jalapa*), we can cross a plant that has red flower petals with another that has white flower petals; the offspring will have pink flower petals. If these pink-flowered F_1 plants are crossed, the F_2 plants appear in a ratio of 1:2:1, having red, pink, or white flower petals, respectively (fig. 2.10). The pink-flowered plants are heterozygotes that have a petal color intermediate between the red and white colors of the homozygotes. In this case, one allele (R_1) specifies red pigment color, and another allele specifies no color (R_2; the flower petals have a white background color). Flowers in heterozygotes (R_1R_2) have about half the red pigment of the flowers in red homozygotes (R_1R_1) because the heterozygotes have only one copy of the allele that produces color, whereas the homozygotes have two copies.

As technology has improved, we have found more and more cases in which we can differentiate the heterozygote. It is now clear that dominance and recessiveness are phenomena dependent on which alleles are interacting and on what phenotypic level we are studying. For example, in Tay-Sachs disease, homozygous recessive children usually die before the age of three after suffering severe nervous system degeneration; heterozygotes seem to be normal. As biologists have discovered how the disease works, they have made the detection of the heterozygotes possible.

As with many genetic diseases, the culprit is a defective **enzyme** (protein catalyst). Afflicted homozygotes have no enzyme activity, heterozygotes have about half the normal level, and, of course, homozygous normal individuals have the full level. In the case of Tay-Sachs disease, the defective enzyme is hexoseaminidase-A, needed for proper lipid metabolism. Modern techniques allow technicians to assay the blood for this enzyme and to identify heterozygotes by their intermediate level of enzyme activity. Two heterozygotes can now know that there is a 25% chance that any child they bear will have the disease. They can make an educated decision as to whether or not to have children.

The other category in which the heterozygote is discernible occurs when the heterozygous phenotype is not on a scale somewhere between the two homozygotes, but actually expresses both phenotypes simultaneously. We refer to this situation as **codominance.** For example, people with blood type AB are heterozygotes who express both the *A* and *B* alleles for blood type (see the section entitled "Multiple Alleles" for more information about blood types). Electrophoresis (a technique described in chapter 5) lets us see proteins directly and also gives us many examples of codominance when we can see the protein products of both alleles.

NOMENCLATURE

Throughout the last century, botanists, zoologists, and microbiologists have adopted different methods for naming alleles. Botanists and mammalian geneticists tend to prefer the capital-lowercase scheme. *Drosophila* geneticists and microbiologists have adopted schemes that relate to the **wild-type.** The wild-type is the phenotype of the organism commonly found in nature. Though other naturally occurring phenotypes of the same species may also be present, there is usually an agreed-upon common

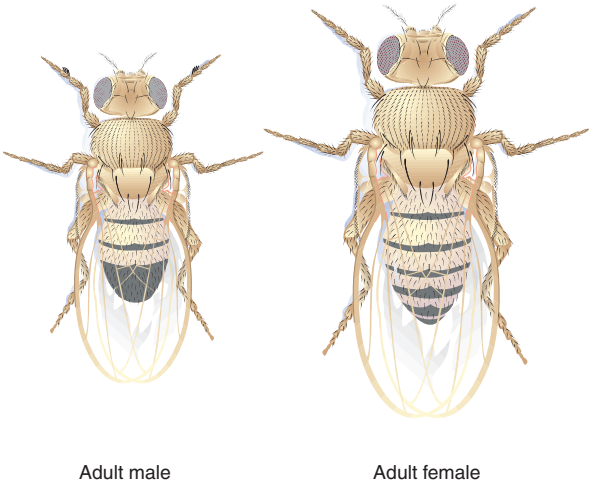


Figure 2.11 Wild-type fruit fly, *Drosophila melanogaster.*

Table 2.1 **Some Mutants of *Drosophila***

Mutant Designation	Description	Dominance Relationship to Wild-Type
abrupt (*ab*)	Shortened, longitudinal, median wing vein	Recessive
amber (*amb*)	Pale yellow body	Recessive
black (*b*)	Black body	Recessive
Bar (*B*)	Narrow, vertical eye	Dominant
dumpy (*dp*)	Reduced wings	Recessive
Hairless (*H*)	Various bristles absent	Dominant
white (*w*)	White eye	Recessive
white-apricot (w^a)	Apricot-colored eye (allele of white eye)	Recessive

phenotype that is referred to as the wild-type. For fruit flies (*Drosophila*), organisms commonly used in genetic studies, the wild-type has red eyes and round wings (fig. 2.11). Alternatives to the wild-type are referred to as **mutants** (fig. 2.12). Thus, red eyes are wild-type, and white eyes are mutant. Fruit fly genes are named after the mutant, beginning with a capital letter if the mutation is dominant and a lowercase letter if it is recessive. Table 2.1 gives some examples. The wild-type allele often carries the symbol of the mutant with a + added as a superscript; by definition, every mutant has a wild-type allele as an alternative. For example, *w* stands for the white-eye allele, a recessive mutation. The wild-type (red eyes) is thus assigned the symbol w^+. Hairless is a dominant allele with the symbol *H.* Its wild-type allele is denoted as H^+. Sometimes geneticists use the + symbol alone for the wild-type, but only when there will be no confusion about its use. If we are discussing eye color only, then + is clearly the same as w^+: both mean red eyes. However, if we are discussing both eye color and bristle morphology, the + alone could refer to either of the two aspects of the phenotype and should be avoided.

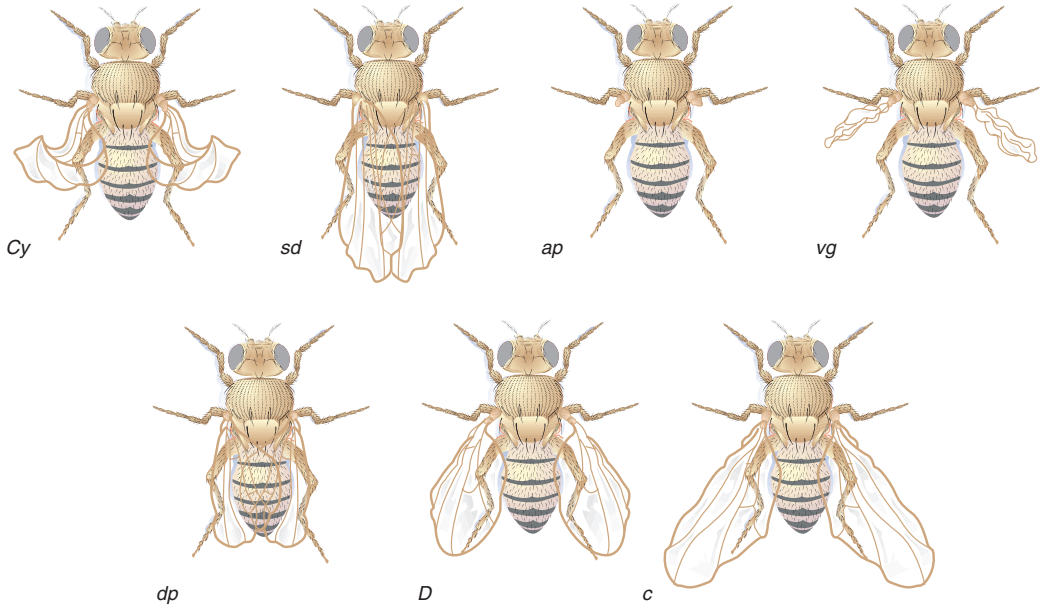


Figure 2.12 Wing mutants of *Drosophila melanogaster* and their allelic designations: *Cy,* curly; *sd,* scalloped; *ap,* apterous; *vg,* vestigial; *dp,* dumpy; *D,* Dichaete; *c,* curved.

Table 2.2 ABO Blood Types with Immunity Reactions

Blood Type Corresponding to Antigens on Red Blood Cells	Antibodies in Serum	Genotype	Reaction of Red Cells to Anti-A Antibodies	Reaction of Red Cells to Anti-B Antibodies
O	Anti-A and anti-B	*ii*	−	−
A	Anti-B	I^AI^A or I^Ai	+	−
B	Anti-A	I^BI^B or I^Bi	−	+
AB	None	I^AI^B	+	+

MULTIPLE ALLELES

A given gene can have more than two alleles. Although any particular individual can have only two, many alleles of a given gene may exist in a population. The classic example of multiple human alleles is in the ABO blood group, which Karl Landsteiner discovered in 1900. This is the best known of all the red-cell antigen systems primarily because of its importance in blood transfusions. There are four blood-type phenotypes produced by three alleles (table 2.2). The I^A and I^B alleles are responsible for the production of the A and B antigens found on the surface of the erythrocytes (red blood cells). Antigens are substances, normally foreign to the body, that induce the immune system to produce antibodies (proteins that bind to the antigens). The ABO system is unusual because antibodies can be present (e.g., anti-B antibodies can exist in a type A person) without prior exposure to the antigen. Thus, people with a particular ABO antigen on their red cells will have in their serum the antibody against the other antigen: type A persons have A antigen on their red cells and anti-B antibody in their serum; type B persons have B antigen on their red cells and anti-A antibody in their serum; type O persons do not have either antigen but have both antibodies in their serum; and type AB persons have both A and B antigens and form neither anti-A or anti-B antibodies in their serum.

The I^A and I^B alleles, coding for glycosyl transferase enzymes, each cause a different modification to the terminal sugars of a mucopolysaccharide (H structure) found on the surface of red blood cells (fig. 2.13). They are codominant because both modifications (antigens) are present in a heterozygote. In fact, whichever enzyme (product of the I^A or I^B allele) reaches the H structure first will modify it. Once modified, the H structure will not respond to the other enzyme. Therefore, both A and B antigens will be produced in the heterozygote in roughly equal proportions. The *i* allele causes no change to the H structure: because of a mutation it produces a nonfunctioning enzyme. The *i* allele and its phenotype are recessive; the presence of the I^A or I^B allele, or both,

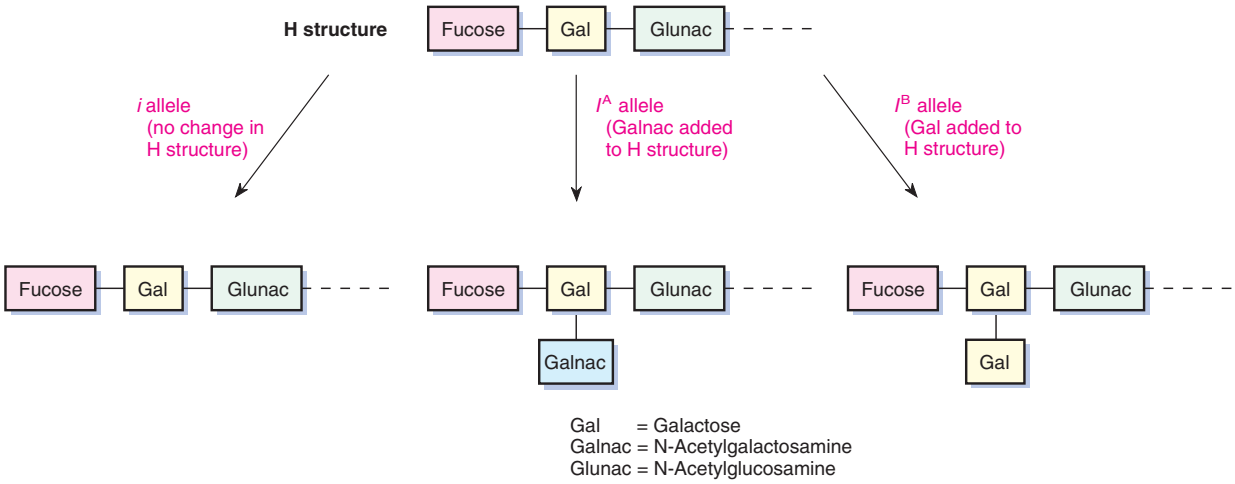


Figure 2.13 Function of the I^A, I^B, and *i* alleles of the ABO gene. The gene products of the I^A and I^B alleles of the ABO gene affect the terminal sugars of a mucopolysaccharide (H structure) found on red blood cells. The gene products of the I^A and I^B alleles are the enzymes alpha-3-N-acetyl-D-galactosaminyltransferase and alpha-3-D-galactosyltransferase, respectively.

will modify the H product, thus masking the fact that the *i* allele was ever there.

Adverse reactions to blood transfusions primarily occur because the antibodies in the recipient's serum react with the antigens on the donor's red blood cells. Thus, type A persons cannot donate blood to type B persons. Type B persons have anti-A antibody, which reacts with the A antigen on the donor red cells and causes the cells to clump.

Since both I^A and I^B are dominant to the *i* allele, this system not only shows multiple allelism, it also demonstrates both codominance and simple dominance. (As with virtually any system, intense study yields more information, and subgroups of type A are known. We will not, however, deal with that complexity here.) According to the American Red Cross, 46% of blood donors in the United States are type O, 40% are type A, 10% are type B, and 4% are type AB.

Many other genes also have multiple alleles. In some plants, such as red clover, there is a gene, the S gene, with several hundred alleles that prevent self-fertilization. This means that a pollen grain is not capable of forming a successful pollen tube in the style if the pollen grain or its parent plant has a self-incompatibility allele that is also present in the plant to be fertilized. Thus, pollen grains from a flower falling on its own stigma are rejected. Only a pollen grain with either a different self-incompatibility allele or from a parent plant with different self-incompatibility alleles is capable of fertilization; this avoids inbreeding. Thus, over evolutionary time, there has been selection for many alleles of this gene. Presumably, a foreign plant would not want to be mistaken for the same plant, providing the selective pressure for many alleles to survive in a population. Recent research has indicated that the products of the *S* alleles are ribonuclease enzymes, enzymes that destroy RNA. Researchers are interested in discovering the molecular mechanisms for this pollen rejection.

In *Drosophila,* numerous alleles of the white-eye gene exist, and people have numerous hemoglobin alleles. In fact, multiple alleles are the rule rather than the exception.

INDEPENDENT ASSORTMENT

Mendel also analyzed the inheritance pattern of traits observed two at a time. He looked, for instance, at plants that differed in the form and color of their peas: he crossed true-breeding (homozygous) plants that had seeds that were round and yellow with plants that produced seeds that were wrinkled and green. Mendel's results appear in figure 2.14. The F_1 plants all had round, yellow seeds, which demonstrated that round was dominant to wrinkled and yellow was dominant to green. When these F_1 plants were self-fertilized, they produced an F_2 generation that had all four possible combinations of the two seed characteristics: round, yellow seeds; round, green seeds; wrinkled, yellow seeds; and wrinkled, green seeds. The numbers Mendel reported in these categories were 315, 108, 101, and 32, respectively. Dividing each number by 32 gives a 9.84 to 3.38 to 3.16 to 1.00 ratio, which is very close to a 9:3:3:1 ratio. As you will see, this is the ratio we would expect if the genes governing these two traits behaved independently of each other.

In figure 2.14, the letter *R* is assigned to the dominant allele, round, and *r* to the recessive allele, wrinkled; *Y* and *y* are used for yellow and green color, respectively. In figure 2.15, we have rediagrammed the cross in figure 2.14. The P_1 plants in this cross produce only one type of gamete each, *RY* for the parent with the dominant traits and *ry* for the parent with the recessive traits. The resulting F_1 plants are heterozygous for both genes **(dihybrid).** Self-fertilizing the dihybrid (*RrYy*) produces the F_2 generation.

In constructing the **Punnett square** in figure 2.15 to diagram the F_2 generation, we make a critical assumption: The four types of gametes from each parent will be produced in equal numbers, and hence every offspring category, or "box," in the square is equally likely. Thus, because sixteen boxes make up the Punnett square (named after its inventor, Reginald C. Punnett), the ratio of F_2 offspring should be in sixteenths. Grouping the F_2 offspring by phenotype, we find there are 9/16 that have round, yellow seeds; 3/16 that have round, green seeds; 3/16 that have wrinkled, yellow seeds; and 1/16 that have wrinkled, green seeds. This is the origin of the expected 9:3:3:1 F_2 ratio.

Reginald C. Punnett (1875–1967).
From *Genetics,* 58 (1968): frontispiece.
Courtesy of the Genetics Society of America.

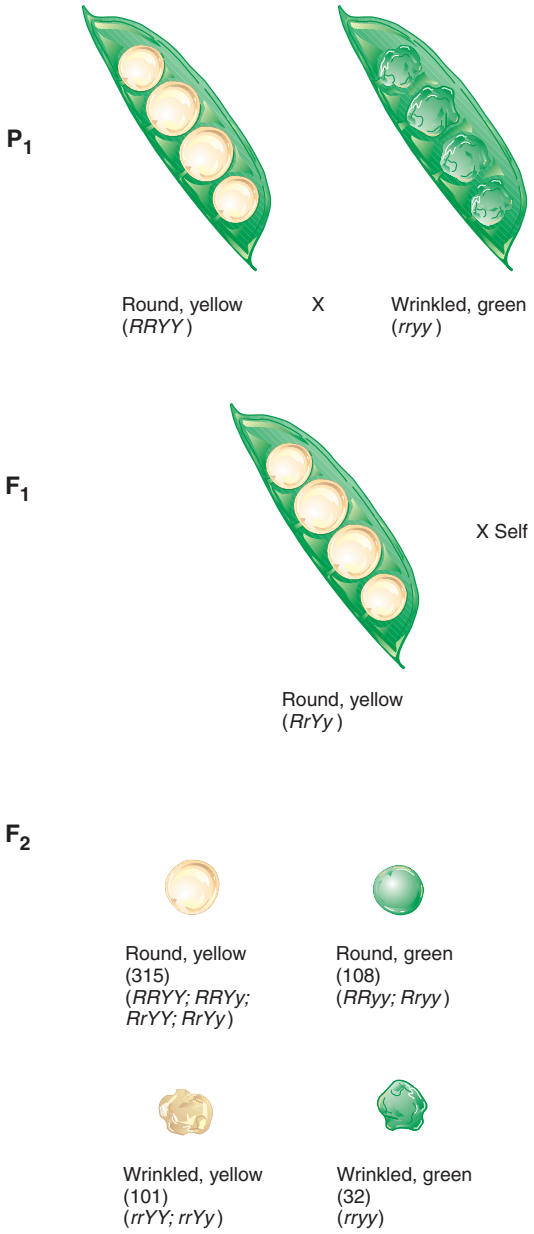


Figure 2.14 Independent assortment in garden peas.

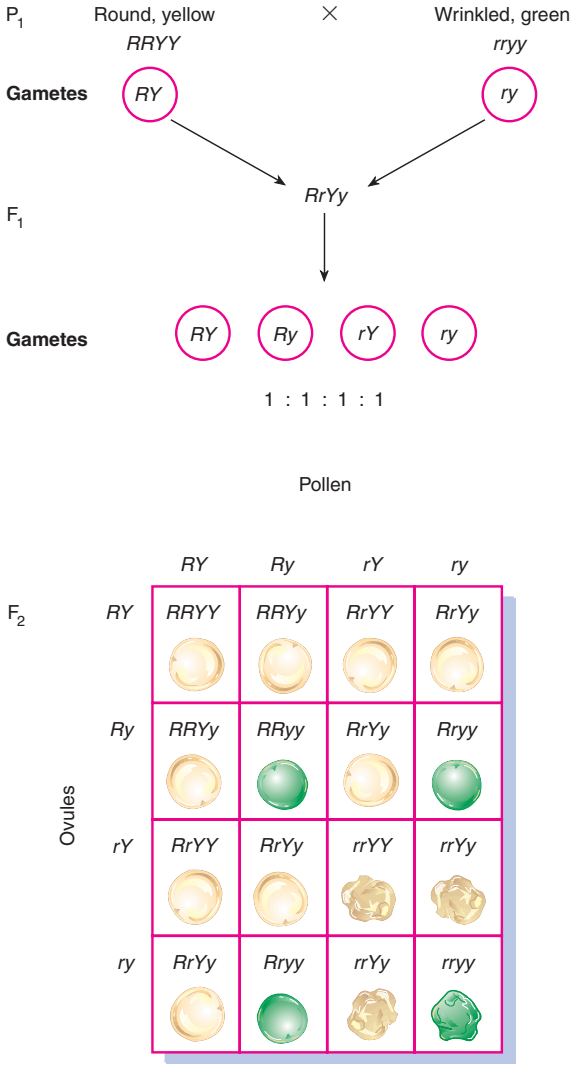


Figure 2.15 Assigning genotypes to the cross in figure 2.14.

Rule of Independent Assortment

This ratio comes about because the two characteristics behave independently. The F_1 plants produce four types of gametes (check fig. 2.15): *RY, Ry, rY,* and *ry.* These gametes occur in equal frequencies. Regardless of which seed shape allele a gamete ends up with, it has a 50:50 chance of getting either of the alleles for color—the two genes are segregating, or assorting, independently. This is the essence of Mendel's second rule, the **rule of independent assortment,** which states that alleles for one gene can segregate independently of alleles for other genes. Are the alleles for the two characteristics of color and form segregating properly according to Mendel's first principle?

If we look only at seed shape (see fig. 2.14), we find that a homozygote with round seeds was crossed with a homozygote with wrinkled seeds in the P_1 generation (*RR* × *rr*). This cross yields only heterozygous plants with round seeds (*Rr*) in the F_1 generation. When these

BOX 2.1

Historical Perspectives

Excerpts from Mendel's Original Paper

In February and March of 1865, Mendel delivered two lectures to the Natural History Society of Brünn. These were published as a single forty-eight-page article handwritten in German. The article appeared in the 1865 *Proceedings of the Society,* which came out in 1866. It was entitled "*Versuche über Pflanzen-Hybriden,*" which means "Experiments in Plant Hybridization." Following are some paragraphs from the English translation to give us some sense of the original.

In his introductory remarks, Mendel writes:

> That, so far, no generally applicable law governing the formation and development of hybrids has been successfully formulated can hardly be wondered at by anyone who is acquainted with the extent of the task, and can appreciate the difficulties with which experiments of this class have to contend. A final decision can only be arrived at when we shall have before us the results of detailed experiments made on plants belonging to the most diverse orders.
>
> Those who survey the work done in this department will arrive at the conviction that among all the numerous experiments made, not one has been carried out to such an extent and in such a way as to make it possible to determine the number of different forms under which the offspring of hybrids appear, or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their statistical relations. . . .
>
> The paper now presented records the results of such a detailed experiment. This experiment was practically confined to a small plant group, and is now, after eight years' pursuit, concluded in all essentials. Whether the plan upon which the separate experiments were conducted and carried out was the best suited to attain the desired end is left to the friendly decision of the reader.

After discussing the origin of his seeds and the nature of the experiments, Mendel discusses the F_1, or hybrid, generation:

> This is precisely the case with the Pea hybrids. In the case of each of the seven crosses the hybrid-character resembles that of one of the parental forms so closely that the other either escapes observation completely or cannot be detected with certainty. This circumstance is of great importance in the determination and classification of the forms under which the offspring of the hybrids appear. Henceforth in this paper those characters which are transmitted entire, or almost unchanged in the hybridization, and therefore in themselves constitute the characters of the hybrid, are termed the *dominant,* and those which become latent in the process, *recessive.* The expression "recessive" has been chosen because the characters thereby designated withdraw or entirely disappear in the hybrids, but nevertheless reappear unchanged in their progeny, as will be demonstrated later on.

He then writes about the F_2 generation:

> In this generation there reappear, together with the dominant characters, also the recessive ones with their peculiarities fully developed, and this occurs in the definitely expressed average proportion of three to one, so that among each four plants of this generation three display the dominant character and one the recessive. This relates without exception to all the characters which were investigated in the experiments. The angular wrinkled form of the seed, the green colour of the albumen, the white colour of the seed-coats and the flowers, the constrictions of the pods, the yellow colour of the unripe pod, of the stalk, of the calyx, and of the leaf venation, the umbel-like form of the inflorescence, and the dwarfed stem, all reappear in the numerical proportion given, without any essential alteration. *Transitional forms were not observed in any experiment. . . .*

F_1 plants are self-fertilized, the result is 315 + 108 round seeds (*RR* or *Rr*) and 101 + 32 wrinkled seeds (*rr*) in the F_2 generation. This is a 423:133 or a 3.18:1.00 phenotypic ratio—very close to the expected 3:1 ratio. So the gene for seed shape is segregating normally. In a similar manner, if we look only at the gene for color, we see that the F_2 ratio of yellow to green seeds is 416:140, or 2.97:1.00—again, very close to a 3:1 ratio. Thus, when two genes are segregating normally according to the rule of segregation, their independent behavior demonstrates the rule of independent assortment (box 2.1).

From the Punnett square in figure 2.15, you can see that because of dominance, all phenotypic classes except the homozygous recessive one—wrinkled, green

Expt. 1. Form of seed.—From 253 hybrids 7,324 seeds were obtained in the second trial year. Among them were 5,474 round or roundish ones and 1,850 angular wrinkled ones. Therefrom the ratio 2.96 to 1 is deduced.

If *A* be taken as denoting one of the two constant characters, for instance the dominant, *a* the recessive, and *Aa* the hybrid form in which both are conjoined, the expression

$$A + 2Aa + a$$

shows the terms in the series for the progeny of the hybrids of two differentiating characters.

Mendel used a notation system different from ours. He designated heterozygotes with both alleles (e.g., *Aa*) but homozygotes with only one allele or the other (e.g., *A* for our *AA*). Thus, whereas he recorded $A + 2Aa + a$, we would record $AA + 2Aa + aa$. Mendel then went on to discuss the dihybrids. He mentions the genotypic ratio of 1:2:1:2:4:2:1:2:1 and the principle of independent assortment:

The fertilized seeds appeared round and yellow like those of the seed parents. The plants raised therefrom yielded seeds of four sorts, which frequently presented themselves in one pod. In all, 556 seeds were yielded by 15 plants, and of those there were:

315 round and yellow,
101 wrinkled and yellow,
108 round and green,
32 wrinkled and green.

Consequently the offspring of the hybrids, if two kinds of differentiating characters are combined therein, are represented by the expression

$$AB + Ab + aB + ab + 2ABb + 2aBb + 2AaB + 2Aab + 4AaBb.$$

(In today's notation, we would write: $AABB + AAbb + aaBB + aabb + 2AABb + 2aaBb + 2AaBB + 2Aabb + 4AaBb$.)

This expression is indisputably a combination series in which the two expressions for the characters *A* and *a*, *B* and *b* are combined. We arrive at the full number of the classes of the series by the combination of the expressions

$$A + 2Aa + a$$

$$B + 2Bb + b$$

(In today's notation we would write

$$AA + 2Aa + aa$$

$$BB + 2Bb + bb.)$$

There is therefore no doubt that for the whole of the characters involved in the experiments the principle applies that *the offspring of the hybrids in which several essentially different characters are combined exhibit the terms of a series of combinations, in which the developmental series for each pair of differentiating characters are united.* It is demonstrated at the same time that *the relation of each pair of different characters in hybrid union is independent of the other differences in the two original parental stocks.*

Table 1 is a summary of all the data Mendel presented on monohybrids (the data from only one dihybrid and one trihybrid cross were presented):

Table 1 Mendel's Data

	Dominant Phenotype	Recessive Phenotype	Ratio
Seed form	5,474	1,850	2.96:1
Cotyledon color	6,022	2,001	3.01:1
Seed coat color	705	224	3.15:1
Pod form	882	299	2.95:1
Pod color	428	152	2.82:1
Flower position	651	207	3.14:1
Stem length	787	277	2.84:1
Total	14,949	5,010	2.98:1

Source: Copyright The Royal Horticultural Society. Taken from the *Journal of the Royal Horticultural Society*, vol. 26. Pg. 1–32. 1901.

seeds—are actually genetically heterogeneous, with phenotypes made up of several genotypes. For example, the dominant phenotypic class, with round, yellow seeds, represents four genotypes: *RRYY, RRYy, RrYY,* and *RrYy.* When we group all the genotypes by phenotype, we obtain the ratio shown in figure 2.16. Thus, with complete dominance, a self-fertilized dihybrid gives a 9:3:3:1 phenotypic ratio in its offspring (F_2). A 1:2:1:2:4:2:1:2:1 genotypic ratio also occurs in the F_2 generation. If the two genes exhibited incomplete dominance or codominance, the latter would also be the phenotypic ratio. What ratio would be obtained if one gene exhibited dominance and the other did not? An example of this case appears in figure 2.17.

BOX 2.2

Historical Perspectives

Did Mendel Cheat?

Overwhelming evidence gathered during this century has proven the correctness of Mendel's conclusions. However, close scrutiny of Mendel's paper has led some to suggest that (1) Mendel failed to report the inheritance of traits that did not show independent assortment and (2) Mendel fabricated numbers. Both these claims are, on the surface, difficult to ignore; both have been countered effectively.

The first claim—that Mendel failed to report crosses involving traits that did not show independent assortment—arises from the observation that all seven traits that Mendel studied do show independent assortment and that the pea plant has precisely seven pairs of chromosomes. For Mendel to have chosen seven genes, one located on each of the seven chromosomes, by chance alone seems extremely unlikely. In fact, the probability would be

$$7/7 \times 6/7 \times 5/7 \times 4/7 \times 3/7 \times 2/7 \times 1/7 = 0.006$$

That is, Mendel had less than one chance in one hundred of randomly picking seven traits on the seven different chromosomes. However, L. Douglas and E. Novitski in 1977 analyzed Mendel's data in a different way. To understand their analysis, you have to know that two genes sufficiently far apart on the same chromosome will appear to assort independently (to be discussed in chapter 6). Thus, Mendel's choice of characters showing independent assortment has to be viewed in light of the lengths of the chromosomes. That is, Mendel could have chosen two genes on the same chromosome that would still show independent assortment. In fact, he did. For example, stem length and pod texture (wrinkled or smooth) are on the fourth chromosome pair in peas. In their analysis, Douglas and Novitski report that the probability of randomly choosing seven characteristics that appear to assort independently is actually between one in four and one in three. So it seems that Mendel did not have to manipulate his choice of characters in order to hide the failure of independent assortment. He had a one in three chance of naively choosing the seven characters that he did, thereby uncovering no deviation from independent assortment.

The second claim—that Mendel fabricated data—comes from a careful analysis of Mendel's paper by R. A. Fisher, a brilliant English statistician and population geneticist. In a paper in 1936, Fisher pointed out two problems in Mendel's work. First, all of Mendel's published data taken together fit their expected ratios better than chance alone would predict. Second, some of Mendel's data fit incorrect expected ratios. This second "error" on Mendel's part came about as follows.

Testcrossing Multihybrids

A simple test of Mendel's rule of independent assortment is the testcrossing of the dihybrid plant. We would predict, for example, that if we crossed an *RrYy* F_1 individual with an *rryy* individual, the results would include four phenotypes in a 1:1:1:1 ratio, as shown in figure 2.18. Mendel's data verified this prediction (box 2.2). We will proceed to look at a **trihybrid** cross in order to develop general rules for **multihybrids.**

A trihybrid Punnett square appears in figure 2.19. From this we can see that when a homozygous dominant and a homozygous recessive individual are crossed in the P_1 generation, plants in the F_1 generation are capable of producing eight gamete types. When these F_1 individuals are selfed, they in turn produce F_2 offspring of twenty-seven different genotypes in a ratio of sixty-fourths. By extrapolating from the monohybrid through the trihybrid, or simply by the rules of probability, we can construct table 2.3, which contains the rules for F_1 gamete production and F_2 zygote formation in a multihybrid cross. For example, from this table we can figure out the F_2 offspring when a dodecahybrid (twelve segregating genes: *AA BB CC . . . LL* × *aa bb cc . . . ll*) is selfed. The F_1 organisms in that cross will produce gametes with 2^{12}, or 4,096, different genotypes. The proportion of homozygous recessive offspring in the F_2 generation is $1/(2^n)^2$ where $n = 12$, or 1 in 16,777,216. With complete dominance, there will be 4,096 different phenotypes in the F_2 generation. If dominance is incomplete, there can be 3^{12}, or 531,441, different phenotypes in the F_2 generation.

GENOTYPIC INTERACTIONS

Often, several genes contribute to the same phenotype. An example occurs in the combs of fowl (fig. 2.20). If we cross a rose-combed hen with a pea-combed rooster (or vice versa), all the F_1 offspring are walnut-combed. If we

Mendel determined whether a dominant phenotype in the F_2 generation was a homozygote or a heterozygote by self-fertilizing it and examining ten offspring. In an F_2 generation composed of 1*AA*:2*Aa*:1*aa,* he expected a 2:1 ratio of heterozygotes to homozygotes within the dominant phenotypic class. In fact, this ratio is not precisely correct because of the problem of misclassification of heterozygotes. It is probable that some heterozygotes will be classified as homozygotes because all their offspring will be of the dominant phenotype. The probability that one offspring from a selfed *Aa* individual has the dominant phenotype is 3/4, or 0.75: the probability that ten offspring will be of the dominant phenotype is $(0.75)^{10}$ or 0.056. Thus, Mendel misclassified heterozygotes as dominant homozygotes 5.6% of the time. He should have expected a 1.89:1.11 ratio instead of a 2:1 ratio to demonstrate segregation. Mendel classified 600 plants this way in one cross and got a ratio of 201 homozygous to 399 heterozygous offspring. This is an almost perfect fit to the presumed 2:1 ratio and thus a poorer fit to the real 1.89:1.11 ratio. This bias is consistent and repeated in Mendel's trihybrid analysis.

Fisher, believing in Mendel's basic honesty, suggested that Mendel's data do not represent an experiment but more of a hypothetical demonstration. In 1971, F. Weiling published a more convincing case in Mendel's defense. Pointing out that the data of Mendel's rediscoverers are also suspect for the same reason, he suggested that the problem lies with the process of pollen formation in plants, not with the experimenters. In an *Aa* heterozygote, two *A* and two *a* cells develop from a pollen mother cell. These cells tend to stay together on the anther. Thus, pollen cells do not fertilize in a strictly random fashion. A bee is more likely to take equal numbers of *A* and *a* pollen than chance alone would predict. The result is that the statistics Fisher used are not applicable. By using a different statistic, Weiling showed that, in fact, Mendel need not have manipulated any numbers (nor would have his rediscoverers) in order to get data that fit the expected ratios well. By the same reasoning, very little misclassification of heterozygotes would have occurred.

More recently, Weiling and others have made several additional points. First, for Mendel to be sure of ten offspring, he probably examined more than ten, and thus he probably kept his misclassification rate lower than 5.6%. Second, despite Fisher's brilliance as a statistician, several have made compelling arguments that Fisher's statistical analyses were incorrect. In other words, for subtle statistical reasons, many of his analyses involved methods and conclusions that were in error.

We conclude that there is no compelling evidence to suggest that Mendel in any way manipulated his data to demonstrate his rules. In fact, taking into account what is known about him personally, it is much more logical to believe that he did not "cheat."

cross the hens and roosters of this heterozygous F_1 group, we will get, in the F_2 generation, walnut-, rose-, pea-, and single-combed fowl in a ratio of 9:3:3:1. Can you figure out the genotypes of this F_2 population before reading further? An immediate indication that two allelic pairs are involved is the fact that the 9:3:3:1 ratio appeared in the F_2 generation. As we have seen, this ratio comes about when we cross dihybrids in which both genes have alleles that control traits with complete dominance.

Figure 2.21 shows the analysis of this cross. When dominant alleles of both genes are present in an individual (*R- P-*), the walnut comb appears. (The dash indicates any second allele; thus, *R- P-* could be *RRPP, RrPP, RRPp,* or *RrPp.*) A dominant allele of the rose gene (*R-*) with

Table 2.3 **Multihybrid Self-Fertilization, Where *n* Equals Number of Genes Segregating Two Alleles Each**

	Monohybrid $n = 1$	Dihybrid $n = 2$	Trihybrid $n = 3$	General Rule
Number of F_1 gametic genotypes	2	4	8	2^n
Proportion of recessive homozygotes among the F_2 individuals	1/4	1/16	1/64	$1/(2^n)^2$
Number of different F_2 phenotypes, given complete dominance	2	4	8	2^n
Number of different genotypes (or phenotypes, if no dominance exists)	3	9	27	3^n

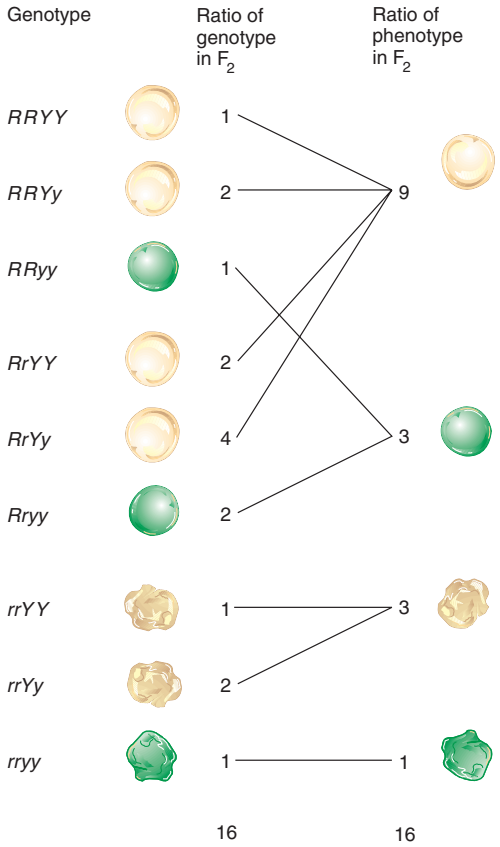


Figure 2.16 The phenotypic and genotypic ratios of the offspring of dihybrid peas.

recessive alleles of the pea gene (*pp*) gives a rose comb. A dominant allele of the pea gene (*P-*) with recessive alleles of the rose gene (*rr*) gives pea-combed fowl. When both genes are homozygous for the recessive alleles, the fowl are single-combed. Thus, a 9:3:3:1 F_2 ratio arises from crossing dihybrid individuals even though different expressions of the same phenotypic characteristic, the comb, are involved. In our previous 9:3:3:1 example (see fig. 2.15), we dealt with two separate characteristics: shape and color of peas.

In corn (or maize, *Zea mays*), several different field varieties produce white kernels on the ears. In certain crosses, two white varieties will result in an F_1 generation with all purple kernels. If plants grown from these purple kernels are selfed, the F_2 individuals have both purple and white kernels in a ratio of 9:7. How can we explain this? We must be dealing with the offspring of dihybrids with each gene segregating two alleles, because the ratio is in sixteenths. Furthermore, we can see that the F_2 9:7 ratio is a variation of the 9:3:3:1 ratio. The 3, 3, and 1 categories here are producing the same phenotype and thus make up 7/16 of the F_2 offspring. Figure 2.22 outlines the cross. We can see from this figure that the purple color appears only when dominant alleles of both genes are present. When one or both genes have only recessive alleles, the kernels will be white.

Epistasis

The color of corn kernels illustrates the concept of **epistasis,** the interaction of nonallelic genes in the formation

Figure 2.17 Independent assortment of two blood systems in human beings. In the ABO system, the I^A and I^B alleles are codominant. In a simplified view of the Rhesus system, the Rh^+ phenotype (*D* allele) is dominant to the Rh^- phenotype (*d* allele).

P_1 I^AI^ADD X I^BI^Bdd

F_1 I^AI^BDd (F_1 X F_1)

F_2

Female \ Male	I^AD	I^Ad	I^BD	I^Bd
I^AD	I^AI^ADD	I^AI^ADd	I^AI^BDD	I^AI^BDd
I^Ad	I^AI^ADd	I^AI^Add	I^AI^BDd	I^AI^Bdd
I^BD	I^AI^BDD	I^AI^BDd	I^BI^BDD	I^BI^BDd
I^Bd	I^AI^BDd	I^AI^Bdd	I^BI^BDd	I^BI^Bdd

F_2 Summary

Phenotype	A Rh^+	A Rh^-	B Rh^+	B Rh^-	AB Rh^+	AB Rh^-
Frequency	3	1	3	1	6	2

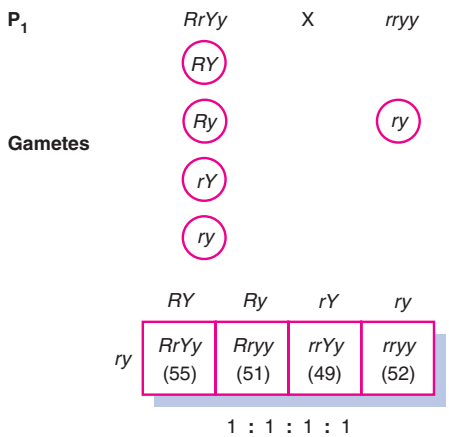


Figure 2.18 Testcross of a dihybrid. A 1:1:1:1 ratio is expected in the offspring.

of the phenotype. This is a process analogous to dominance among alleles of one gene. For example, the recessive apterous (wingless) gene in fruit flies is epistatic to any gene that controls wing characteristics; hairy wing is hypostatic to apterous (that is, the recessive apterous gene, when homozygous, masks the presence of the hairy wing gene, because, obviously, without wings, no wing characteristics can be expressed). Note that the genetic control of comb type in fowl does not involve epistasis. There are no allelic combinations at one locus that mask genotypes at another locus: the 9:3:3:1 ratio is not an indication of epistasis. To illustrate further the principle of epistasis, we can look at the control of coat color in mice.

In one particular example, a pure-breeding black mouse is crossed with a pure-breeding albino mouse (pure white because all pigment is lacking); all of the offspring are agouti (the typical brownish-gray mouse color). When the F_1 agouti mice are crossed with each other, agouti, black, and albino offspring appear in the F_2 generation in a ratio of 9:3:4. What are the genotypes in this cross? The answer appears in figure 2.23. By now it should be apparent that the F_2 ratio of 9:3:4 is also a variant of the 9:3:3:1 ratio; it indicates epistasis in a dihybrid cross. What is the mechanism producing this 9:3:4 ratio? Of a potential 9:3:3:1 ratio, one of the 3/16 classes and the 1/16 class are combined to create a 4/16 class. Any genotype that includes $c^a c^a$ will be albino, masking the A gene, but as long as at least one dominant *C* allele is present, the A gene can express itself. Mice with dominant alleles of both genes (*A-C-*) will have the agouti color, whereas mice that are homozygous recessive at the A gene (*aaC-*) will be black. So, at the A gene, *A* for agouti

P_1 *AA BB CC* × *aa bb cc*

F_1 *Aa Bb Cc* × Self

	A B C	*A B c*	*A b C*	*A b c*	*a B C*	*a B c*	*a b C*	*a b c*
A B C	AA BB CC	AA BB Cc	AA Bb CC	AA Bb Cc	Aa BB CC	Aa BB Cc	Aa Bb CC	Aa Bb Cc
A B c	AA BB Cc	AA BB cc	AA Bb Cc	AA Bb cc	Aa BB Cc	Aa BB cc	Aa Bb Cc	Aa Bb cc
A b C	AA Bb CC	AA Bb Cc	AA bb CC	AA bb Cc	Aa Bb CC	Aa Bb Cc	Aa bb CC	Aa bb Cc
A b c	AA Bb Cc	AA Bb cc	AA bb Cc	AA bb cc	Aa Bb Cc	Aa Bb cc	Aa bb Cc	Aa bb cc
a B C	Aa BB CC	Aa BB Cc	Aa Bb CC	Aa Bb Cc	aa BB CC	aa BB Cc	aa Bb CC	aa Bb Cc
a B c	Aa BB Cc	Aa BB cc	Aa Bb Cc	Aa Bb cc	aa BB Cc	aa BB cc	aa Bb Cc	aa Bb cc
a b C	Aa Bb CC	Aa Bb Cc	Aa bb CC	Aa bb Cc	aa Bb CC	aa Bb Cc	aa bb CC	aa bb Cc
a b c	Aa Bb Cc	Aa Bb cc	Aa bb Cc	Aa bb cc	aa Bb Cc	aa Bb cc	aa bb Cc	aa bb cc

Figure 2.19 Trihybrid cross.

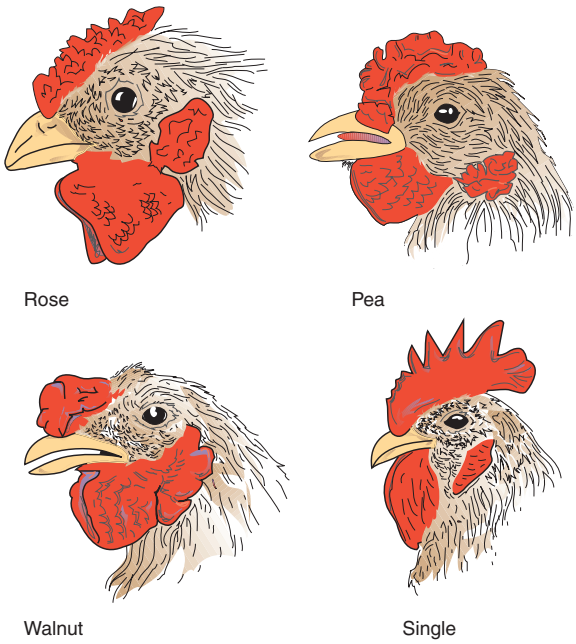


Figure 2.20 Four types of combs in fowl.

P_1 Rose comb *RRpp* × Pea comb *rrPP*

F_1 Walnut comb *RrPp*

$F_1 \times F_1$

F_2

	RP	*Rp*	*rP*	*rp*
RP	*RRPP* Walnut	*RRPp* Walnut	*RrPP* Walnut	*RrPp* Walnut
Rp	*RRPp* Walnut	*RRpp* Rose	*RrPp* Walnut	*Rrpp* Rose
rP	*RrPP* Walnut	*RrPp* Walnut	*rrPP* Pea	*rrPp* Pea
rp	*RrPp* Walnut	*Rrpp* Rose	*rrPp* Pea	*rrpp* Single

F_2 **Summary** Walnut : Rose : Pea : Single
9 : 3 : 3 : 1

Figure 2.21 Independent assortment in the determination of comb type in fowl.

P_1 First white variety *AAbb* × Second white variety *aaBB*

F_1 Purple *AaBb* × Self

F_2

	AB	*Ab*	*aB*	*ab*
AB	*AABB*	*AABb*	*AaBB*	*AaBb*
Ab	*AABb*	*AAbb*	*AaBb*	*Aabb*
aB	*AaBB*	*AaBb*	*aaBB*	*aaBb*
ab	*AaBb*	*Aabb*	*aaBb*	*aabb*

F_2 **Summary** Purple : White
9 : 7

Figure 2.22 Color production in corn.

is dominant to *a* for black. The albino gene (c^a), when homozygous, is epistatic to the A gene; the A gene is hypostatic to the gene for albinism.

Mechanism of Epistasis

In this case, the physiological mechanism of epistasis is known. The pigment melanin is present in both the black and agouti phenotypes. The agouti is a modified black hair in which yellow stripes (the pigment phaeomelanin) have been added. Thus, with melanin present, agouti is dominant. Without melanin, we get an albino regardless of the genotype of the agouti gene because both agouti and black depend on melanin. Albinism is the result of one of several defects in the enzymatic pathway for the synthesis of melanin (fig. 2.24).

Knowing that epistatic modifications of the 9:3:3:1 ratio come about through gene interactions at the biochemical level, we can look for a biochemical explanation for the 9:7 ratio in corn kernel color (fig. 2.22). Two possible mechanisms for a 9:7 ratio are shown in figure 2.25. Either a two-step process takes a precursor molecule and turns it into purple pigment, or two precursors that must be converted to final products then combine to produce purple pigment. The dominant alleles from the two genes control the two steps in the process. Recessive alleles are ineffective. Thus, dominants are necessary for both steps to complete the pathways for a purple pig-

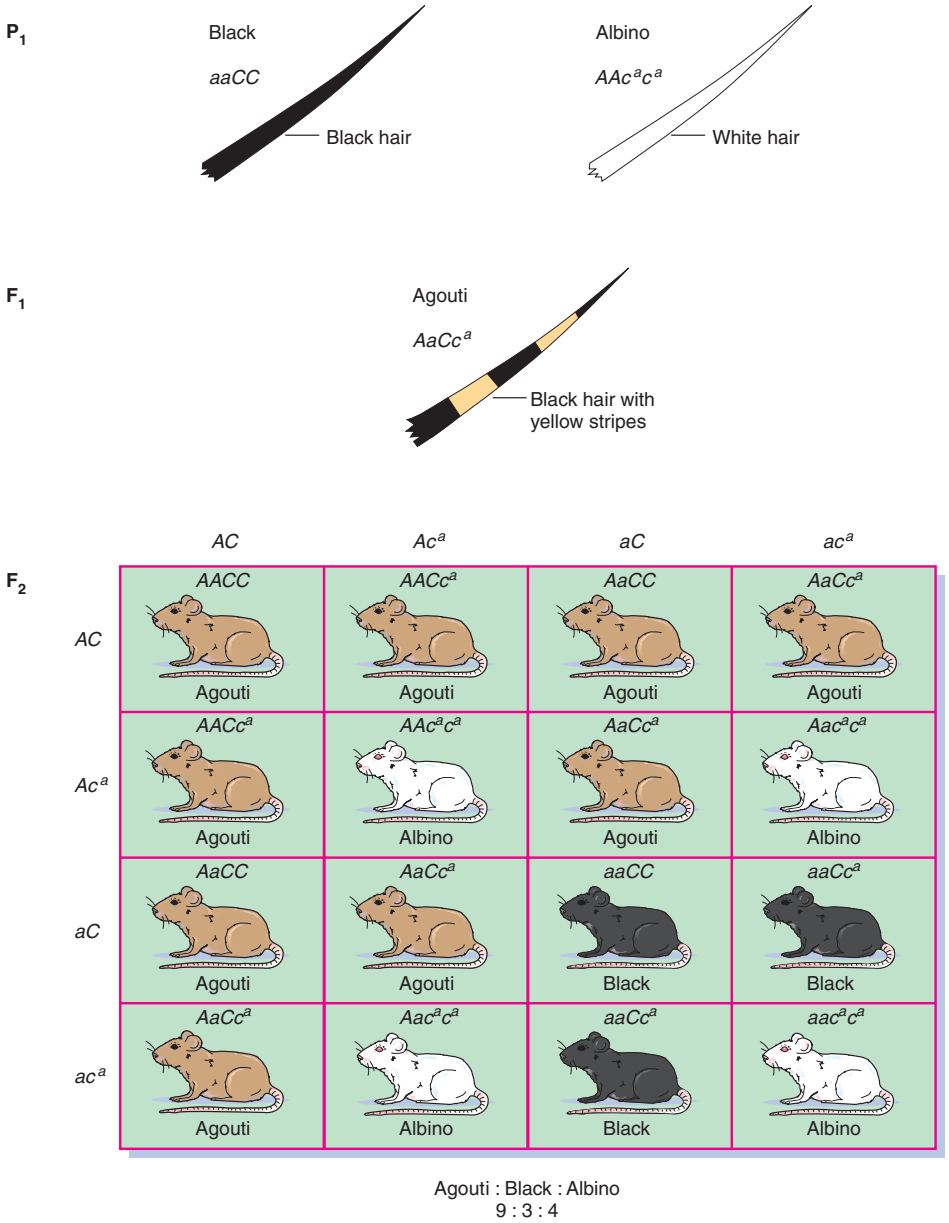


Figure 2.23 Epistasis in the coat color of mice.

ment. Stopping the process at any point prevents the production of purple color.

Another example of epistasis occurs in the snapdragon (*Antirrhinum majus*). There, a gene called *nivea* has alleles that determine whether any pigment is produced; the *nn* genotype prevents pigment production, whereas the *NN* or *Nn* genotypes permit pigment color genes to express themselves. The *eosinea* gene controls the production of a red anthocyanin pigment. In the presence of the *N* allele of the *nivea* gene, the genotypes *EE* or *Ee* of the *eosinea* gene produce red flowers; the *ee* genotype produces pink flowers. When dihybrids are self-fertilized, red-, pink-, and white-flowered plants are produced in a ratio of 9:3:4 (fig. 2.26). The epistatic interaction is the *nn* genotype masking the expression of alleles at the *eosinea* gene. In other words, regardless of the genotypes of the *eosinea* gene (*EE, Ee,* or *ee*), the flowers will be white if the *nivea* gene has the *nn*

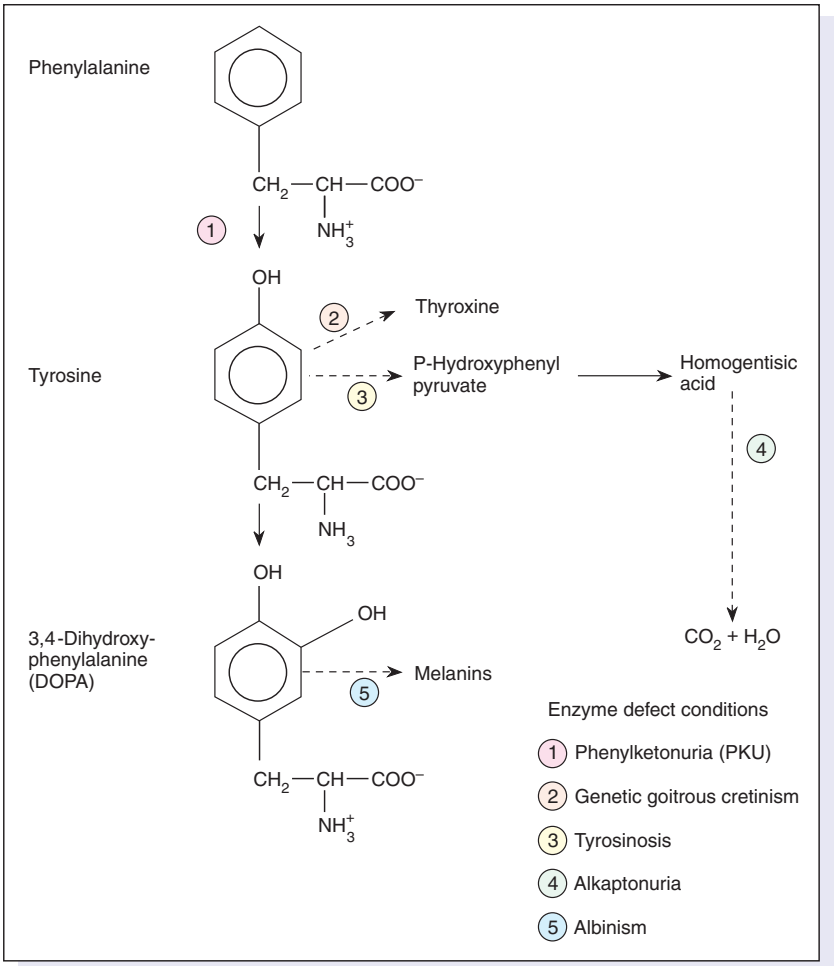


Figure 2.24 In humans, errors in melanin synthesis produce different physical conditions and diseases, depending on which part of the tyrosine (an amino acid) metabolic pathway is disrupted. The broken arrows indicate that there is more than one step in the pathways; the conditions listed occur only in homozygous recessives.

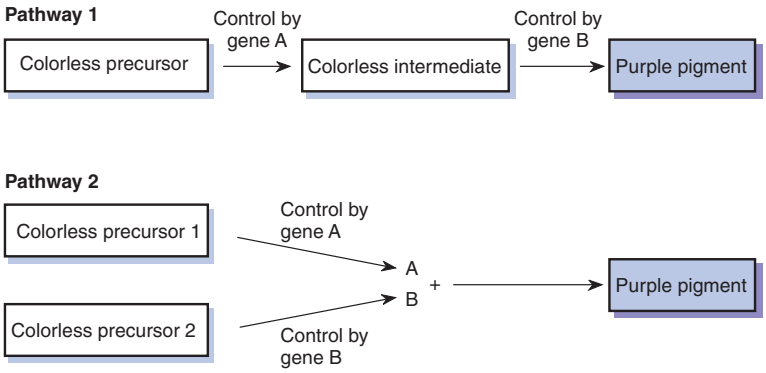


Figure 2.25 Possible metabolic pathways of color production that would yield 9:7 ratios in the F_2 generation of a self-fertilized dihybrid.

combination of alleles. Thus, *nivea* is epistatic to *eosinea,* and *eosinea* is hypostatic to *nivea.* (We should add that at least seven major colors occur in snapdragons, along with subtle shade differences, all genetically controlled by the interactions of at least seven genes.)

Other types of epistatic interactions occur in other organisms. Table 2.4 lists several. We do not know the exact physiological mechanisms in many cases, especially when developmental processes are involved (e.g., size and shape). However, from an analysis of crosses, we can know the number of genes involved and the general nature of their interactions.

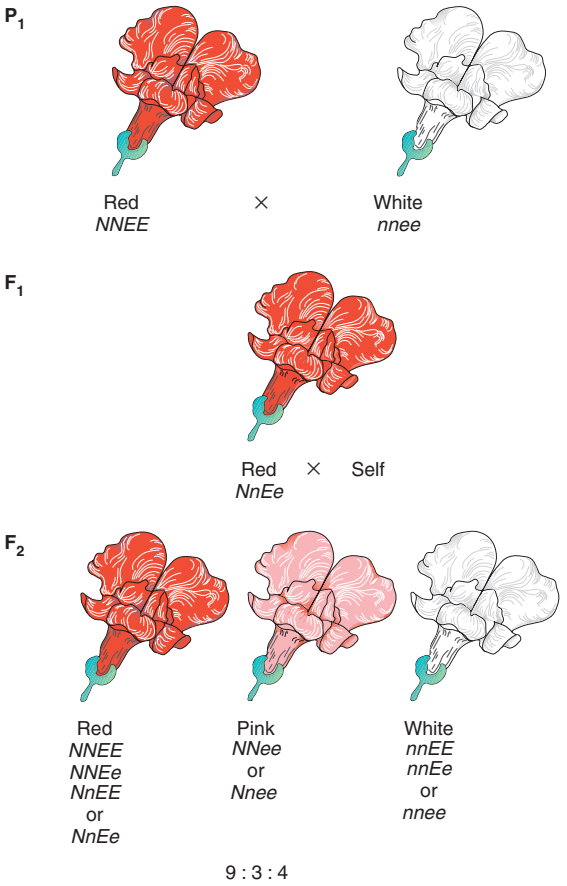


Figure 2.26 Flower color inheritance in snapdragons. This is an example of epistasis: an *nn* genotype masks the expression of alleles (*EE, Ee,* or *ee*) at the *eosinea* gene.

BIOCHEMICAL GENETICS

Inborn Errors of Metabolism

The examples of mouse coat color, corn kernel color, and snapdragon flower petal color demonstrate that genes control the formation of enzymes, proteins that control the steps in biochemical pathways. For the most part, dominant alleles control functioning enzymes that catalyze biochemical steps. Recessive alleles often produce nonfunctioning enzymes that cannot catalyze specific steps. Often a heterozygote is normal because one allele produces a functional enzyme; usually only half the enzyme quantity of the dominant homozygote is enough. The study of the relationship between genes and enzymes is generally called **biochemical genetics** because it involves the genetic control of biochemical pathways. A. E. Garrod, a British physician, pointed out this general concept of human gene action in *Inborn Errors of Metabolism,* published in 1909. Only nine years after Mendel was rediscovered, Garrod described several human conditions, such as albinism and alkaptonuria, that occur in individuals who are homozygous for recessive alleles (see fig. 2.24).

Table 2.4 **Some Examples of Epistatic Interactions Among Alleles of Two Genes**

Characteristic	Phenotype of F_1 Dihybrid (*AaBb*)	Phenotypic F_2 Ratio
Corn and sweet pea color	Purple	Purple:white 9:7
Mouse coat color	Agouti	Agouti:black:albino 9:3:4
Shepherd's purse seed capsule shape	Triangular	Triangular:oval 15:1
Summer squash shape	Disk	Disk:sphere:elongate 9:6:1
Fowl color	White	White:colored 13:3

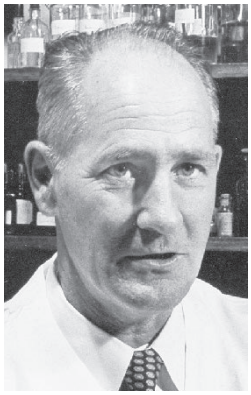
For example, people normally degrade homogentisic acid (alkapton) into maleylacetoacetic acid. Persons with the disease alkaptonuria are homozygous for a nonfunctional form of the enzyme essential to the process: homogentisic acid oxidase, found in the liver. Absence of this enzyme blocks the degradation reaction so that homogentisic acid builds up. This acid darkens upon oxidation. Thus, affected persons can be identified by the black color of their urine after its exposure to air. Eventually, alkaptonuria causes problems in the joints and a darkening of cartilage that is visible in the ears and the eye sclera.

One-Gene-One-Enzyme Hypothesis

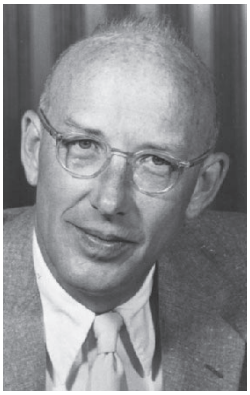
Pioneering work in the concept that genes control the production of enzymes, which in turn control the steps in biochemical pathways, was done by George Beadle and Edward Tatum, who eventually shared the Nobel Prize for their work. They not only put forth the **one-gene-one-enzyme hypothesis,** but also used mutants to work out the details of biochemical pathways. In 1941, Beadle and Tatum were the first scientists to isolate mutants with nutritional requirements that defined steps in a biochemical pathway. In the early 1940s, they united the fields of biochemistry and genetics by using strains of a bread mold with specific nutritional requirements to discover the steps in biochemical pathways in that organism.

Through this century, the study of mutations has been the driving force in genetics. The process of mutation produces alleles that differ from the wild-type and shows us that a particular aspect of the phenotype is under genetic control. Beadle and Tatum used mutants to work out the steps in the biosynthesis of niacin (vitamin B_3) in pink bread mold, *Neurospora crassa.*

Normally, *Neurospora* synthesizes niacin via the pathway shown in figure 2.27. Beadle and Tatum isolated mutants that could not grow unless niacin was provided in the culture medium; these mutants had enzyme deficiencies in the synthesizing pathway that ends with niacin. Thus, although wild-type *Neurospora* could grow on a medium without additives, the mutants could not. Beadle and Tatum had a general idea, based on the structure of niacin, as to what substances would be in the niacin biosynthesis pathway. They could thus make educated guesses as to what substances they might add to the culture medium to enable the mutants to grow. Mutant B (table 2.5), for example, could grow if given niacin or, alternatively, 3-hydroxyanthranilic acid. It could not grow if given only kynurenine. Thus, Beadle and Tatum knew that the B mutation affected the pathway between kynurenine and 3-hydroxyanthranilic acid. Similarly, mutant A could grow if given 3-hydroxyanthranilic acid or kynurenine instead of niacin. Therefore, these two prod-



George W. Beadle (1903–89). Courtesy of the Archives, California Institute of Technology.



Edward L. Tatum (1909–75). Courtesy of the Proceedings for the National Academy of Sciences.

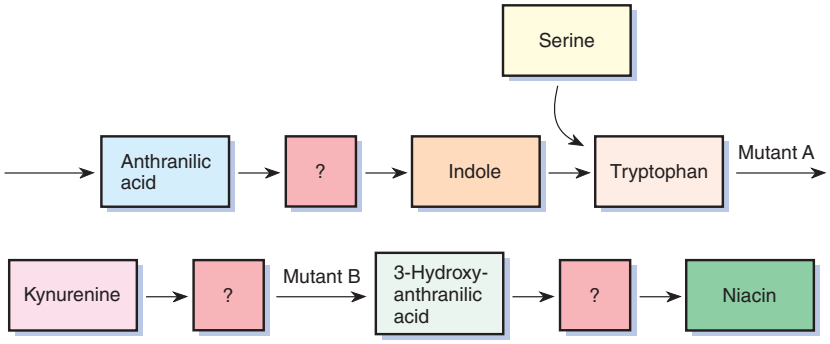


Figure 2.27 Pathway of niacin synthesis in *Neurospora.* Each *arrow* represents an enzyme-mediated step. Each *question mark* represents a presumed but (at the time Beadle and Tatum were working) unknown compound.

Table 2.5 **Growth Performance of *Neurospora* Mutants (plus sign indicates growth; minus sign indicates no growth)**

	Additive			
	Tryptophan	Kynurenine	3-Hydroxyanthranilic Acid	Niacin
Wild-type	+	+	+	+
Mutant A	−	+	+	+
Mutant B	−	−	+	+

ucts must be in the pathway *after* the step interrupted in mutant A. Conversely, since neither of these mutant organisms could grow when given only tryptophan, Beadle and Tatum knew that tryptophan occurred in the pathway before the steps with the deficient enzymes. By this type of analysis, they discovered the steps in several biochemical pathways of *Neurospora.* Many biochemical pathways are similar in a huge range of organisms, and thus Beadle and Tatum's work was of general importance. (We will spend more time studying *Neurospora* in chapter 6.)

Beadle and Tatum could further verify their work by observing which substances accumulated in the mutant organisms. If a biochemical pathway is blocked at a certain point, then the substrate at that point cannot convert into the next product, and it builds up in the cell. For example, in the niacin pathway (fig. 2.27), if a block occurs just after 3-hydroxyanthranilic acid, that substance will build up in the cell because it cannot convert into the next substance on the way to niacin.

This analysis could be misleading, however, if the built-up substance is being "siphoned off" into other biochemical pathways in the cell. Also, the cell might attempt to break down or sequester toxic substances. This would mean there might not be an obvious buildup of the substance just before the blocked step.

Beadle and Tatum concluded from their studies that one gene controls the production of one enzyme. The one-gene-one-enzyme hypothesis is an oversimplification that we will clarify later in the book. As a rule of thumb, however, the hypothesis is valid, and it has served to direct attention to the functional relationship between genes and enzymes in biochemical pathways.

Although a change in a single enzyme usually disrupts a single biochemical pathway, it frequently has more than one effect on phenotype. Multiple effects are referred to as **pleiotropy.** A well-known example is sickle-cell anemia, caused by a mutation in the gene for the β chain of the hemoglobin molecule. In a homozygote, this mutation causes a sickling of red blood cells (fig. 2.28). The sickling of these cells has two major ramifications.

First, the liver destroys the sickled cells, causing anemia. The phenotypic effects of this anemia include physical weakness, slow development, and hypertrophy of the bone marrow, resulting in the "tower skull" seen in some of those afflicted with the disease. The second major effect of sickle-cell anemia is that the sickled cells interfere with capillary blood flow, clumping together and resulting in damage to every major organ. The individual can suffer pain, heart failure, rheumatism, and other ill effects. Hence, a single mutation shows itself in many aspects of the phenotype.

Figure 2.28 Sickle-shaped red blood cells from a person with sickle-cell anemia. Red blood cells are about 7 to 8 μm in diameter. (Courtesy of Dr. Patricia N. Farnsworth.)

SUMMARY

STUDY OBJECTIVE 1: To understand that genes are discrete units that control the appearance of an organism 17–18

Genes control phenotypic traits such as size and color. They are inherited as discrete units.

STUDY OBJECTIVE 2: To understand Mendel's rules of inheritance: segregation and independent assortment 18–22

Higher organisms contain two alleles of each gene, but only one allele enters each gamete. Zygote formation restores the double number of alleles in the cell. This is Mendel's rule of segregation.

Alleles of different genes segregate independently of each other. Mendel was the first to recognize the 3:1 phenotypic ratio as a pattern of inheritance; the 9:3:3:1 ratio demonstrates independent assortment in hybrids. Mendel was successful in his endeavor because he performed careful experiments using discrete characteristics, large numbers of offspring, and an organism (the pea plant) amenable to controlled fertilizations.

STUDY OBJECTIVE 3: To understand that dominance is a function of the interaction of alleles; similarly, epistasis is a function of the interaction of nonallelic genes 22–37

There can be many alleles for one gene, although each individual organism has only two alleles for each gene. A phenotype is dominant if it is expressed when one or two copies of its allele are present (heterozygote or homozygote). Dominance depends, however, on the level of the phenotype one looks at.

Genes usually control the production of enzymes, which control steps in metabolic pathways. Many human metabolic diseases are due to homozygosity of an allele that produces a nonfunctioning enzyme.

Nonallelic genes can interact in producing a phenotype so that alleles of one gene mask the expression of alleles of another gene. This process, termed *epistasis,* alters the expected phenotypic ratios.

STUDY OBJECTIVE 4: To define how genes generally control the production of enzymes and thus the fate of biochemical pathways 37–39

Beadle and Tatum used mutants with mutations in the niacin biosynthesis pathway to work out the steps in the pathway. A single mutation can have many phenotypic effects (pleiotropy).

SOLVED PROBLEMS

PROBLEM 1: In corn, rough sheath (*rs*) is recessive to smooth sheath (*Rs*), midrib absent (*mrl*) is recessive to midrib present (*Mrl*), and crinkled leaf (*cr*) is recessive to smooth leaf (*Cr*). (Alleles are named for the mutants, which are all recessive.) What are the results of testcrossing a trihybrid?

Answer: The trihybrid has the genotype *Rsrs Mrlmrl Crcr.* This parent is capable of producing eight different gamete types in equal frequencies, all combinations of one allele from each gene (*Rs Mrl Cr, Rs Mrl cr, Rs mrl Cr, Rs mrl cr, rs Mrl Cr, rs Mrl cr, rs mrl Cr,* and *rs mrl cr*). In a testcross, the other parent is a recessive homozygote with the genotype *rsrs mrlmrl crcr,* capable of producing only one type of gamete, with the alleles *rs mrl cr.* Thus, this cross can produce zygotes of eight different genotypes (and phenotypes), one for each of the gamete types of the trihybrid parent: *Rsrs Mrlmrl Crcr* (smooth sheath, midrib present, smooth leaf); *Rsrs Mrlmrl crcr* (smooth sheath, midrib present, crinkled leaf); *Rsrs mrlmrl Crcr* (smooth sheath, midrib absent, smooth leaf); *Rsrs mrlmrl crcr* (smooth sheath, midrib absent, crinkled leaf); *rsrs Mrlmrl Crcr* (rough sheath, midrib present, smooth leaf); *rsrs Mrlmrl crcr* (rough sheath, midrib present, crinkled leaf); *rsrs mrlmrl Crcr* (rough sheath, midrib absent, smooth leaf); and *rsrs mrlmrl crcr* (rough sheath, midrib absent, crinkled leaf). Each should make up one-eighth of the total number of offspring.

PROBLEM 2: Summer squash come in three shapes: disk, spherical, and elongate. In one experiment, researchers crossed two squash plants with disk-shaped fruits. The first 160 seeds planted from this cross produced plants with fruit shapes as follows: 89 disk, 61 sphere, and 10 elongate. What is the mode of inheritance of fruit shape in summer squash?

Answer: The numbers are very close to a ratio of 90:60:10, or 9:6:1, an epistatic variant of the 9:3:3:1, with the two 3/16ths categories combined. If this is the case, then the parent plants with disk-shaped fruits were dihybrids (*AaBb*). Among the offspring, 9/16ths had disk-

shaped fruit, indicating that it takes at least one dominant allele of each gene to produce disk-shaped fruits (*A-B-*: *AABB, AaBB, AABb,* or *AaBb*). The 1/16th category of plants with elongate fruits indicates that this fruit shape occurs in homozygous recessive plants (*aabb*). The plants with spherical fruit are thus plants with a dominant allele of one gene but a homozygous recessive combination at the other gene (*AAbb, Aabb, aaBB,* or *aaBb*). In summary, then, two genes combine to control fruit shape in summer squash. The epistatic interactions between the two genes produce a 9:6:1 ratio of offspring phenotypes when dihybrids are crossed.

PROBLEM 3: A geneticist studying the pathway of synthesis of phenylalanine in *Neurospora* isolated several mutants that require phenylalanine to grow. She tested whether each mutant would grow when provided additives that she believed were in the pathway of phenylalanine synthesis (see table); a plus indicates growth and minus indicates the lack of growth in the three mutants tested.

	Additive			
	Phenylpyruvate	Prephenate	Chorismate	Phenylalanine
Wild-type	+	+	+	+
Mutant 1	−	−	−	+
Mutant 2	+	+	−	+
Mutant 3	+	−	−	+

Where in the pathway to phenylalanine synthesis does each of the additives belong, if at all?

Answer: The wild-type grows in the presence of all additives. This is not surprising since the wild-type can grow, by definition, in the absence of all the additives because it can synthesize phenylalanine *de novo.* Mutant 1 cannot grow in the presence of any additive except phenylalanine, indicating that its mutation affects the step just before the end of the pathway at phenylalanine. In other words, each of the other additives occurs in the phenylalanine pathway before the point of the mutation in mutant 1. Mutant 2 can grow if given any additive but chorismate, indicating that chorismate is at the beginning of the pathway, and the mutation affects the pathway just after that step. Finally, mutant 3 can grow if given phenylpyruvate or phenylalanine, indicating that its mutation affects the step before phenylpyruvate and phenylalanine, but after the earlier part of the pathway. Putting all of this information together indicates that the pathway to phenylalanine, with mutants indicated, is:

$$\text{chorismate} \xrightarrow{2} \text{prephenate} \xrightarrow{3} \text{phenylpyruvate} \xrightarrow{1} \text{phenylalanine}$$

EXERCISES AND PROBLEMS*

SEGREGATION

1. Mendel crossed tall pea plants with dwarf ones. The F_1 plants were all tall. When these F_1 plants were selfed to produce the F_2 generation, he got a 3:1 tall-to-dwarf ratio in the offspring. Predict the genotypes and phenotypes and relative proportions of the F_3 generation produced when the F_2 generation was selfed.
2. What properties of fruit flies and corn made them the organisms of choice for geneticists during most of the first half of the twentieth century? (Molecular geneticists have made great strides working with bacteria and viruses. You could begin thinking at this point about the properties that have made these organisms so valuable to geneticists.)
3. State precisely the rules of segregation and independent assortment. (See also the Exercises and Problems section on Independent Assortment.)
4. In *Drosophila,* a cross between a dark-bodied fly and a tan-bodied fly yields seventy-six tan and eighty dark flies. Diagram the cross.
5. If two black mice are crossed, ten black and three white mice result.
   a. Which allele is dominant?
   b. Which allele is recessive?
   c. What are the genotypes of the parents?
6. In *Drosophila,* two red-eyed flies mate and yield 110 red-eyed and 35 brown-eyed offspring. Diagram the cross and determine which allele is dominant.

DOMINANCE IS NOT UNIVERSAL

7. Explain how Tay-Sachs disease can be both a recessive and an incomplete dominant trait. What are the differences between incomplete dominance and codominance?
8. How does the biochemical pathway in figure 2.13 explain how alleles I^A and I^B are codominant, yet both dominant to allele i?

*Answers to selected exercises and problems are on page A-1.

9. Two short-eared pigs are mated. In the progeny, three have no ears, seven have short ears, and four have long ears. Explain these results by diagramming the cross.

10. A plant with red flowers is crossed with a plant with white flowers. All the progeny are pink. When the plants with pink flowers are crossed, the progeny are eleven red, twenty-three pink, and twelve white. What is the mode of inheritance of flower color?

NOMENCLATURE

11. In fruit flies, a new dominant trait, washed eye, was discovered. Describe different ways of naming the alleles of the washed-eye gene.

12. The following is a list of ten genes in fruit flies, each with one of its alleles given. Are the alleles shown dominant or recessive? Are they mutant or wild-type? What is the alternative allele for each? Is the alternative allele dominant or recessive in each case?

Name of Gene	Allele
yellow	y^+
Hairy wing	*Hw*
Abruptex	Ax^+
Confluens	*Co*
raven	rv^+
downy	*dow*
Minute(2)e	$M(2)e^+$
Jammed	*J*
tufted	tuf^+
burgundy	*bur*

MULTIPLE ALLELES

13. In the ABO blood system in human beings, alleles I^A and I^B are codominant, and both are dominant to the *i* allele. In a paternity dispute, a type AB woman claimed that one of four men, each with different blood types, was the father of her type A child. Which of the following could be the blood type of the father of the child on the basis of the evidence given?
   a. Type A
   b. Type B
   c. Type O
   d. Type AB

14. Under what circumstances can the phenotypes of the ABO system be used to refute paternity?

15. In blood transfusions, one blood type is called the "universal donor" and one the "universal recipient" because of their ABO compatibilities. Which is which?

16. Among the genes having the greatest number of alleles are those involved in self-incompatibility in plants. In some cases, hundreds of alleles exist for a single gene. What types of constraints might exist to set a limit on the number of alleles a gene can have?

17. In the human ABO blood system, the alleles I^A and I^B are dominant to *i*. What possible phenotypic ratios do you expect from a mating between a type A individual and a type B individual?

18. In screech owls, crosses between red and silver individuals sometimes yield all red; sometimes 1/2 red:1/2 silver; and sometimes 1/2 red:1/4 white:1/4 silver offspring. Crosses between two red owls yield either all red, 3/4 red:1/4 silver, or 3/4 red:1/4 white offspring. What is the mode of inheritance?

19. A premed student, Steve, plans to marry the daughter of the dean of nursing. The dean's husband was sterile, and the daughter was conceived by artificial insemination. Steve's father puts pressure on Steve to marry someone else. Having served as an anonymous sperm donor, he is concerned that Steve and his fiancé may be half brother and sister. Given the following information, deduce whether Steve and his fiancé could be related. (The MN and Ss systems are two independent, codominant blood-type systems.)

	Blood Type
Dean	A, MN, Ss
Her daughter	O, M, S
Steve's father	A, MN, Ss
Steve	O, N, s
Steve's mother	B, N, s

INDEPENDENT ASSORTMENT

20. Mendel self-fertilized dihybrid plants (*RrYy*) with round and yellow seeds and got a 9:3:3:1 ratio in the F_2 generation. As a test of Mendel's hypothesis of independent assortment, predict the kinds and numbers of progeny produced in testcrosses of these F_2 offspring.

21. Four o'clock plants have a gene for color and a gene for height with the following phenotypes:

RR: red flower	*TT:* tall plant
Rr: pink flower	*Tt:* medium height plant
rr: white flower	*tt:* dwarf plant

Give the proportions of genotypes and phenotypes produced if a dihybrid plant is self-fertilized.

22. A particular variety of corn has a gene for kernel color and a gene for height with the following phenotypes:

CC, Cc: purple kernels	*TT:* tall stem
cc: white kernels	*Tt:* medium height stem
	tt: dwarf stem

Give the proportions of genotypes and phenotypes produced if a dihybrid plant is selfed.

23. To determine the genotypes of the offspring of a cross in which a corn trihybrid (*Aa Bb Cc*) was selfed, a geneticist has three choices. He or she can take a sample of the progeny and (a) self-fertilize the individual plants, (b) testcross the plants, or (c) cross the individuals with a trihybrid (backcross). Which method is preferable?

24. In figure 2.17, the F_2 phenotypic ratio is 3:1:3:1:6:2. What are the phenotypic segregation ratios for each blood system (AB, Rh) separately? Are they segregating properly? What phenotypic ratio in the F_2 generation would indicate interference with independent assortment?

25. Assume that Mendel looked simultaneously at four traits of his pea plants (and each trait exhibited dominance). If he crossed a homozygous dominant plant with a homozygous recessive plant, all the F_1 offspring would be of the dominant phenotype. If he then selfed the F_1 plants, how many different types of gametes would these F_1 plants produce? How many different phenotypes would appear in the F_2 generation? How many different genotypes would appear? What proportion of the F_2 offspring would be of the fourfold recessive phenotype?

26. A geneticist crossed two corn plants, creating an F_1 decahybrid (ten segregating loci). He then self-fertilized this decahybrid. How many different kinds of gametes did the F_1 plant produce? What proportion of the F_2 offspring were recessive homozygotes? How many different kinds of genotypes and phenotypes were generated in the F_2 offspring? What would your answer be if the geneticist testcrossed the decahybrid instead?

27. Consider the following crosses in pea plants and determine the genotypes of the parents in each cross. Yellow and green refer to seed color; tall and short refer to plant height.

	Progeny			
Cross	**Yellow, Tall**	**Yellow, Short**	**Green, Tall**	**Green, Short**
a. Yellow, tall × yellow, tall	89	31	33	10
b. Yellow, short × yellow, short	0	42	0	15
c. Green, tall × yellow, short	21	20	24	22

28. A brown-eyed, long-winged fly is mated with a red-eyed, long-winged fly. The progeny are

51 long, red	18 short, red
53 long, brown	16 short, brown

What are the genotypes of the parents?

29. True-breeding flies with long wings and dark bodies are mated with true-breeding flies with short wings and tan bodies. All the F_1 progeny have long wings and tan bodies. The F_1 progeny are allowed to mate and produce:

44 tan, long	14 tan, short
16 dark, long	6 dark, short

What is the mode of inheritance?

30. In peas, tall (*T*) is dominant to short (*t*), yellow (*Y*) is dominant to green (*y*), and round (*R*) is dominant to wrinkled (*r*). From a cross of two triple heterozygotes, what is the chance of getting a plant that is

a. tall, yellow, round?
b. short, green, wrinkled?
c. short, green, round?

31. In corn, the genotype *A- C- R-* is colored. Individuals homozygous for at least one recessive allele are colorless. Consider the following crosses involving colored plants, all with the same genotype. Based on the results, deduce the genotypes of the colored plants.

colored × *aa cc RR* → 1/2 colored; 1/2 colorless
colored × *aa CC rr* → 1/4 colored; 3/4 colorless
colored × *AA cc rr* → 1/2 colored; 1/2 colorless

32. Consider the following crosses in *Drosophila.* Based on the results, deduce which alleles are dominant and the genotypes of the parents. Orange and red are eye colors; crossveins occur on the wings.

	Progeny			
Parents	**Orange, crossveins**	**Orange, crossveinless**	**Red, crossveins**	**Red crossveinless**
a. Orange, crossveins × orange, crossveins	83	26	0	0
b. Red, crossveins × red, crossveinless	20	18	65	63
c. Red, crossveinless × red, crossveins	0	0	74	81
d. Red, crossveins × red, crossveins	28	11	93	34

33. In *Drosophila melanogaster,* a recessive autosomal gene, *ebony,* produces a dark body color when homozygous, and an independently assorting autosomal

gene, *black,* has a similar effect. If homozygous *ebony* flies are crossed with homozygous *black* flies,

a. what will be the phenotype of the F_1 flies?

b. what phenotypes and what proportions would occur in the F_2 generation?

c. what phenotypic ratios would you expect to find in the progeny of the backcrosses of $F_1 \times$ *ebony*? $F_1 \times$ *black*?

34. *A, B,* and *C* are independently assorting Mendelian factors (genes) controlling the production of black pigment in a rodent species. Alleles of these genes are indicated as *a, b,* and *c,* respectively. Assume that *A, B,* and *C* act in this pathway:

$$\begin{array}{lcl} \text{colorless} & \xrightarrow{\text{control by } A} & \text{red} \\ & & \downarrow \\ & & + \xrightarrow{\text{control by } C} \text{black} \\ & & \uparrow \\ \text{colorless} & \xrightarrow{\text{control by } B} & \text{red} \end{array}$$

A black *AA BB CC* individual is crossed with a colorless *aa bb cc* to give black F_1 individuals. The F_1 individuals are selfed to give F_2 progeny.

a. What proportion of the F_2 generation is colorless?

b. What proportion of the F_2 generation is red?

35. In a particular *Drosophila* species, there are four strains differing in eye color: wild-type, orange-1, orange-2, and pink. The following matings of true-breeding individuals were performed.

Cross	F_1
wild-type × orange-1	all wild-type
wild-type × orange-2	all wild-type
orange-1 × orange-2	all wild-type
orange-2 × pink	all orange-2
F_1 (orange-1 × orange-2) × pink	1/4 orange-2: 1/4 pink:1/4 orange-1: 1/4 wild-type

What F_2 ratio would you expect if the F_1 progeny from orange-1 × orange-2 were selfed?

GENOTYPIC INTERACTIONS

36. In a variety of onions, three bulb colors segregate: red, yellow, and white. A plant with a red bulb is crossed to a plant with a white bulb, and all the offspring have red bulbs. When these are selfed, the following plants are obtained:

Red-bulbed	119
Yellow-bulbed	32
White-bulbed	9

What is the mode of inheritance of bulb color, and how do you account for the ratio?

37. When studying an inherited phenomenon, a geneticist discovers a phenotypic ratio of 9:6:1 among offspring of a given mating. Give a simple, genetic explanation for this result. How would you test this hypothesis?

38. You notice a rooster with a pea comb and a hen with a rose comb in your chicken coop. Outline how you would determine the nature of the genetic control of comb type. How would you proceed if both your rooster and hen had rose combs?

39. Suggest possible mechanisms for the epistatic ratios given in table 2.4. Can you add any further ratios?

40. What are the differences among dominance, epistasis, and pleiotropy? How can you determine that pleiotropic effects, such as those seen in sickle-cell anemia, are not due to different genes?

41. You are working with the exotic organism *Phobia laboris* and are interested in obtaining mutants that work hard. Normal phobes are lazy. Perseverance finally pays off, and you successfully isolate a true-breeding line of hard workers. You begin a detailed genetic analysis of this trait. To date you have obtained the following results:

hard worker × nonworker
↓
F_1: all nonworkers of both sexes
F_1 female × worker male
↓
3/4 hard workers:1/4 nonworkers of both sexes

From these results, predict the expected phenotypic ratio from crossing two F_1 nonworkers.

BIOCHEMICAL GENETICS

42. The following is a pathway from substance Q to substance U, with each step numbered:

$$Q \xrightarrow{1} R \xrightarrow{2} S \xrightarrow{3} T \xrightarrow{4} U$$

Which product should build up in the cell and which products should never appear if the pathway is blocked at point 1? At 2? At 3? At 4?

43. The following chart shows the growth (+) or lack of growth (−) of four mutant strains of *Neurospora* with various additives. The additives are in the pathway of niacin biosynthesis. Diagram the pathway and show which steps the various mutants block. Which compound would each mutant accumulate? When you complete this problem, compare your results with figure 2.27. What effect on growth would you observe following a mutation in the pathway of serine biosynthesis?

	Mutants			
Additives	**1**	**2**	**3**	**4**
Nothing	−	−	−	−
Niacin	+	+	+	+
Tryptophan	+	+	−	−
Kynurenine	+	+	−	−
3-Hydroxyanthranilic acid	+	+	+	−
Indole	−	+	−	−

44. The following shows the growth (+) or lack of growth (−) of various mutants in another biosynthesis pathway. Determine this pathway, the point of blockage for each mutant, and the substrate each mutant accumulates.

	Mutants				
Additives	**1**	**2**	**3**	**4**	**5**
Nothing	−	−	−	−	−
A	−	+	+	+	+
B	−	+	−	+	−
C	−	+	−	+	+
D	−	−	−	+	−
E	+	+	+	+	+

45. Maple sugar urine disease is a rare inborn error of human metabolism in which the urine of affected individuals smells like maple sugar.

a. If two unaffected individuals have an affected child, what is the probable mode of inheritance of the disease?

b. What is the chance that the second child will be unaffected?

CRITICAL THINKING QUESTIONS

1. In the shepherd's purse plant, the seed capsule comes in two forms, triangular and rounded. If two dihybrids are crossed, the resulting ratio of capsules is 15:1 in favor of triangular seed capsules. What type of biochemical pathway might generate that ratio?

2. Assume Mendel made the cross of two true-breeding plants that differed in all seven traits under study, one with all dominant traits, the other with all recessive traits. What would the ratio of phenotypes be in the F_2 generation?

Suggested Readings for chapter 2 are on page B-1.

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3

MITOSIS AND MEIOSIS

STUDY OBJECTIVES

1. To observe the morphology of chromosomes 48
2. To understand the processes of mitosis and meiosis 50
3. To analyze the relationships between meiosis and Mendel's rules 61

STUDY OUTLINE

Chromosomes 48
The Cell Cycle 50
Mitosis 52
The Mitotic Spindle 52
Prophase 53
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Anaphase 54
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Meiosis 55
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Metaphase I and Anaphase I 59
Telophase I and Prophase II 59
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Meiosis in Animals 63
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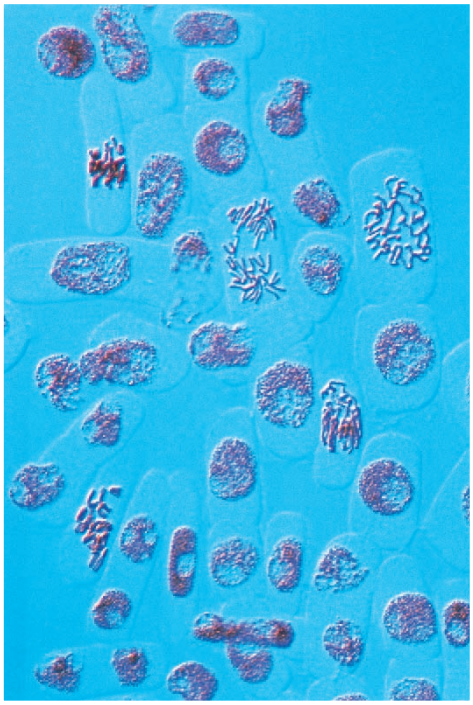


Onion (*Allium cepa*) cells in various stages of mitosis.

(© Andrew Syred/Tony Stone Images.)

The zygote, or fertilized egg of higher organisms, is the starting point of most life cycles. This zygote then divides many times to produce an adult organism. In animals, the adults then produce gametes that combine to start the cycle again. In higher plants, the adult is a sporophyte that produces spores by genetic reduction. These spores develop into gametophytes, which may or may not be independent, and gametophytes produce gametes that fuse to form the zygote (fig. 3.1). (Numerous variations on these themes exist, some of which we will discuss later in this chapter or others.) The process of cell division includes a nuclear and a cytoplasmic component. Nuclear division **(karyokinesis)** has two forms, a nonreductional **mitosis** in which the mother and daughter cells have exactly the same genetic complement, and a reductional **meiosis** in which the products, gametes in animals and spores in higher plants, have approximately half the genetic material as the parent cell. Halving the amount ensures that, when the gametes recombine, the amount of genetic material in a zygote is the same from generation to generation. The division of the cytoplasm, resulting in two cells from one original cell, is termed **cytokinesis.** In this chapter, we examine the processes of mitosis and meiosis, which allow chromosomes, the gene vehicles, to properly apportion among daughter cells. We will discuss the engineering difficulties these processes pose and the relationship of meiosis to Mendel's rules.

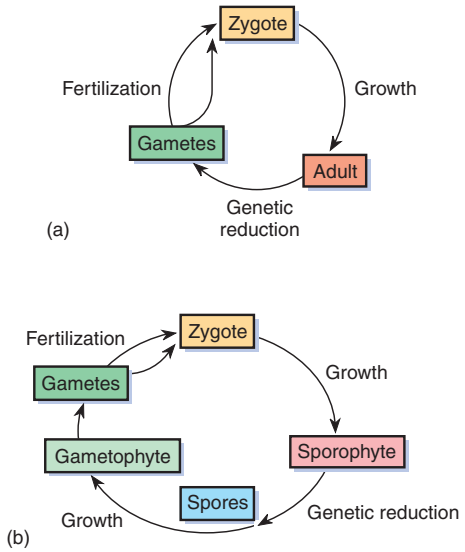


Figure 3.1 Generalized life cycle of (*a*) animals and (*b*) plants.

Mendel's work was rediscovered at the turn of the century after being ignored for thirty-four years. One of the major reasons scientists could appreciate it in 1900 was that many of the processes that chromosomes undergo had been described. With those discoveries, a physical basis for genes had been found. That is, chromosomal behavior during gamete formation precisely fits Mendel's predictions for gene behavior during gamete formation. In this chapter, we look at the morphology of chromosomes and their behavior during somatic-cell division and gamete and spore formation.

Modern biologists classify organisms into two major categories: **eukaryotes,** organisms that have true, membrane-bound nuclei, and **prokaryotes,** organisms that lack true nuclei (table 3.1). Bacteria and blue-green algae are prokaryotes. All other organisms are eukaryotes. In most prokaryotes, the genetic material is a circle of double-stranded DNA (deoxyribonucleic acid) with some associated proteins; ancillary circles of double-stranded DNA called plasmids are also found frequently (see chapters 13 and 17). In eukaryotes, the genetic material, located in the nucleus (fig. 3.2), is linear, double-stranded DNA highly complexed with protein **(nucleoprotein).** In this chapter, we concentrate on the nuclear division processes of eukaryotes.

Table 3.1 Differences Between Prokaryotic and Eukaryotic Cells

	Prokaryotic Cells	Eukaryotic Cells
Taxonomic groups	Bacteria	All plants, fungi, animals, protists
Size*	Usually less than 5 μm in greatest dimension	Usually greater than 5 μm in smallest dimension
Nucleus	No true nucleus, no nuclear membrane	Nuclear membrane
Genetic material	One circular molecule of DNA, little protein	Linear DNA molecules complexed with histones
Mitosis and meiosis	Absent	Present

* See table 3.2 on page 48.

Table 3.2 **Metric Units of Linear Measurement**

Unit	Abbreviation	Size
meter	m	39.37 U.S. inches
centimeter	cm	10^{-2} meter
millimeter	mm	10^{-3} meter
micrometer	μm	10^{-6} meter
nanometer	nm	10^{-9} meter
Angstrom	Å	10^{-10} meter

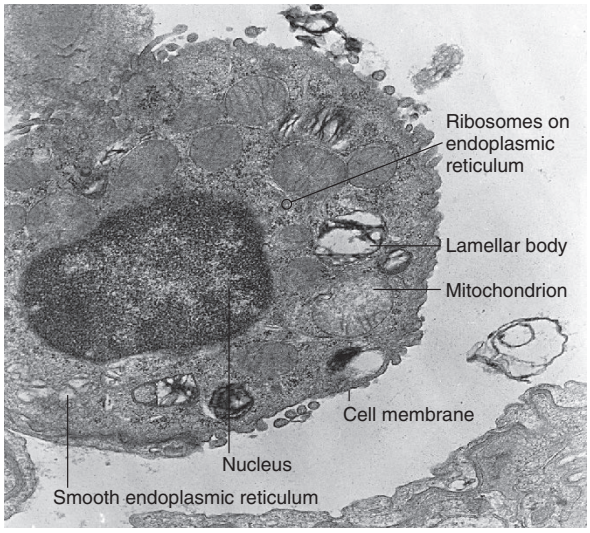


Figure 3.2 Mouse lung cell magnified 4,270x. (Courtesy of Wayne Rosenkrans.)

CHROMOSOMES

Chromosomes were discovered by C. von Nägeli in 1842. The term **chromosome,** which W. Waldeyer coined in 1888, means "colored body." Von Nägeli discovered chromosomes after staining techniques were developed that made them visible. The nucleoprotein material of the chromosomes is referred to as **chromatin.** When diffuse, chromatin is referred to as **euchromatin;** when condensed and readily visible, as **heterochromatin.**

Although all eukaryotes have chromosomes, in the **interphase** between divisions, they are spread out or diffused throughout the nucleus and are usually not identifiable. Each chromosome, with very few exceptions, has a distinct attachment point for fibers **(microtubules)** that make up the mitotic and meiotic spindle apparatuses. The attachment point occurs at a constriction in the chromosome termed the **centromere,** which is composed of several specific DNA sequences (see chapter 15). The **kinetochore** is the proteinaceous structure on the surface of the centromere to which microtubules of the spindle attach. Chromosomes can be classified according to whether the centromere is in the middle of the chromosome **(metacentric),** at the end of the chromosome **(telocentric),** very near the end of the chromosome **(acrocentric),** or somewhere in between (**subtelocentric** or **submetacentric;** figs. 3.3 and 3.4). For any particular chromosome, the position of the centromere is fixed. In various types of preparations, dark bands **(chromomeres)** are visible (see chapter 15).

Most higher eukaryotic cells are **diploid;** that is, all their chromosomes occur in pairs. One member of each pair came from each parent. **Haploid** cells, which include the reproductive cells (gametes), have only one copy of each chromosome. In the diploid state, members of the same chromosome pair are referred to as **homologous chromosomes** (homologues); the two make up a homologous pair.

The total chromosomal complement of a cell, the **karyotype,** can be photographed during mitosis and rearranged in pairs to make a picture called a karyotype or **idiogram** (fig. 3.5). From the idiogram it is possible to see whether the chromosomes have any abnormalities and to identify the sex of the organism. As you can see from figure 3.5, all of the homologous pairs are made up of identical partners, and are thus referred to as **homomorphic chromosome pairs.** A potential exception is the sex chromosomes, which in some species are of un-

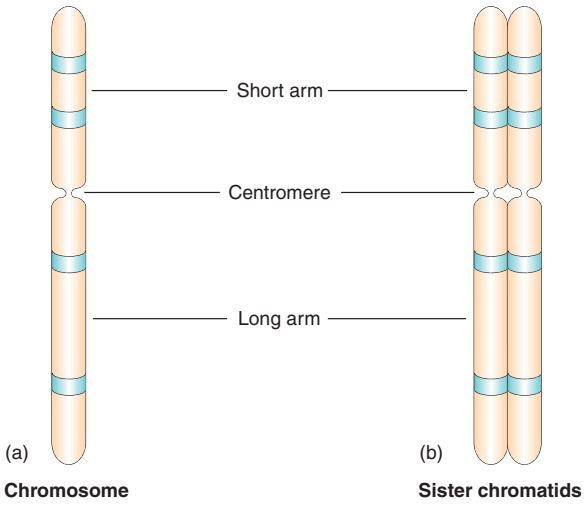


Figure 3.3 (*a*) Submetacentric chromosome and (*b*) submetacentric chromosome in mitosis. The chromosome is best seen after it has duplicated but before the identical halves (sister chromatids) separate.

Figure 3.4 (*a*) Metacentric, (*b*) submetacentric, and (*c*) acrocentric chromosomes in human beings. Except in telocentric chromosomes, the centromere divides the chromosome into two arms. (Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

equal size and are therefore called a **heteromorphic chromosome pair.**

The number of chromosomes individuals of a particular species possess is constant. Some species exist mostly in the haploid state or have long haploid intervals in their life cycle. For example, pink bread mold, *Neurospora crassa,* a fungus, has a chromosome number of seven ($n = 7$) in the haploid state. Its diploid number is, of course, fourteen ($2n = 14$). The diploid chromosome numbers of several species appear in table 3.3.

In eukaryotes, two processes partition the genetic material into offspring, or daughter, cells. One is the simple division of one cell into two. In this process, the two daughter cells must each receive an exact copy of the genetic material in the parent cell. The cellular process is simple cell division, and the nuclear process accompanying it is mitosis. In the other partitioning process, the genetic material must precisely halve so that fertilization will restore the diploid complement. The cellular process is gamete formation in animals and spore formation in higher plants, and the nuclear process is meiosis. The term *mitosis* comes from the Greek word for "thread," referring to a chromosome. The term *meiosis* comes from the Greek meaning "to lessen."

Chromosomes separate in both processes of nuclear division. The division of the cytoplasm of the cell, *cytokinesis,*

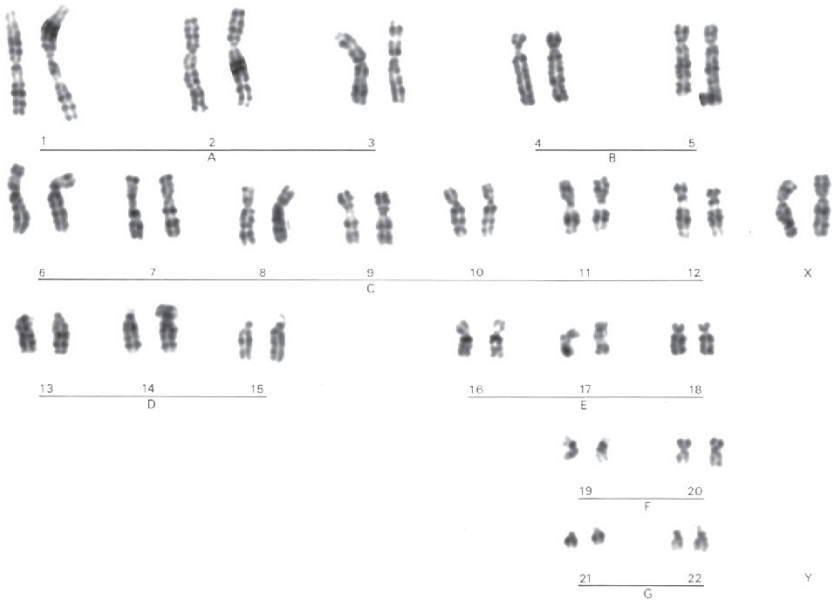


Figure 3.5 Idiogram or karyotype of a human female (two X chromosomes, no Y chromosome). A male would have one X and one Y chromosome. The chromosomes are grouped into categories (A–G, X, Y) by length and centromere position. Similar chromosomes are often distinguished by their chromomeres. (Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

Table 3.3 Chromosome Number for Selected Species

Species	2*n*
Human being (*Homo sapiens*)	46
Garden pea (*Pisum sativum*)	14
Fruit fly (*Drosophila melanogaster*)	8
House mouse (*Mus musculus*)	40
Roundworm (*Ascaris* sp.)	2
Pigeon (*Columba livia*)	80
Boa constrictor (*Constrictor constrictor*)	36
Cricket (*Gryllus domesticus*)	22
Lily (*Lilium longiflorum*)	24
Indian fern (*Ophioglossum reticulatum*)	1,260

Note: 2*n* is the diploid complement. The fern has the highest known diploid chromosome number.

is less organized. In animals, a constriction of the cell membrane distributes the cytoplasm. In plants, the growth of a cell plate accomplishes the same purpose.

THE CELL CYCLE

The continuity of life depends on cells growing, replicating their genetic material, and then dividing, a process called the **cell cycle** (fig. 3.6). Although cells usually divide when they have doubled in volume, the control of this process is very complex and precise. Not only do all the steps have to occur in sequence, but the cell must also "know" when to proceed and when to wait. Continuing at inappropriate moments—for example, before the DNA has replicated or when the chromosomes or spindle are damaged—could have catastrophic consequences to a cell or a whole organism. Numerous stops occur during the cycle to assess whether the next step should proceed.

Early research into the cell cycle involved fusing cells in different stages of the cycle (such as the G_1, S, and G_2 phases; see fig. 3.6) to determine whether the cytoplasmic components of one cell would affect the behavior of the other. Results of these experiments led to the discovery of a protein complex called the **maturation-promoting factor (MPF)** because of its role in causing oocytes to mature. It is now also referred to as the **mitosis-promoting factor** since it initiates the mitosis phase of the cell cycle. Further research has shown that MPF is made of two proteins, one that oscillates in quantity during the cell cycle and one whose quantity is constant. The oscillating component is referred to as **cyclin;** the constant gene product is an enzyme controlled by the *cdc2* gene (*cdc* stands for *c*ell *d*ivision *c*ycle) called Cdc2p. Cdc2p is a kinase, an enzyme that phosphorylates other proteins, transferring a phosphate group from ATP to an amino acid of the protein it is acting on. (Phosphorylation controls many of the processes in mitosis and in metabolism in general; for example, the nuclear membrane begins to break down when its subunits are phosphorylated.) Because the Cdc2p kinase works when combined with cyclin, it is referred to as a **cyclin-dependent kinase (CDK).** Several of these kinase-cyclin combinations control stages of the cell cycle; the cyclin of the mitosis-promoting factor is called cyclin B. In general, cylin-dependent kinases are regulated by phosphorylation and dephosphorylation, cyclin levels, and activation or deactivation of inhibitors.

Normally, Cdc2p remains at high levels in the cell but does not initiate mitosis for two reasons. First, phosphate groups block its active site, the place on the enzyme that actually does the phosphorylating. Second, the enzyme can only function when it combines with a molecule of cyclin B, the protein that oscillates during the cell cycle. Cyclin B is at very low levels when mitosis ends. During ensuing cell growth, numbers of cyclin B molecules increase, combining with Cdc2p proteins until a critical quantity is reached. However, Cdc2p-cyclin B complexes are still not active. That requires the product of another gene to dephosphorylate the Cdc2p-cyclin B complex. At that point, the Cdc2p-cyclin B complex goes into action, initiating the changes that begin mitosis (fig. 3.7). Presumably the cell is ready for mitosis at that point, having

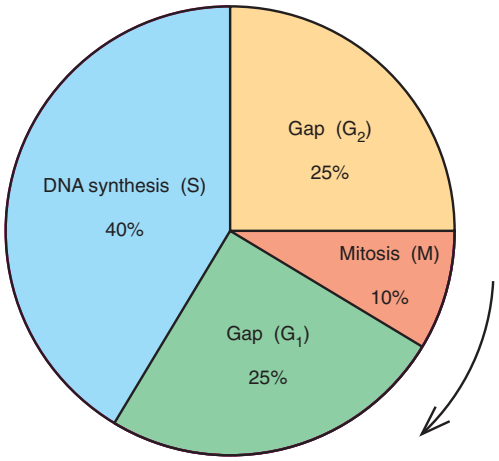


Figure 3.6 Cell cycle in the broad bean, *Vicia faba*. Total time in the cycle is under twenty hours. The DNA content of the cell doubles during the S phase and is then reduced back to its original value by mitosis.

gone through G1, S, and G2 phases (which we will discuss in detail later in the chapter).

Once mitosis has been initiated, cyclin B, along with other proteins that have served their purpose by this point in the cell cycle, breaks down with the help of a protein complex called the **anaphase-promoting complex (APC),** also called the **cyclosome.** The cyclosome works by attaching a **ubiquitin** molecule to the proteins that are to be broken down. (Ubiquitin is a polypeptide of 76 amino acids; it directs the attached protein into a breakdown pathway discussed in chapter 16.) Cdc2p is then phosphorylated to block its active site. The cell now completes mitosis and enters G_1; quantities of cyclin B are very low, and virtually no functioning Cdc2p-cyclin B remains (fig. 3.7). Thus, active Cdc2p is the kinase that controls the initiation of mitosis.

Some points in the cell cycle, such as the initiation of mitosis, can be delayed until all necessary conditions are in place. These **checkpoints** allow the cell to make sure that various events have been "checked off" as completed before the next phase begins. **Surveillance mechanisms** that involve dozens of proteins, many just discovered, oversee these checkpoints. In the cell cycle, three checkpoints involve cyclin-dependent kinases; each has its own specific cyclin that initiates either the G_1, S, or mitosis phase. In addition, other checkpoints that don't involve cyclin-dependent kinases occur at other transition phases in the cell cycle.

Cell cycle control is of particular interest because the cell cycle routinely halts if there is genetic damage, giving the cell a chance to repair the damage before committing to cell division. If the damage is too extreme, the cell can enter a programmed cell death sequence, discussed in chapter 16. If these mechanisms fail, cancer may result. The genetic control of the cell cycle is one of the most active areas of current research.

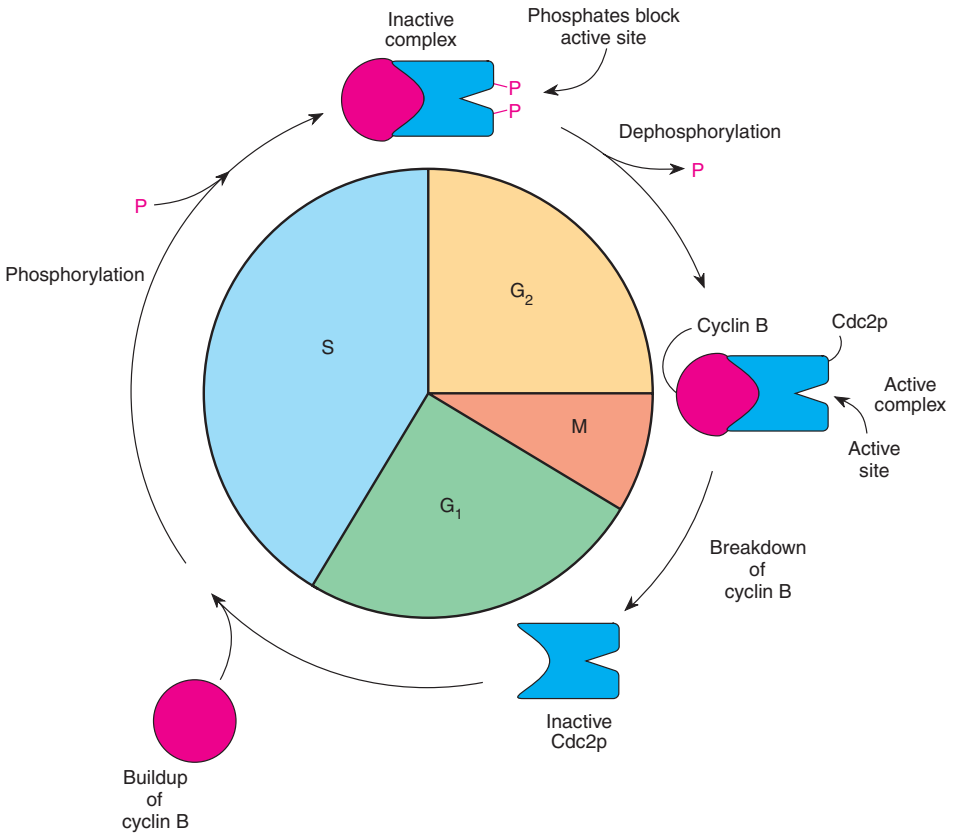


Figure 3.7 The proteins Cdc2p (CDK1) and cyclin B combine to form the maturation-promoting (or mitosis-promoting) factor. During mitosis, cyclin B is broken down. During G_1 and S phases, cyclin B builds up and combines with Cdc2P, which is then phosphorylated at the active site to render it inactive. Dephosphorylation, a process that begins to take place only after DNA replication is finished, produces an active maturation-promoting factor.

MITOSIS

Consider the engineering problem that mitosis must solve. Identical **chromatids,** called **sister chromatids,** the result of chromosomal replication, must separate so that each goes into a different daughter cell (see fig. 3.3). These chromatids are the visible manifestation of the chromosomal replication that has taken place in the S phase of the cell cycle. The chromatids are initially held together; each will be called a chromosome when it separates and becomes independent. Each of the two daughter cells then ends up with a chromosome complement identical to that of the parent cell. Mitosis is nature's elegant process to achieve that end—surely an engineering marvel.

Mitosis is a continuous process. However, for descriptive purposes, we can break it into four stages: **prophase, metaphase, anaphase,** and **telophase** (Greek: *pro-,* before; *meta-,* mid; *ana-,* back; *telo-,* end). Replication (duplication) of the genetic material occurs during the S phase of the cell cycle (see fig. 3.6). The timing of the four stages varies from species to species, from organ to organ within a species, and even from cell to cell within a given cell type.

The Mitotic Spindle

The process of mitosis involves an apparatus called the **spindle.** This structure is composed of microtubules, hollow cylinders made of protein subunits; each subunit is composed of one molecule of α tubulin and one of β tubulin; and each tubulin is the product of a different gene. (The spindle is named for the rounded rods, tapered at each end, once commonly used to hold yarn or thread.) Microtubules provide shape and structure to a eukaryotic cell as well as allow the cell to move its internal components and to move the cell itself with cilia and flagella. Motion occurs as the microtubules slide past each other, a vesicle of some kind slides along the microtubules, and the microtubules shorten. Two proteins make up the microtubule motors that allow motion: **kinesin** and **dynein.** Scientists have studied microtubules through protein chemistry, through mutant organisms, and through innovative methods such as by coupling tubulin subunits with fluorescing dyes to observe the microtubules in action.

Microtubules are in a dynamic equilibrium, with subunits constantly being added or removed at both ends. On any microtubule, more activity occurs at one end than the other. The more active end of the tubule is called the plus end, the less active end the minus end (fig. 3.8). Both ends may be adding or removing subunits, or the plus end may be adding while the minus end is removing subunits. Generally, dynein causes movement toward the minus end, whereas kinesin causes movement toward the plus end of a microtubule, although exceptions exist.

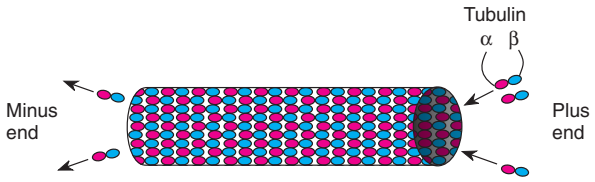


Figure 3.8 Microtubules are hollow tubes made of α and β tubulin subunits that are constantly being added or removed.

Microtubules are formed from active centers called **microtubule organizing centers. Centrioles,** composed of two cylinders—themselves composed of microtubules—are microtubule organizing centers for cilia and flagella. Under those circumstances, the centrioles are referred to as **basal bodies.** The centrioles were also originally believed to organize spindles. However, for most organisms, the microtubule organizing center is called the **centrosome.** In some organisms, such as fungi, a different cell organelle, the **spindle pole body,** serves this function. In most animals, the centrosome contains a centriole (fig. 3.9). However, the centriole is absent in most higher plants. Moreover, innovative ex-

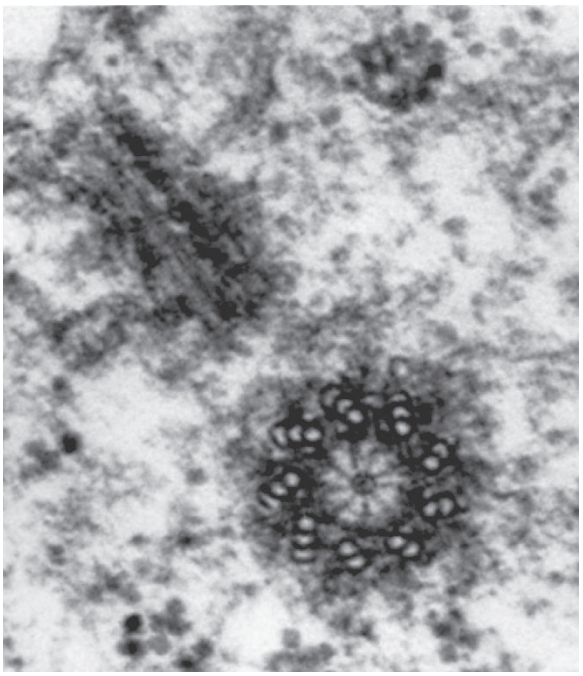


Figure 3.9 A centriole is composed of two barrels at right angles to each other. Each barrel is composed of nine tripartite units and a central cartwheel. Each of the three parts of a tripartite unit is a microtubule. Magnification 111,800×. (Reproduced from *The Journal of Cell Biology,* 1968, Vol. 37, p. 381, by copyright permission of The Rockefeller University Press.)

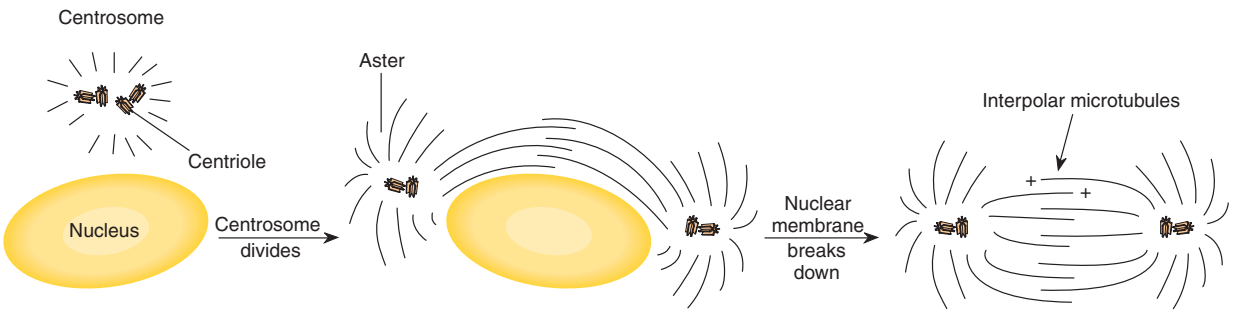


Figure 3.10 Early in mitosis, the centrosome divides, and the separating halves move to opposite poles of the cell. This creates a spindle in the middle of the cell after the nuclear membrane breaks down.

periments that removed the centrioles from cells that normally had them demonstrated that the centriole is not necessary for spindle formation. So, although we used to believe that the centriole formed the spindle in many organisms, we now know that the spindle is usually organized around the centrosome, which can function in this capacity without a centriole.

The centriole, when present, replicates during the S and G_2 phases. When mitosis begins, the centrosome divides and moves to opposite poles of the cell, around the nucleus (fig. 3.10). The centrosomes trail microtubules, forming the spindle, that at this point begin at each centrosome and overlap in the middle of the cell. These are called **interpolar microtubules.** Microtubules also spread out from the centrosome in the opposite direction from the spindle itself, forming an **aster** (see fig. 3.10). The minus ends of microtubules emanate from the centrosome and the plus ends overlap in the middle of the cell. A third form of tubulin, γ tubulin, is needed to begin the formation of a microtubule.

Prophase

This stage of mitosis is characterized by the formation of the spindle and a shortening and thickening of the chromosomes so that individual chromosomes become visible. (We will discuss details of the molecular structure of the eukaryotic chromosome and the processes of coiling and shortening in chapter 15.) At this time also, the nuclear envelope (membrane) disintegrates and the **nucleolus** disappears (fig. 3.11). The nucleolus is a darkly stained body in the nucleus that is involved in ribosome construction and that forms around a **nucleolar organizer** locus on one of the chromosome pairs. The number of nucleoli varies in different species, but in the simplest case there are two nucleolar organizers per nucleus, one each on the two members of a homologous pair of chromosomes. Nucleoli re-form after mitosis.

As prophase progresses, each chromosome is composed of two identical (sister) chromatids (see fig. 3.3); the chromosomes continue to shorten and thicken. The centromeres have already divided, and no new DNA synthesis is needed for the process to be completed. At this point, the sister chromatids are kept together by a complex, called **cohesin,** made up of at least four different protiens.

Spindle fibers are initially nucleated at the centrosome and grow outward into the cytoplasm (fig. 3.12). Some of these fibers "capture" a kinetochore, the proteinaceous complex at the centromere of each sister chromatid; these fibers are called **kinetochore microtubules.** At first, one kinetochore or the other randomly attaches to a spindle fiber. As the microtubules further move the chromosomes and as new microtubules attach and old microtubules break, each sister kinetochore eventually attaches to microtubules emanating from different poles. This ensures that sister chromatids move to opposite poles during anaphase. The number of microtubules that attach to each kinetochore differs in different species. It seems that 1 attaches to each kinetochore in yeast, 4 to 7 attach to each kinetochore in the cells of a rat fetus, and 70 to 150 attach in the plant *Haemanthus* (see fig. 3.12).

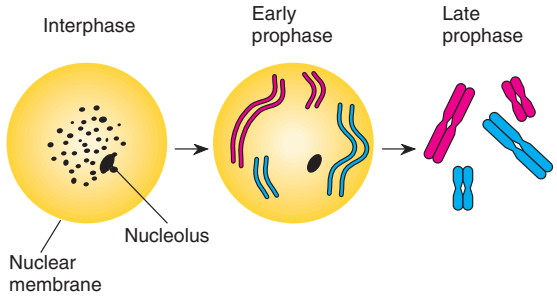


Figure 3.11 Nuclear events during interphase and prophase of mitosis. In this cell, $2n = 4$, consisting of one pair of long and one pair of short metacentric chromosomes. Maternal chromosomes are *red;* paternal chromosomes are *blue.* Note that each chromosome consists of two chromatids when the cell enters mitosis.

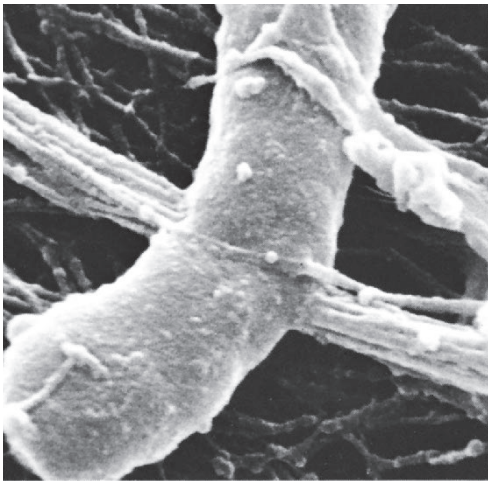


Figure 3.12 Scanning electron micrograph of the centromeric region of a metaphase chromosome from the plant *Haemanthus katherinae*. Spindle fiber bundles on either side of the centromere extend in opposite directions. A fiber not connected to the kinetochore is visible lying over the centromere. These fibers are 60 to 70 nm in diameter. (Waheeb K. Heneen, "The centromeric region in the scanning electron microscope," *Hereditas,* 97 (1982): 311–14. Reproduced by permission.)

Metaphase

During metaphase, the chromosomes move to the equator of the cell. With the attachment of the spindle fibers and the completion of the spindle itself, the chromosomes jockey into position in the equatorial plane of the spindle, called the **metaphase plate.** This happens as kinetochore microtubules exert opposing tension on the two sister kinetochores. Alignment of the chromosomes on this plate marks the end of metaphase (fig. 3.13).

Anaphase

During anaphase, the sister chromatids separate and move toward opposite poles on the spindle. The physical separation of the sister chromatids and their movement to opposite poles are two separate activities. Chromatid separation represents a checkpoint in the process of mitosis; a surveillance mechanism will not allow the process to continue until all chromosomes are lined up on the metaphase plate with their sister kinetochores held by microtubules from opposite poles. The surveillance mechanism somehow checks the physical tension the spindle fibers exert on a pair of sister chromatids; an unpaired chromatid can delay or stop the process. Initially, an inhibitory protein called **securin** binds an enzyme called **separin** that can break down cohesin, the complex holding the chromatids together. At the correct moment, the cyclosome ubiquitinates the inhibitor, causing it to break down and freeing the separin to break down cohesin. This liberates the sister chromatids from each other (and is the instant when chromatids become chromosomes).

The spindle then separates the sister chromatids in two stages, called **anaphase A** and **anaphase B.** In anaphase A, the chromosomes move toward the poles (fig. 3.14). During this process, the kinetochore itself acts as a microtubule motor, disassembling microtubules as it moves down them, pulling the chromosomes along (fig. 3.15). Thus, metacentric chromosomes appear V-shaped (as in fig. 3.15), subtelocentrics appear J-shaped, and telocentrics appear rod-shaped. In anaphase B, the spindle itself elongates as overlapping interpolar microtubules slide apart. The general elongation of the spindle pulls the chromosomes apart.

Telophase

At the end of anaphase (fig. 3.16), the separated sister chromatids (now full-fledged chromosomes) have been pulled to opposite poles of the cell. The cell now reverses the steps of prophase to return to the interphase state (fig. 3.17). The chromosomes uncoil and begin to direct protein synthesis. A nuclear envelope re-forms around each set of chromosomes, nucleoli re-form, and cytokinesis takes place. The spindle breaks down into tubulin subunits; a residual of microtubules remains at

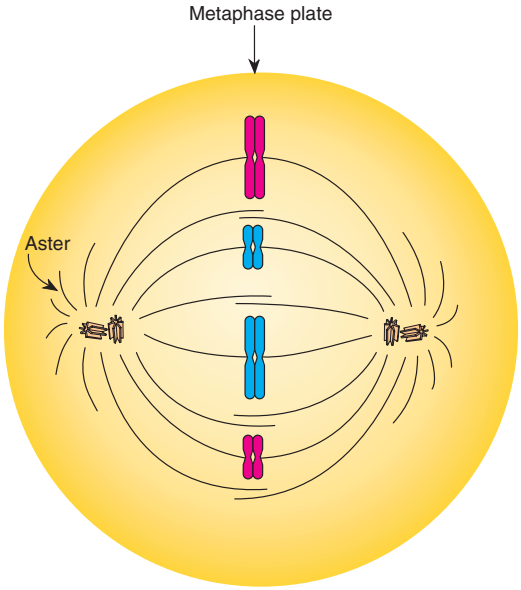
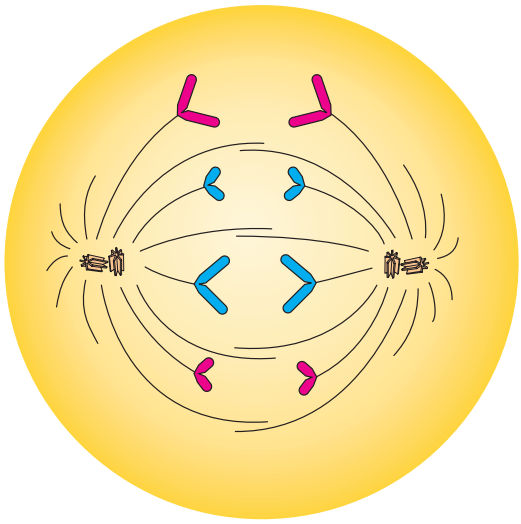


Figure 3.13 Metaphase of mitosis. In this cell, $2n = 4$. Maternal chromosomes are *red;* paternal chromosomes are *blue*.

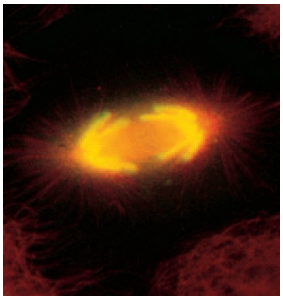
the center of the cell and seems to be involved in the formation of a constricting ring in animal cells or in the growth of a cell plate in plant cells. The cell has now entered the G_1 phase of the cell cycle (see fig. 3.6). Figure 3.18 summarizes mitosis.

The Significance of Mitosis

Cytokinesis and mitosis result in two daughter cells, each with genetic material identical to that of the parent cell. This exact distribution of the genetic material, in the form of chromosomes, to the daughter cells, ensures the stability of cells and the inheritance of traits from one cell generation to the next. Cells have evolved complex life functions; with mitosis, they will produce offspring cells with these same capabilities. With this stability assured, single-celled organisms could thrive and multicellular organisms could evolve.



(a)



(b)

Figure 3.14 (*a*) The mitotic spindle during anaphase. In this cell, $2n = 4$. Maternal chromosomes are *red;* paternal chromosomes are *blue*. (*b*) Fluorescent microscope image of a cultured cell in anaphase. Microtubules are *red;* chromosomes (DNA) are stained *yellow*. ([*b*] John M. Murray, Department of Anatomy, University of Pennsylvania. Cover of *BioTechniques,* volume 7, number 3, March 1989. Reproduced with permission.)

MEIOSIS

Gamete formation presents an entirely new engineering problem to be solved. To form gametes in animals (and, for the most part, to form spores in plants), a diploid organism with two copies of each chromosome must form daughter cells that have only one copy of each chromosome. In other words, the genetic material must be reduced by half so that when gametes recombine to form zygotes, the original number of chromosomes is restored, not doubled.

If we were to try to engineer this task, we would first need to be able to recognize homologous chromosomes. We could then push one member of each pair into one daughter cell and the other into the other daughter cell. If we were unable to recognize homologues, we would not be able to ensure that each daughter cell received one and only one member of each pair. The cell solves this problem by pairing up homologous chromosomes during an extended prophase. The spindle apparatus then separates members of the homologous chromosome pairs, just as it separates sister chromatids during mitosis. But there is one complication. As in mitosis, cells entering meiosis have already replicated their chromosomes. Therefore, two nuclear divisions without an intervening chromosome replication are necessary to produce haploid gametes or

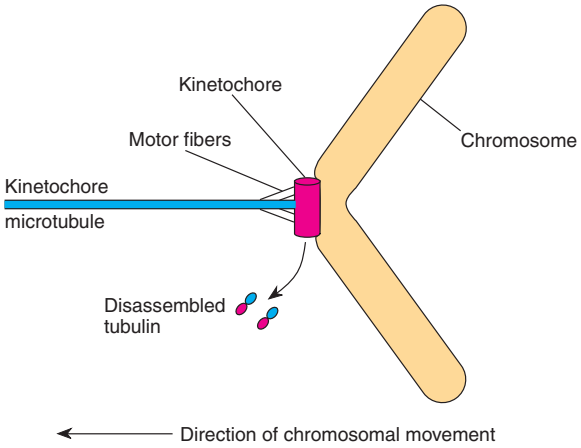


Figure 3.15 The kinetochore acts as a microtubule motor, pulling the chromosome along the kinetochore microtubules toward the pole. One microtubule is shown, although many may be present.

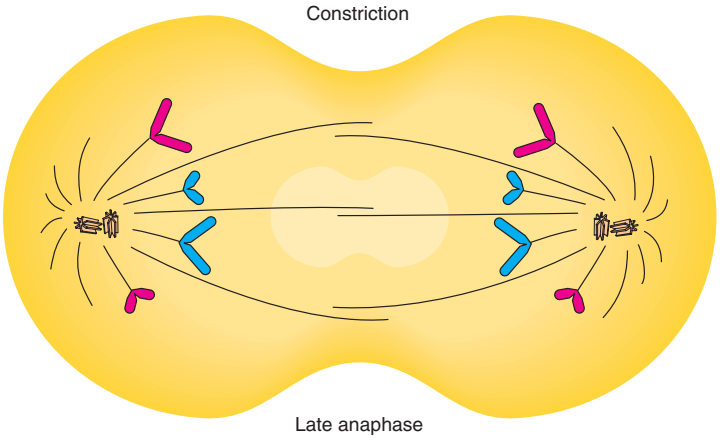


Figure 3.16 Late anaphase of mitosis; $2n = 4$. A constriction begins to form in the middle of the cell (in animals). Maternal chromosomes are *red;* paternal chromosomes are *blue*.

spores. Meiosis is, then, a two-division process that produces four cells from each original parent cell. The two divisions are known as meiosis I and meiosis II.

Unlike mitosis, meiosis occurs only in certain kinds of cells. In animals, meiosis begins in the primary gametocytes; in higher plants, the process takes place only in the spore-mother cells of the sporophyte generation (see fig. 3.1). At the end of this chapter, we review the processes of gamete and spore formation in animals and plants, respectively.

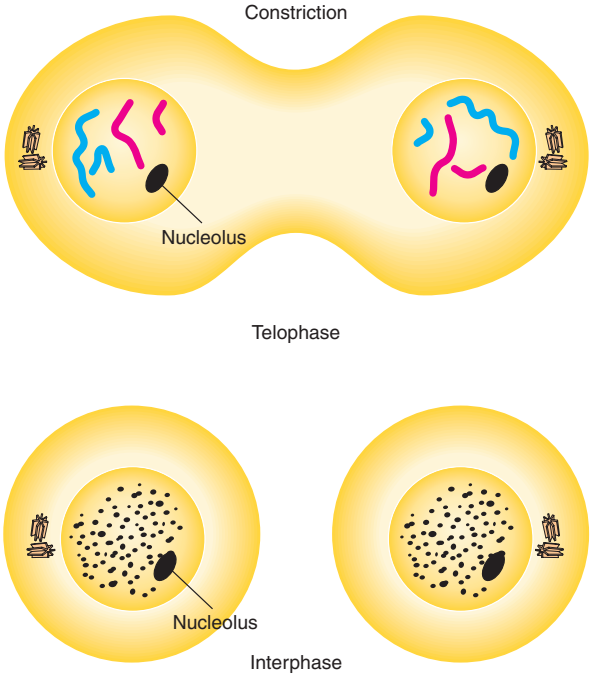


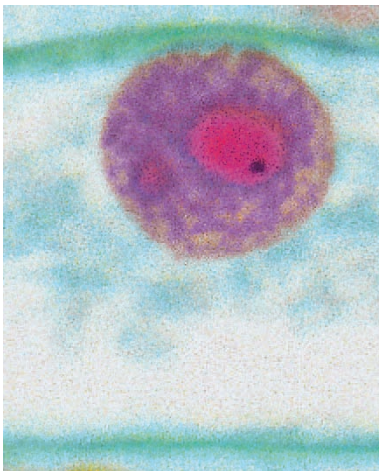
Figure 3.17 Telophase and interphase of mitosis; $2n = 4$. Maternal chromosomes are *red;* paternal chromosomes are *blue*.

Prophase I

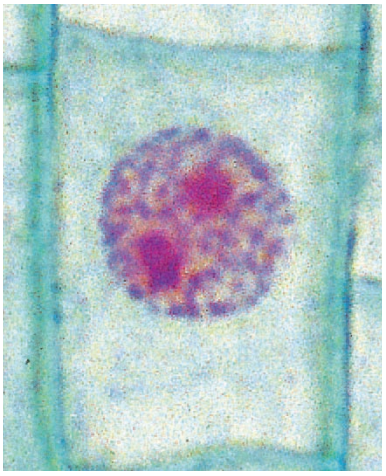
Cytogeneticists have divided the prophase of meiosis I into five stages: **leptonema, zygonema, pachynema, diplonema,** and **diakinesis** (Greek: *lepto-,* thin; *zygo-,* yoke-shaped; *pachy-,* thick; *diplo-,* double; *dia-,* across). A cell entering prophase I (leptotene stage) behaves similarly to one entering prophase of mitosis, with the centrosome duplicated and the spindle forming around the intact nucleus. (Note the adjectival forms—*leptotene*—versus the noun forms—*leptonema*—of the stage names.) As the chromosomes coil down in size during leptonema, they are visible as individual threads: sister chromatids are in such close apposition that they are not distinct. The chromosomes are more spread out than they are in mitosis, with dark spheres or bands called chromomeres interspersed.

The tips of the chromosomes are attached to the nuclear membrane in the leptotene stage (fig. 3.19). In the leptotene to zygotene transition, the tips of the chromosomes move until most end up in a limited region near each other. This forms an arrangement called a **bouquet stage.** Presumably, this arrangement helps homologous chromosomes find each other and begin the pairing process without becoming entangled.

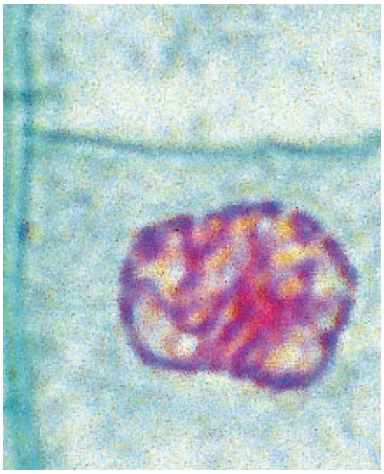
The pairing of homologous chromosomes marks the zygotene stage. Initial contact between identical regions of homologous chromosomes leads to a point-for-point pairing along their lengths. This process is referred to as **synapsis.** A proteinaceous complex, referred to as a



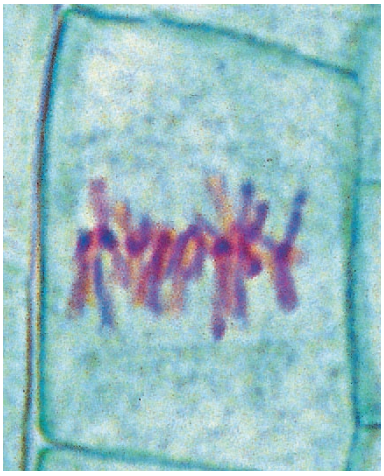
(a) Interphase



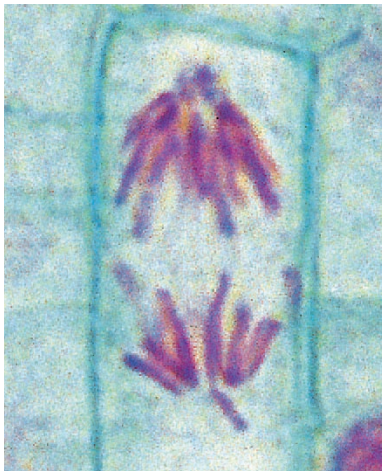
(b) Early prophase



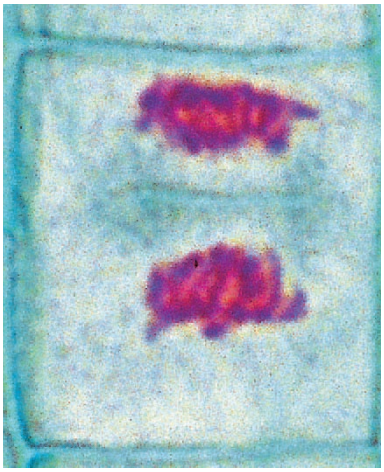
(c) Late prophase



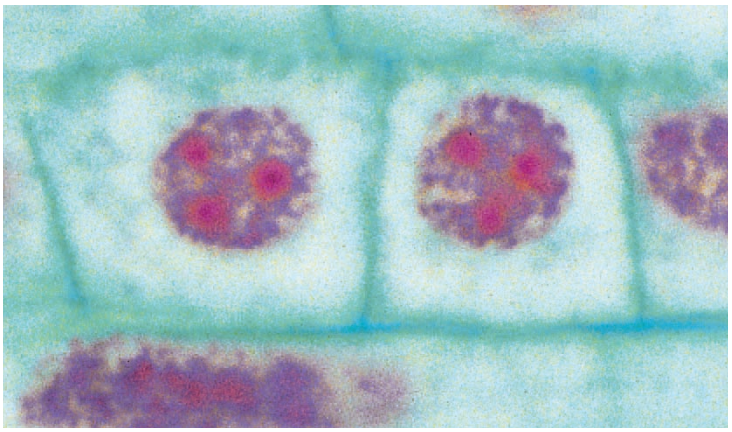
(d) Metaphase



(e) Anaphase



(f) Telophase



(g) Daughter cells

Figure 3.18 Cells in interphase and in various stages of mitosis in the onion root tip. The average cell is about 50 μm long. (© The McGraw-Hill Companies, Inc./Kingsley Stern, photographer.)

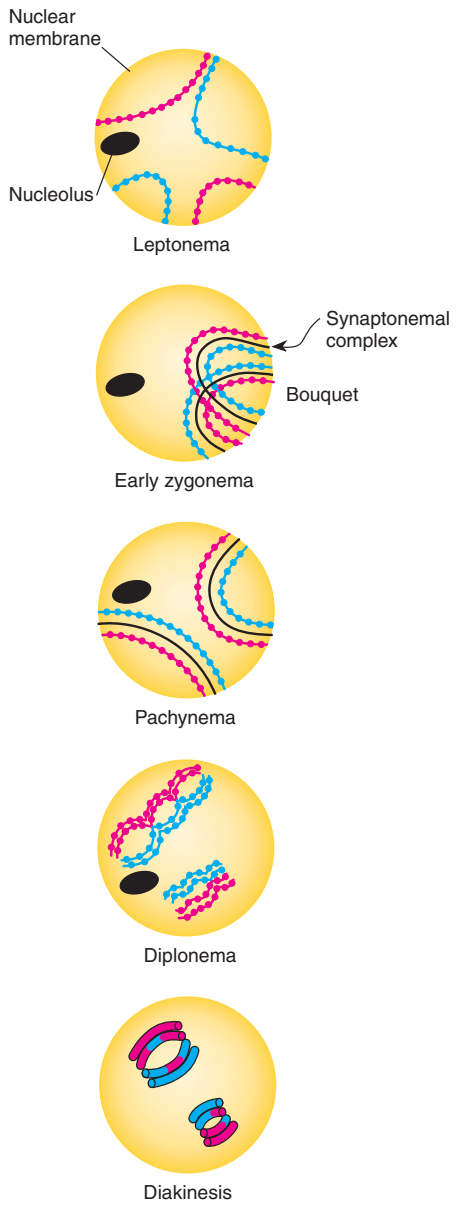


Figure 3.19 Prophase I of meiosis; $2n = 4$ (nuclei shown). Maternal chromosomes are *red;* paternal chromosomes are *blue*. Note that crossing over is evident at diplonema.

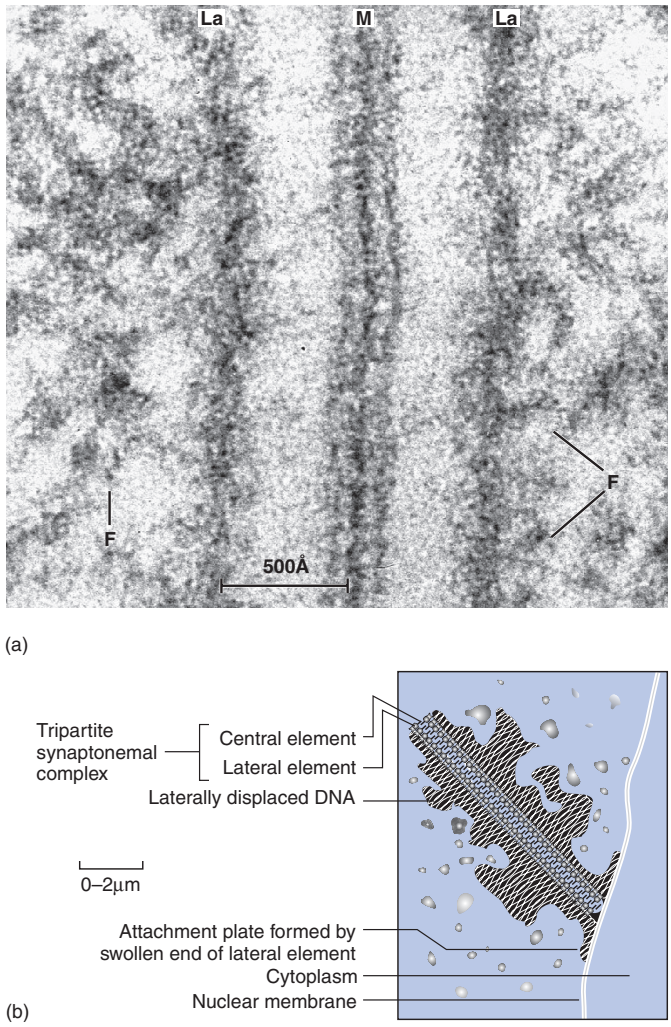


Figure 3.20 The synaptonemal complex. *(a)* In the electron micrograph, *M* is the central element, *La* are lateral elements, and *F* are chromosome fibers. Magnification 400,000×. *(b)* Diagram of the structure. ([*a*] R. Wettstein and J. R. Sotelo, "The molecular architecture of synaptonemal complexes," in E. J. DuPraw, ed., *Advances in Cell and Molecular Biology,* vol. 1 (New York: Academic Press, 1971), p. 118. Reproduced by permission. [*b*] From B. John and K. R. Lewis, *Chromosome Hierarchy.* Copyright © 1975 Oxford University Press, London, England. Reprinted by permission of the Oxford University Press.)

synaptonemal complex (fig. 3.20), appears between the homologous chromosomes and mediates synapsis in an unknown way. At this point, the chromosome figures are referred to as **bivalents,** one bivalent per homologous pair. The synapsis of all chromosomes marks the end of zygonema.

The chromosomes now continue to shorten and thicken, giving pachynema its name. During the entire prophase, **crossing over** takes place. When two chromatids come to lie in close proximity, enzymes can break both chromatid strands and reattach them differently (fig. 3.21). Thus, although genes have a fixed position on a chromosome, alleles that started out attached to a paternal centromere can end up attached to a maternal cen-

Figure 3.21 Crossing over in a tetrad during prophase of meiosis I. Maternal chromosomes are *red;* paternal chromosomes are *blue.* Note the exchange of chromosome pieces after the process is completed.

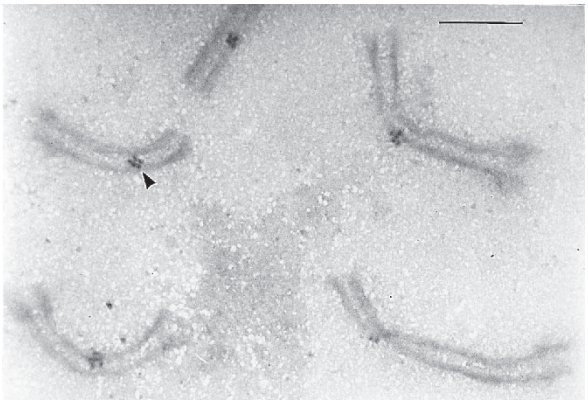
tromere. Crossing over can greatly increase the genetic variability in gametes by associating alleles that were not previously joined. (We examine the molecular mechanism of this process in chapter 12.) Before crossing over takes place, densely staining nodules are visible, first in zygonema and lasting through pachynema. These are called **recombination nodules** (fig. 3.22*a*); they are correlated with crossing over and presumably represent the enzymatic machinery present on the chromosomes.

As the chromosomes shorten and thicken further in diplonema, each chromosome can be seen to be made of two sister chromatids. Now the chromosome figures are referred to as **tetrads** because each is made up of four chromatids (see fig. 3.19). At about this time, the synaptonemal complex disintegrates in all but the areas of the **chiasmata** (singular: chiasma), the X-shaped configurations marking the places of crossing over (fig. 3.22*b*). Virtually all tetrads exhibit chiasmata; in cases in which no crossing over occurs, the tetrads tend to fall apart and segregate randomly. Thus, crossing over not only increases genetic diversity but also ensures the proper separation of homologous chromosomes. A meiosis-specific form of cohesin keeps sister chromatids together.

During the diplotene stage, chromosomes can again uncondense and become active. This is especially obvious in amphibians and birds, which produce a great amount of cytoplasmic nutrient for the future zygote. Recondensation of the chromosomes takes place at the end of diplonema. This stage can be very long; in human females, it begins in the fetus and does not complete until the egg is shed during ovulation, sometimes more than fifty years later. As prophase I moves into diakinesis, the chromosomes become very condensed (see fig. 3.19).

Metaphase I and Anaphase I

Metaphase I is marked by the breakdown of the nuclear membrane and the attachment of kinetochore microtubules to the tetrads. Unlike in mitosis, in which sister chromatids are pulled apart because each sister kinetochore is attached to a different pole, both sister kinetochores become attached to spindle microtubules coming from the same pole in metaphase I (fig. 3.23). During anaphase I, cohesin breaks down every place but at the centromeres, allowing sister chromatids to be pulled to



(a)

(b)

Figure 3.22 (*a*) Recombination nodules (*arrowhead*) in spermatocytes of the pigeon, *Columba livia.* (Bar = 1 μm.) (*b*) A tetrad from the grasshopper, *Chorthippus parallelus,* at diplonema with five chiasmata. ([a] From M. I. Pigozzi and A. J. Solari, "Recombination Nodule Mapping and Chiasma Distribution in Spermatocytes of the Pigeon, *Columba livia,*" in *Genome,* 42: 308–314, 1999. Reprinted by permission. [b] Courtesy of Bernard John.)

the same pole: homologous chromosomes are separated (fig. 3.24). This meiotic division is therefore called a **reductional division** because it reduces the number of chromosomes to half the diploid number in each daughter cell. For every tetrad there is now one chromosome in the form of a chromatid pair, known as a **dyad** or **monovalent,** at each pole of the cell. The initial objective of meiosis, separating homologues into different daughter cells, is accomplished. However, since each dyad consists of two sister chromatids, a second, mitosis-like division is required to reduce each chromosome to a single chromatid.

Telophase I and Prophase II

Depending on the organism, telophase I may or may not be greatly shortened in time. In some organisms, all the expected stages take place; chromosomes enter an interphase configuration as cytokinesis takes place. However, no chromosome duplication (DNA replication) occurs during this abbreviated interphase, termed **interkinesis.** Next, in these organisms, prophase II begins and

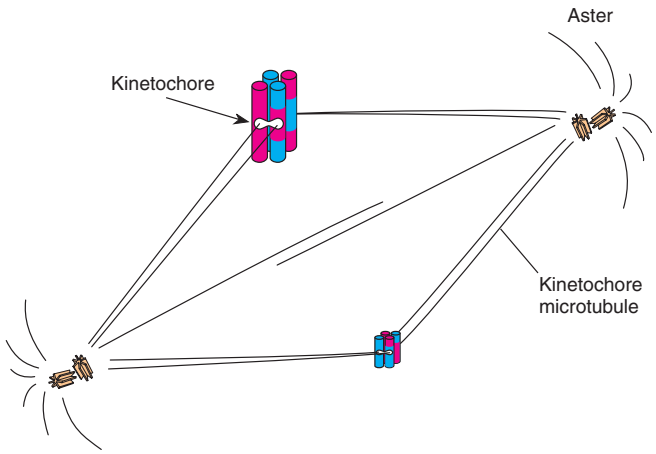
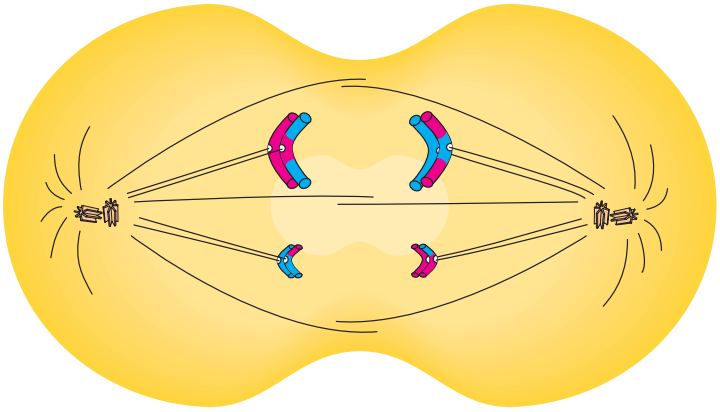


Figure 3.23 Metaphase of meiosis I; $2n = 4$. Maternal chromosomes are *red;* paternal chromosomes are *blue.* Sister kinetochores (effectively single, merged kinetochores) are attached to microtubules from the same pole.



Anaphase I

Figure 3.24 Anaphase of meiosis I; $2n = 4$. Maternal chromosomes are *red;* paternal chromosomes are *blue.* Homologous chromosomes separate and move to opposite poles.

meiosis II proceeds. In still other organisms, the late anaphase I chromosomes go almost directly into metaphase II, virtually skipping telophase I, interphase, and prophase II.

Meiosis II

Meiosis II is basically a mitotic division in which the chromatids of each chromosome are pulled to opposite poles. For each original cell entering meiosis I, four cells emerge at telophase II. Meiosis II is an **equational division;** although it reduces the amount of genetic material per cell by half, it does not further reduce the chromosome number per cell (fig. 3.25). (Sometimes it is simpler to concentrate on the behavior of centromeres during meiosis than on the chromosomes and chromatids. Meiosis I separates maternal from paternal centromeres, and meiosis II separates sister centromeres.) Figure 3.26 summarizes meiosis in corn (*Zea mays*).

In terms of chromosomes, meiosis begins with a diploid cell and produces four haploid cells. In terms of DNA, the process is a bit more complex but has the same

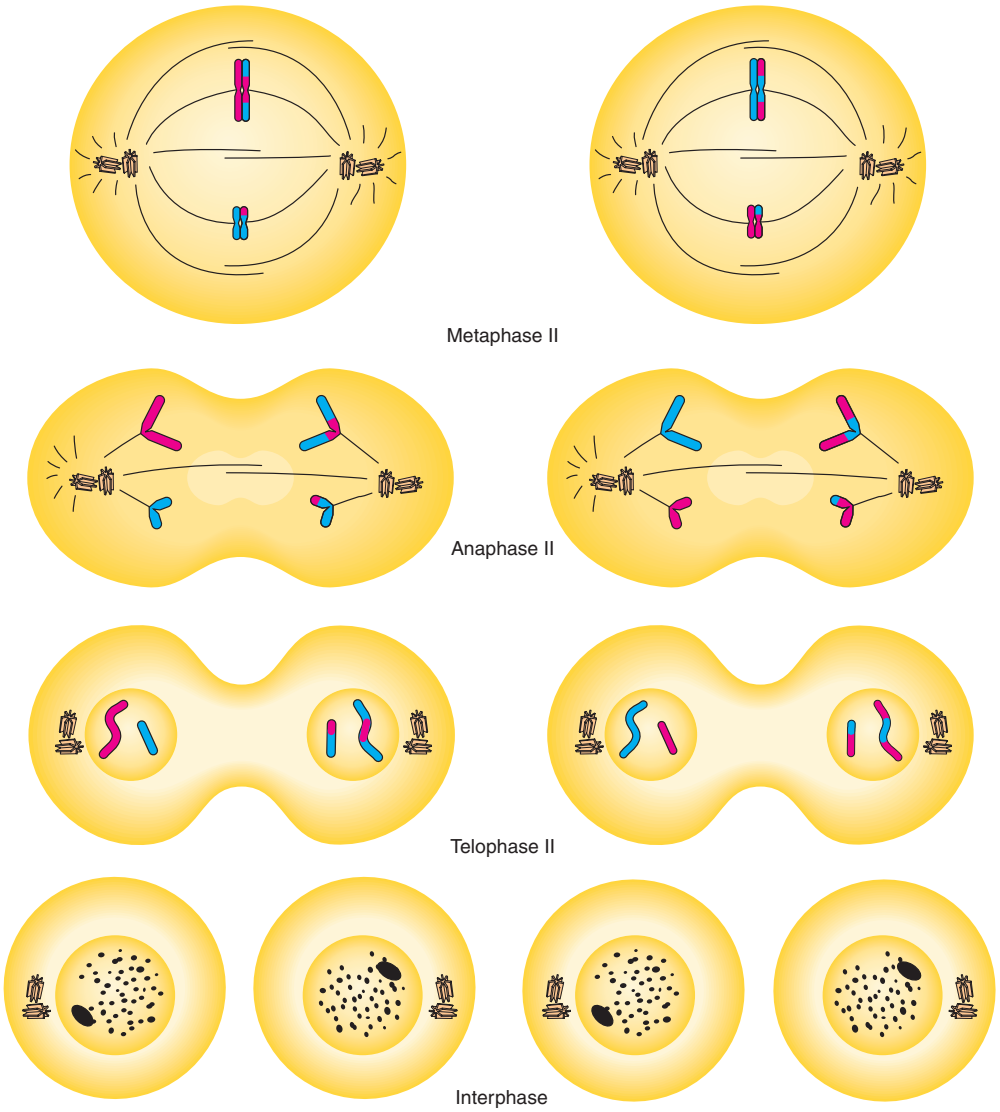


Figure 3.25 Meiosis II; $2n = 4$. Maternal chromosomes are *red;* paternal chromosomes are *blue.*

result. Let us call the quantity of DNA in a gamete "C." A diploid cell before S phase has 2C DNA, and the same cell after S phase, but before mitosis, has 4C DNA. Mitosis reduces the quantity of DNA to 2C. A cell entering meiosis also has 4C DNA. After the first meiotic division, each daughter cell has 2C DNA, and after the second meiotic division, each daughter cell has C DNA, the quantity appropriate for a gamete.

The Significance of Meiosis

Meiosis is significant for several reasons. First, it reduces the diploid number of chromosomes so that each of four daughter cells has one complete haploid chromosome set. Second, because of the randomness of the process of chromosomal separation, a very large number of different chromosomal combinations can form in the gametes. For example, in human beings, if each gamete could get either the maternal or paternal chromosome, and we have twenty-three chromosomal pairs, 2^{23} or 8,388,608 different combinations can occur. Third, because of crossing over, even more allelic combinations are possible. The process of creating new arrangements, either by crossing over or by independent segregation of homologous pairs of chromosomes, is called **recombination.** Assuming one hundred thousand genes in a human

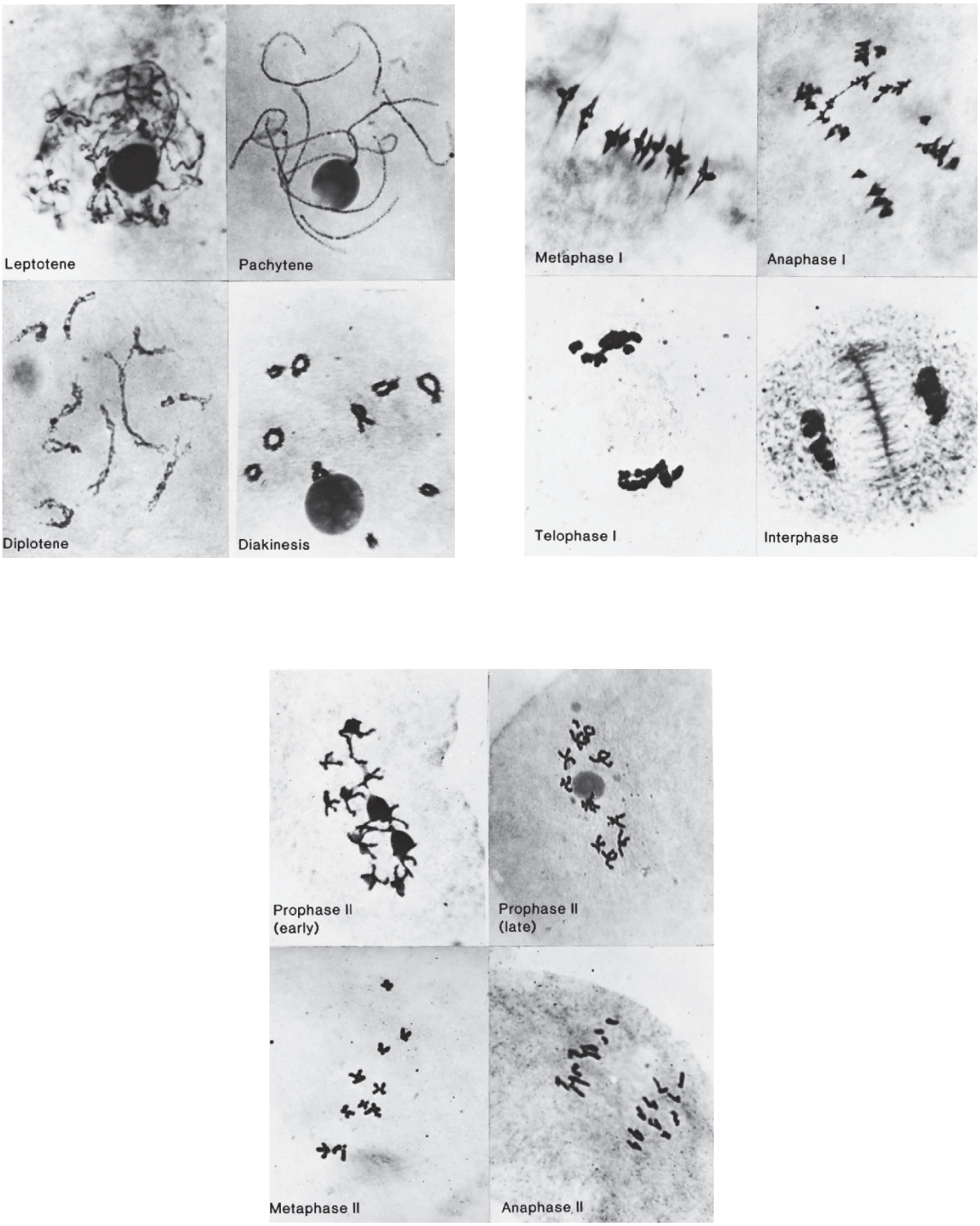


Figure 3.26 Meiosis in corn (*Zea mays*). (Courtesy of Dr. M. M. Rhoades. "Meiosis in maize," *Journal of Heredity*, 41: 59–67, 1950. Reproduced by permission.)

being with two alleles each, $2^{100,000}$ different gametes could potentially arise by meiosis.

The behavior of any tetrad follows the pattern of Mendel's rule of segregation. At spore or gamete formation (meiosis), the diploid number of chromosomes is halved; each gamete receives only one chromosome from a homologous pair. This process, of course, explains Mendel's rule of segregation. Chromosomal behavior at meiosis also explains independent assortment (fig. 3.27). In anaphase I, the direction of separation is independent in different tetrads. Whereas one pole may get the maternal centromere from chromosomal pair number 1, it could get either the maternal or the paternal centromere from chromosomal pair number 2, and so on (see fig. 3.27). Alleles of one gene segregate independently of alleles of other genes. Very shortly after the rediscovery of Mendel's principles in 1900, geneticists were quick to realize this.

MEIOSIS IN ANIMALS

In male animals, each meiosis produces four equal-sized **sperm cells** in a process called **spermatogenesis** (fig. 3.28). In vertebrates, a cell type in the testes known as a **spermatogonium** produces **primary spermatocytes,** as well as additional spermatogonia, by mitosis.

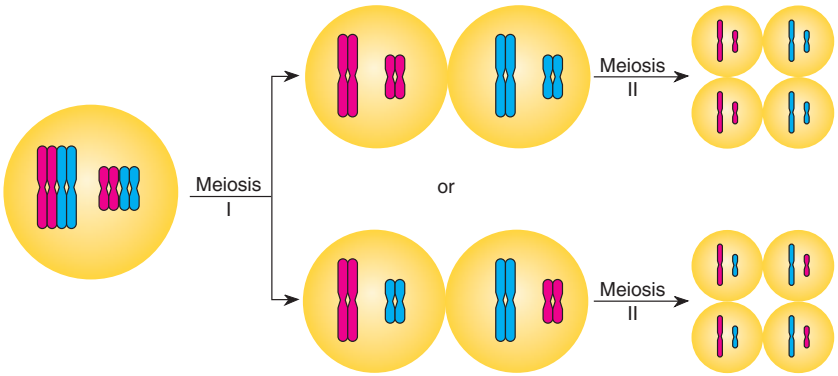


Figure 3.27 Relationship of meiosis to the rule of independent assortment. Maternal (*red*) and paternal (*blue*) chromosomes separate independently in different tetrads.

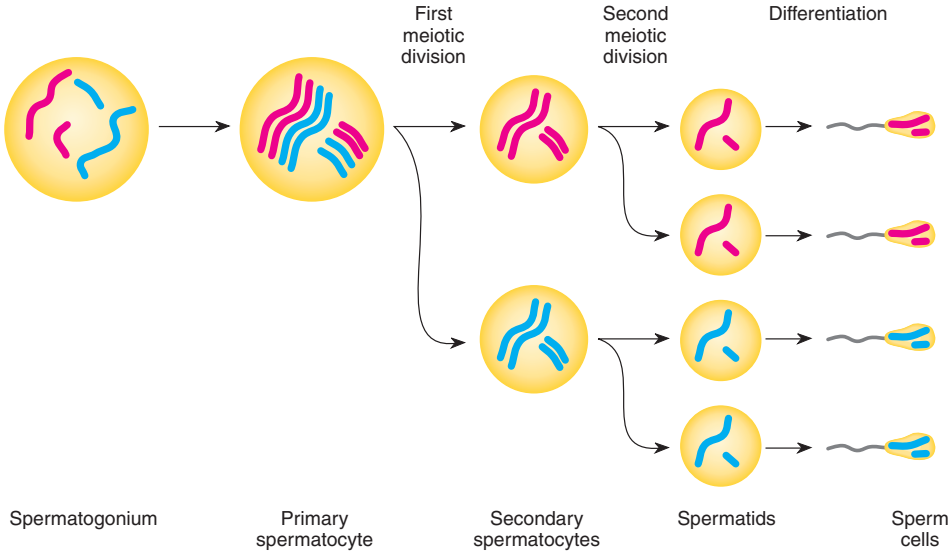


Figure 3.28 Spermatogenesis; $2n = 4$. Maternal chromosomes are *red;* paternal chromosomes are *blue.*

The primary spermatocytes then undergo meiosis. After the first meiotic division, these cells are known as **secondary spermatocytes;** after the second meiotic division, they are known as **spermatids.** The spermatids mature into spermatozoa by a process called **spermiogenesis**—with four sperm cells resulting from each primary spermatocyte. In human beings and other vertebrates without a specific mating season, the process of spermatogenesis is continuous throughout adult life. A normal human male may produce several hundred million sperm cells per day.

During embryonic development in human females, cells in the ovary, known as **oogonia,** proliferate by numerous mitotic divisions to form **primary oocytes.** About one million form per ovary. These begin the first meiotic division and then stop before the birth of the female in a prolonged diplonema, called the **dictyotene** stage. A primary oocyte does not resume meiosis until the female is past puberty, when, under hormonal control, ovulation takes place. This process usually occurs for only one oocyte per month during the female's reproductive life span (from about twelve to fifty years of age). Meiosis only then proceeds in the ovulated oocyte. In the female, the two cells formed by meiosis I are of unequal size. One, termed the **secondary oocyte,** contains almost all the nutrient-rich cytoplasm; the other, a **polar body,** receives very little cytoplasm. The second meiotic division in the larger cell yields another polar body and an **ovum.** The first polar body may or may not divide to form two other polar bodies, which ultimately disintegrate. Thus, **oogenesis** produces cells of unequal size—an ovum and two or three polar bodies (fig. 3.29). Cells of unequal size are produced because the oocyte nucleus and meiotic spindle reside very close to the surface of this large cell.

LIFE CYCLES

For eukaryotes, the basic pattern of the life cycle alternates between a diploid and a haploid state (see fig. 3.1). With the exception of the life cycles of bacteria and viruses, all life cycles are modifications of this general pattern. Bacteria, including blue-green algae, have a single circular chromosome; with exceptions described later, they are always in the haploid state. They divide by replicating their DNA and having the two copies separate into two daughter cells by simple cell division (see chapter 7). Viruses, on the border of being called alive, insert their genetic material into the cells of other organisms, and then manufacture new copies of themselves (see chapter 7).

Most animals are diploids that form gametes by meiosis, then restore the diploid number by fertilization. Exceptions, however, are numerous. For example, in the bees, wasps, and ants (hymenoptera), males are haploid and produce gametes by mitosis; females are diploid. Some fishes exist by **parthenogenesis,** in which the offspring come from unfertilized eggs that do not undergo meiosis. And, in some copepods, the sexual and parthenogenetic stages of their life cycles alternate.

The general pattern of the life cycle of plants alternates between two distinct generations, each of which, depending on the species, may exist independently. In lower plants, the haploid generation predominates, whereas in higher plants, the diploid generation is dominant. In flowering plants **(angiosperms),** the plant you see is the diploid **sporophyte** (see fig. 3.1). It is referred to as a sporophyte because, through meiosis, it will give rise to spores. The spores germinate into the alternate generation, the haploid **gametophyte,** which produces gametes by mitosis. Fertilization then produces the next generation

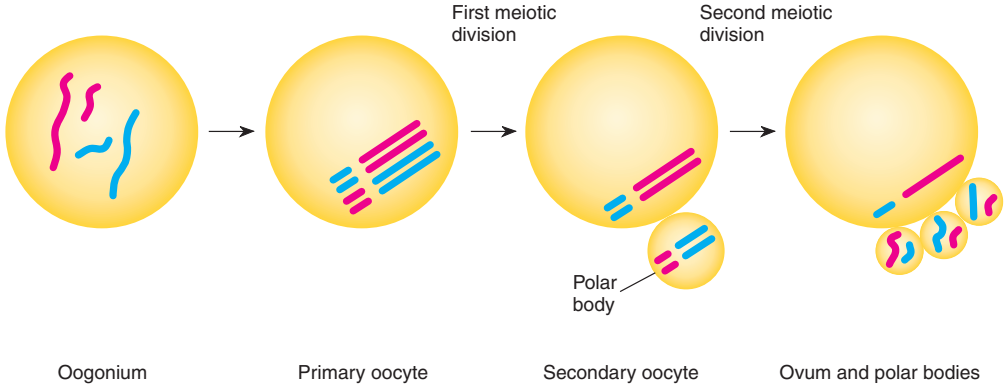


Figure 3.29 Oogenesis; $2n = 4$. Maternal chromosomes are *red;* paternal chromosomes are *blue.*

of diploid sporophytes. In lower plants, the gametophyte has an independent existence; in angiosperms, this generation is radically reduced. For example, in corn (fig. 3.30), an angiosperm, the mature corn plant is the sporophyte. The male flowers produce microspores by meiosis. After mitosis, three cells exist in each spore, a structure that we call a **pollen grain,** the male gametophyte. In female flowers, meiosis produces megaspores. Mitosis within a megaspore produces an embryo sac of seven cells with eight nuclei. This is the female gametophyte. A sperm cell fertilizes the egg cell. The two polar nuclei of the embryo sac are fertilized by a second sperm cell, producing triploid ($3n$) nutritive endosperm tissue. The sporophyte grows from the diploid fertilized egg.

Many fungi and protista are haploid. Fertilization produces a diploid stage, which almost immediately undergoes meiosis to form haploid cells. These cells, in turn, increase in number by mitosis. We will analyze organisms such as *Neurospora,* the pink bread mold, in more detail later (see chapter 6).

Much of our knowledge of genetics derives from the study of specific organisms with unique properties. Mendel found pea plants useful because he could control matings carefully, their generation time was only a year, he could easily grow them in his garden, and they had the discrete traits that he was seeking. Our interest in human beings is obvious. However, we are members of a very difficult species to study experimentally. We have a long generation time and a small number of offspring from matings that we cannot tailor for research purposes. The fruit fly, *Drosophila melanogaster,* is one of the organisms geneticists have studied most extensively. Fruit flies have a short generation time (twelve to fourteen days), which means that many matings can be carried out in a reasonable amount of time. In addition, they do exceptionally well in the laboratory, they have many easily observable mutants, and in several organs they have giant banded chromosomes of great interest to cytogeneticists.

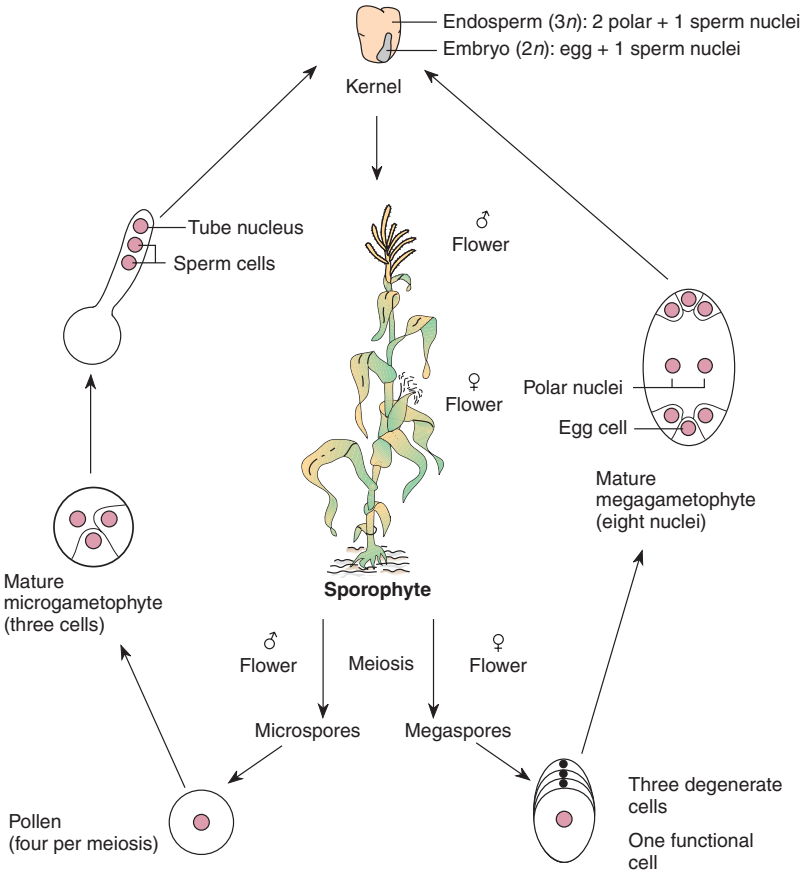


Figure 3.30 Life cycle of the corn plant.

Note that species used in food production tend to be intermediate in their life cycles. That is, many crop plants, such as peas and corn, have only one generation interval per year under normal circumstances. (We use the term *generation interval* here in the broadest sense, as the time it takes to complete an entire life cycle; see also chapter 19.) Crop plants are easier to work with from a genetic standpoint than people, but much more difficult than, say, *Drosophila* or bacteria (table 3.4). Because of their relatively long generation interval, crop plants are limited in their utility for studying basic genetic concepts or applying genetic technology to agriculture.

As you make your way through this book and through other readings on genetics, and as you come across studies involving new organisms, ask yourself the question, What are the properties of this organism that make it ideal for this type of research?

CHROMOSOMAL THEORY OF HEREDITY

In a paper in 1903, cytologist Walter Sutton firmly stated the concepts we have developed here: The behavior of chromosomes during meiosis explains Mendel's principles. Genes, then, must be located on chromosomes. This idea, which several other biologists were also developing at the time, was immediately accepted, ushering in the era of the **chromosomal theory of inheritance.** During this era, intensive effort was devoted to studying the relationships between genes and chromosomes. The major portion of the first section of this book is devoted to classical studies of **linkage** and **mapping.** Linkage deals with the association of genes to each other and to specific chromosomes. Mapping deals with the sequence of genes on a chromosome and the distances between genes on the same chromosome. This is basic information for a study of the structure and function of genes. Here we introduce a new term for the gene. The term **locus** (plural: *loci*), meaning "place" in Latin, refers to the location of a gene on the chromosome.

Table 3.4 Approximate Generation Intervals of Some Organisms of Genetic Interest

Organism	Approximate Generation Interval
Intestinal bacterium (*Escherichia coli*)	20 minutes
Bacterial virus (*lambda*)	1 hour
Pink bread mold (*Neurospora crassa*)	2 weeks
Fruit fly (*Drosophila melanogaster*)	2 weeks
House mouse (*Mus musculus*)	2 months
Corn (*Zea mays*)	6 months
Sheep (*Ovus aries*)	1 year
Cattle (*Bos taurus*)	2 years
Human being (*Homo sapiens*)	14 years

SUMMARY

STUDY OBJECTIVE 1: To observe the morphology of chromosomes 48–50

Chromosomes are made of chromatin and divided by centromeres. Within centromeres are kinetochores, attachment points for spindle fibers. Structure within the chromosomes is evident from bands on chromosomes called chromomeres.

STUDY OBJECTIVE 2: To understand the processes of mitosis and meiosis 50–61

During eukaryotic cell division, the processes of mitosis and meiosis apportion the chromosomes to daughter cells. Both processes are preceded by chromosome replication during the S phase of the cell cycle, which is under genetic control. In mitosis, the two sister chromatids making up each replicated chromosome separate into two daughter cells. Sex cells—gametes in animals and spores in plants—are produced by the two-stage process of meiosis. In meiosis, homologous chromosomes are first separated into two daughter cells, and then the sister chromatids making up each chromosome are distributed to two new daughter cells. We end up with four cells, each with the haploid chromosomal complement. The spindle is the apparatus that separates chromosomes in both mitosis and meiosis.

STUDY OBJECTIVE 3: To analyze the relationships between meiosis and Mendel's rules 61–65

The behavior of chromosomes during meiosis explains Mendel's two principles, segregation and independent assortment.

At the end of this chapter, we define the chromosomal theory of inheritance, the concept that shapes the first section of this book. This theory states that genes are located on chromosomes; their positions and order on the chromosomes can be discovered by mapping techniques described in later chapters.

SOLVED PROBLEMS

PROBLEM 1: What are the differences between chromosomes and chromatids?

Answer: In higher organisms, a chromosome is a linear DNA molecule complexed with protein and, generally, with a centromere somewhere along its length. During the cell cycle, in the S phase, the DNA replicates and each chromosome is duplicated. The duplication is visible in the early stages of mitosis and meiosis when chromosomes shorten. At this point, each duplicated chromosome is made up of two chromatids. The chromatids are called chromosomes when their centromeres are pulled to opposite poles of the spindle and each chromatid becomes independent.

PROBLEM 2: What are the relationships between mitosis and meiosis and Mendel's rules of segregation and independent assortment?

Answer: The process of mitosis does not relate directly to Mendel's rules. The behavior of chromosomes during meiosis, however, explains both segregation and independent assortment. Segregation is explained by the fact that only one chromosome from each homologous pair goes into a gamete; this is also true for the maternal and paternal alleles of a given gene. Independent assortment is explained by the independent behavior of each tetrad at meiosis. That is, the separation of maternal and paternal alleles in one tetrad is independent of the separation of maternal and paternal alleles in any other tetrad.

PROBLEM 3: A hypothetical organism has six chromosomes ($2n = 6$). How many different combinations of maternal and paternal chromosomes can appear in the gametes?

Answer: You could figure this empirically by listing all combinations. For example, let A, B, and C = maternal chromosomes and A′, B′, and C′ = paternal chromosomes. Two combinations in the gametes could be A B C′ and A′ B′ C; obviously, several other combinations exist. It is easier to recall that 2^n = number of combinations, where n = the number of chromosome pairs. In this case, $n = 3$, so we expect $2^3 = 8$ different combinations.

EXERCISES AND PROBLEMS*

CHROMOSOMES

1. What are the major differences between prokaryotes and eukaryotes?
2. What is the difference between a centromere and a kinetochore?
3. What is the difference between sister and nonsister chromatids? Between homologous and nonhomologous chromosomes?
4. In human beings, $2n = 46$. How many chromosomes would you find in a
   a. brain cell?
   b. red blood cell?
   c. polar body?
   d. sperm cell?
   e. secondary oocyte?
   (See also MEIOSIS IN ANIMALS)

MITOSIS

5. You are working with a species with $2n = 6$, in which one pair of chromosomes is telocentric, one pair subtelocentric, and one pair metacentric. The *A, B,* and *C* loci, each segregating a dominant and recessive allele (*A* and *a, B* and *b, C* and *c*), are each located on different chromosome pairs. Draw the stages of mitosis.
6. Identify stages *a–f* in the nuclear division shown in figure 1 (on the next page). Include the process, stage, and diploid number (e.g., meiosis I, prophase, $2n = 10$). Keep in mind that one picture could represent more than one process and stage. Chromosomes are drawn as threads, with circles representing kinetochores. (See also MEIOSIS)
7. When during the cell cycle does chromosome replication take place?
8. A mature human sperm cell has *c* amount of DNA. How much DNA (*c, 2c, 4c,* etc.) will a somatic cell have if it is in
   a. G_1?
   b. G_2?
   How much DNA will be in a cell at the end of meiosis I?

*Answers to selected exercises and problems are on page A-3.

MEIOSIS

9. Given the same information as in problem 5, diagram one of the possible meioses. How many different gametes can arise, absent crossing over? What variation in gamete genotype is introduced by a crossover between the *A* locus and its centromere?

10. How many bivalents, tetrads, and dyads would you find during meiosis in human beings? in fruit flies? in the other species of table 3.3?

11. Can you devise a method of chromosome partitioning during gamete formation that would not involve synapsis—that is, can you reengineer meiosis without passing through a synapsis stage?

12. What are the differences between a reductional and an equational division? What do these terms refer to?

13. How does the process of meiosis explain Mendel's two rules of inheritance?

14. *Drosophila* has four pairs of chromosomes. Let chromosomes from the male parent be A, B, C, and D, and those from the female parent be A′, B′, C′, and D′. What fraction of the gametes from an AA′ BB′ CC′ DD′ individual will be

a. all of paternal origin?
b. all of maternal origin?
c. half of maternal origin and half of paternal origin?

15. Wheat has $2n = 42$ and rye has $2n = 14$ chromosomes. Explain why a wheat-rye hybrid is usually sterile.

16. The arctic fox has fifty small chromosomes, and the red fox has thirty-eight larger chromosomes. Hybrids of these two species are sterile, but cytological studies during meiosis in these hybrids reveal both paired and unpaired chromosomes.

a. Account for the sterility of the hybrids.
b. How can you explain the paired chromosomes?

17. An organism has six pairs of chromosomes. In the absence of crossing over, how many different chromosomal combinations are possible in the gametes?

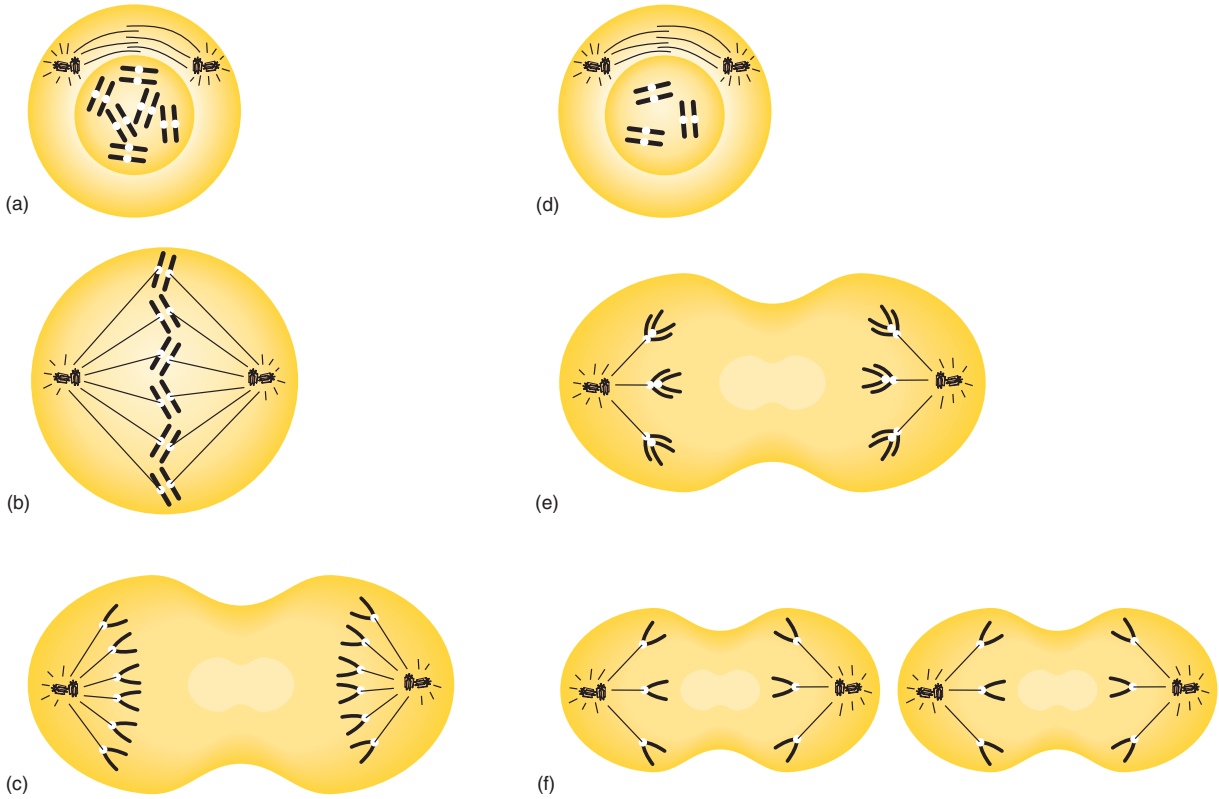


Figure 1 Stages in nuclear division.

MEIOSIS IN ANIMALS

18. How many sperm come from ten primary spermatocytes? How many ova from ten primary oocytes?

19. How do the quantity of genetic material and the ploidy change from stage to stage of spermatogenesis and oogenesis (see figs. 3.28 and 3.29)? (Consider the spermatogonium and the oogonium to be diploid, with the chromosome number arbitrarily set at two.)

20. How many sperm cells will form from
- **a.** fifty primary spermatocytes?
- **b.** fifty secondary spermatocytes?
- **c.** fifty spermatids?

21. In human beings, how many eggs will form from
- **a.** fifty primary oocytes?
- **b.** fifty secondary oocytes?

LIFE CYCLES

22. In corn (see fig. 3.30), the diploid number is twenty. How many chromosomes would you find in a(n)
- **a.** sporophyte leaf cell?
- **b.** embryo cell?
- **c.** endosperm cell?
- **d.** pollen grain?
- **e.** polar nucleus?

23. If a dihybrid corn plant is self-fertilized, what genotypes of the triploid endosperm can result? If you know the endosperm genotype, can you determine the genotype of the embryo?

24. Change the generalized life cycles of figure 3.1 so they describe the life cycles of human beings, peas, and *Neurospora.*

25. If the cytoplasm rather than nuclear genes controlled inheritance, what might be the relationship in phenotype and genotype between an organism and its parents in
- **a.** *Drosophila*?
- **b.** corn?
- **c.** *Neurospora*?

26. A drone (male) honeybee is haploid (arising from unfertilized eggs), and a queen (female) is diploid. Draw a testcross between a dihybrid queen and a drone. How many different kinds of sons and daughters might result from this cross?

27. The plant *Arabidopsis thaliana* has five pairs of chromosomes: AA, BB, CC, DD, and EE. If this plant is self-fertilized, what chromosome complement would be found in a root cell of the offspring?
- **a.** A B C D E
- **b.** AA BB CC DD EE
- **c.** AAA BBB CCC DDD EEE
- **d.** AAAA BBBB CCCC DDDD EEEE

28. In wheat, the haploid number is twenty-one. How many chromosomes would you expect to find in
- **a.** the tube nucleus?
- **b.** a leaf cell?
- **c.** the endosperm?

CHROMOSOMAL THEORY OF HEREDITY

29. A hypothetical organism has two distinct chromosomes ($2n = 4$) and fifty known genes, each with two alleles. If an individual is heterozygous at all known loci, how many gametes can be produced if
- **a.** all genes behave independently?
- **b.** all genes are completely linked?

CRITICAL THINKING QUESTIONS

1. Can meiosis occur in a haploid cell? can mitosis?

2. What is the minimum number of chromosomes that an organism can have? the maximum number?

Suggested Readings for chapter 3 are on page B-1.

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4

PROBABILITY AND STATISTICS

STUDY OBJECTIVES

1. To understand the rules of probability and how they apply to genetics 71
2. To understand the use of the chi-square statistical test in genetics 74

STUDY OUTLINE

Probability 71
Types of Probabilities 71
Combining Probabilities 71
Use of Rules 72
Statistics 74
Hypothesis Testing 74
Chi-Square 76
Failing to Reject Hypotheses 77
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Critical Thinking Questions 81

An agricultural worker studies variability in plants in a greenhouse. Probability influences the differences among organisms. (© David Joel/Tony Stone Images.)

In an experimental science, such as genetics, scientists make decisions about hypotheses on the basis of data gathered during experiments. Geneticists must therefore have an understanding of probability theory and statistical tests of hypotheses. Probability theory allows geneticists to construct accurate predictions of what to expect from an experiment. Statistical testing of hypotheses, particularly with the chi-square test, allows geneticists to have confidence in their interpretations of experimental data.

PROBABILITY

Part of Gregor Mendel's success was due to his ability to work with simple mathematics. He was capable of turning numbers into ratios and deducing the mechanisms of inheritance from them. Taking numbers that did not exactly fit a ratio and rounding them off to fit lay at the heart of Mendel's deductive powers. The underlying rules that make the act of "rounding to a ratio" reasonable are the rules of probability.

In the **scientific method,** scientists make predictions, perform experiments, and gather data that they then compare with their original predictions (see chapter 1). However, even if the bases for the predictions are correct, the data almost never exactly fit the predicted outcome. The problem is that we live in a world permeated by random, or **stochastic,** events. A bright new penny when flipped in the air twice in a row will not always give one head and one tail. In fact, that penny, if flipped one hundred times, could conceivably give one hundred heads. In a stochastic world, we can guess how often a coin should land heads up, but we cannot know for certain what the next toss will bring. We can guess how often a pea should be yellow from a given cross, but we cannot know with certainty what the next pod will contain. Thus, we need **probability theory** to tell us what to expect from data. This chapter closes with some thoughts on statistics, a branch of mathematics that helps us with criteria for supporting or rejecting our hypotheses.

Types of Probabilities

The **probability** (P) that an event will occur is the number of favorable cases (a) divided by the total number of possible cases (n):

$$P = a/n$$

The probability can be determined either by observation (empirical) or by the nature of the event (theoretical). For example, we observe that about one child in ten thousand is born with phenylketonuria. Therefore, the probability that the next child born will have phenylketonuria is 1/10,000. The odds based on the geometry of an event are, for example, like the familiar toss of dice. A die (singular of dice) has six faces. When that die is tossed, there is no reason one face should land up more often than any other. Thus, the probability of any one of the faces being up (e.g., a four) is one-sixth:

$$P = a/n = 1/6$$

Similarly, the probability of drawing the seven of clubs from a deck of cards is

$$P = 1/52$$

The probability of drawing a spade from a deck of cards is

$$P = 13/52 = 1/4$$

The probability (assuming a 1:1 sex ratio, though the actual ratio is about 1.06 males per female born in the United States) of having a daughter in any given pregnancy is

$$P = 1/2$$

And the probability that an offspring from a self-fertilized dihybrid will show the dominant phenotype is

$$P = 9/16$$

From the probability formula, we can say that an event with certainty has a probability of one, and an event that is an impossibility has a probability of zero. If an event has the probability of P, all the other alternatives combined will have a probability of $Q = 1 - P$; thus $P + Q = 1$. That is, the probability of the completely dominant phenotype in the F_2 of a selfed dihybrid is 9/16. The probability of any other phenotype is 7/16, and when the two are added together, they equal 16/16, or 1.

Combining Probabilities

The basic principle of probability can be stated as follows: If one event has c possible outcomes and a second event has d possible outcomes, then there are cd possible outcomes of the two events. From this principle, we obtain three rules that concern us as geneticists.

To understand these rules of probability requires a few definitions. *Mutually exclusive events* are events in which the occurrence of one possibility excludes the occurrence of the other possibilities. In the throwing of a die, for example, only one face can land up. Thus, if it comes up a four, it precludes the possibility of any of the other faces. Similarly, a blue-eyed daughter is mutually exclusive of a brown-eyed son or any other combination of gender and eye color. *Independent events,* however, are events whose outcomes do not influence one another. For example, if two dice are thrown, the face

value of one die is not able to affect the face value of the other; they are thus independent of each other. Similarly, the gender of one child in a family is generally independent of the gender of the children who have come before or might come after. Finally, *unordered events* are events whose probability of outcome does not depend on the order in which the events occur; the probabilities combine both mutual exclusivity and independence. For example, when two dice (one red, one green) are thrown at the same time, we generally do not specify which die has which value; a seven can occur whether the green die is the four or the red die is the four. Similarly, the probability that a family of several children will have two boys and one girl is the same irrespective of their birth order. If the family has two boys and one girl, it does not matter whether the daughter is born first, second, or third. In general, probabilities differ depending on whether order is specified. With these definitions in mind, let us look at three rules of probability that affect genetics.

1. Sum Rule

When events are mutually exclusive, the **sum rule** is used: The probability that one of several mutually exclusive events will occur is the sum of the probabilities of the individual events. This is also known as the *either-or rule.* For example, what is the probability, when we throw a die, of its showing *either* a four *or* a six? According to the sum rule,

$$P = 1/6 + 1/6 = 2/6 = 1/3$$

2. Product Rule

When the occurrence of one event is independent of the occurrence of other events, the **product rule** is used: The probability that two independent events will both occur is the product of their separate probabilities. This is known as the *and rule.* For example, the probability of throwing a die two times and getting a four *and* then a six, in that order, is

$$P = 1/6 \times 1/6 = 1/36$$

3. Binomial Theorem

The **binomial theorem** is used for unordered events: The probability that some arrangement will occur in which the final order is not specified is defined by the binomial theorem. For example, what is the probability when tossing two pennies simultaneously of getting a head and a tail? We will look more closely at how to use the rules of probability to answer this question.

USE OF RULES

There are several ways to calculate the probability just asked for. To put the problem in the form for rule 3 is the quickest method, but this problem can also be solved by using a combination of rules 1 and 2. For each penny, the probability of getting a head (H) *or* a tail (T) is

$$\text{for H: } P = 1/2$$
$$\text{for T: } Q = 1/2$$

Tossing the pennies one at a time, it is possible to get a head *and* a tail in two ways:

first head, then tail (HT)
or
first tail, then head (TH)

Within a sequence (HT or TH), the probabilities apply to independent events. Thus, the probability for any one of the two sequences involves the product rule (rule 2):

$$1/2 \times 1/2 = 1/4 \text{ for HT or TH}$$

The two sequences (HT or TH) are mutually exclusive. Thus, the probability of getting either of the two sequences involves the sum rule (rule 1):

$$1/4 + 1/4 = 1/2$$

Thus, for unordered events, we can obtain the probability by combining rules 1 and 2. The binomial theorem (rule 3) provides the shorthand method.

To use rule 3, we must state the theorem as follows: If the probability of an event (X) is p and an alternative (Y) is q, then the probability in n trials that event X will occur s times and Y will occur t times is

$$P = \frac{n!}{s!t!} p^s q^t$$

In this equation, $s + t = n$, and $p + q = 1$. The symbol !, as in $n!$, is called **factorial,** as in "n factorial," and is the product of all integers from n down to one. For example, $7! = 7 \times 6 \times 5 \times 4 \times 3 \times 2 \times 1$. Zero factorial equals one, as does anything to the power of zero ($0! = n^0 = 1$).

Now, what is the probability of tossing two pennies at once and getting one head and one tail? In this case, $n = 2$, s and $t = 1$, and p and $q = 1/2$. Thus,

$$P = \frac{2!}{1!1!}(1/2)^1(1/2)^1 = 2(1/2)^2 = 1/2$$

This is, of course, our original answer. Now on to a few more genetically relevant problems. What is the probability that a family with six children will have five girls and one boy? (We assume that the probability of either a

son or a daughter equals 1/2.) Since the order is not specified, we use rule 3:

$$P = \frac{6!}{5!1!}(1/2)^5(1/2)^1 = 6(1/2)^6 = 6/64 = 3/32$$

What would happen if we asked for a specific family order, in which four girls were born, then one boy, and then one girl? This would entail rule 2; for a sequence of six independent events:

$$P = 1/2 \times 1/2 \times 1/2 \times 1/2 \times 1/2 \times 1/2 = 1/64$$

When no order is specified, the probability is six times larger than when the order is specified; the reason is that there are six ways of getting five girls and one boy, and the sequence 4-1-1 is only one of them. Rule 3 tells us that there are six ways. These are (letting B stand for boy and G for girl) as follows:

Birth Order					
1	**2**	**3**	**4**	**5**	**6**
B	G	G	G	G	G
G	B	G	G	G	G
G	G	B	G	G	G
G	G	G	B	G	G
G	G	G	G	B	G
G	G	G	G	G	B

Let us look at yet another problem. If two persons, heterozygous for albinism (a recessive condition), have four children, what is the probability that all four children will be normal? The answer is simply $(3/4)^4$ by rule 2. What is the probability that three will be normal and one albino? If we specify which of the four children will be albino (e.g., the fourth), then the probability is $(3/4)^3(1/4)^1 = 27/256$. If, however, we do not specify order,

$$P = \frac{4!}{3!1!}(3/4)^3(1/4)^1 = 4(3/4)^3(1/4)^1$$
$$= 4(27/256) = 108/256$$

This is precisely four times the ordered probability because the albino child could have been born first, second, third, or last.

The formula for rule 3 is the formula for the terms of the **binomial expansion.** That is, if $(p + q)^n$ is expanded (multiplied out), the formula $(n!/s!t!)p^sq^t$ gives the probability for any one of these terms, given that $p + q = 1$ and that $s + t = n$. Since there are $(n + 1)$ terms in the binomial, the formula gives the probability for the term numbered $(t + 1)$. Two bits of useful information come from recalling that rule 3 is in reality the binomial expansion formula. First, if you have difficulty calculating the term, you can use **Pascal's triangle** to get the coefficients:

$$\begin{array}{ccccccccccc}
 & & & & & 1 & & & & & \\
 & & & & 1 & & 1 & & & & \\
 & & & 1 & & 2 & & 1 & & & \\
 & & 1 & & 3 & & 3 & & 1 & & \\
 & 1 & & 4 & & 6 & & 4 & & 1 & \\
1 & & 5 & & 10 & & 10 & & 5 & & 1
\end{array}$$

Pascal's triangle is a triangular array made up of coefficients in the binomial expansion. It is calculated by starting any row with a 1, proceeding by adding two adjacent terms from the row above, and then ending with a 1. For example, the next row would be

$$1, (1 + 5), (5 + 10), (10 + 10), (10 + 5), (5 + 1), 1$$
$$\text{or } 1, 6, 15, 20, 15, 6, 1$$

These numbers give us the combinations for any p^sq^t term. That is, in our previous example, $n = 4$; so we use the $(n + 1)$, or fifth, row of Pascal's triangle. (The second number in any row of the triangle gives the power of the expansion, or n. Here, 4 is the second number in the row.) We were interested in the case of one albino child in a family of four children, or p^3q^1, where p is the probability of the normal child (3/4) and q is the probability of an albino child (1/4). Hence, we are interested in the $(t + 1)$—that is, the $(1 + 1)$—or the second term of the fifth row of Pascal's triangle, which will tell us the number of ways of getting a four-child family with one albino child. That number is 4. Thus, using Pascal's triangle, we see that the solution to the problem is

$$4(3/4)^3(1/4)^1 = 108/256$$

This is the same as the answer we obtained the conventional way.

The second advantage from knowing that rule 3 is the binomial expansion formula is that we can now generalize to more than two outcomes. The general form for the **multinomial expansion** is $(p + q + r + \ldots)^n$ and the general formula for the probability is

$$P = \frac{n!}{s!t!u!\ \ldots}p^sq^tr^u\ldots$$

where $s + t + u + \ldots = n$ and $p + q + r + \ldots = 1$ For example, our albino-carrying heterozygous parents may want an answer to the following question: If we have five children, what is the probability that we will have two normal sons, two normal daughters, and one albino son? (This family will have no albino daughters.) By rule 2, the probability of

a normal son = (3/4)(1/2) = 3/8
a normal daughter = (3/4)(1/2) = 3/8
an albino son = (1/4)(1/2) = 1/8
an albino daughter = (1/4)(1/2) = 1/8

Thus:

$$P = \frac{5!}{2!2!1!0!}(3/8)^2(3/8)^2(1/8)^1(1/8)^0$$
$$= 30(3/8)^4(1/8)^1 = 30(3)^4/(8)^5 = 2{,}430/32{,}768$$
$$= 0.074$$

STATISTICS

In one of Mendel's experiments, F_1 heterozygous pea plants, all tall, were self-fertilized. In the next generation (F_2), he recorded 787 tall offspring and 277 dwarf offspring for a ratio of 2.84:1. Mendel saw this as a 3:1 ratio, which supported his proposed rule of inheritance. In fact, is 787:277 "roundable" to a 3:1 ratio? From a brief discussion of probability, we expect some deviation from an exact 3:1 ratio (798:266), but how much of a deviation is acceptable? Would 786:278 still support Mendel's rule? Would 785:279 support it? Would 709:355 (a 2:1 ratio) or 532:532 (a 1:1 ratio)? Where do we draw the line? It is at this point that the discipline of statistics provides help.

We can never speak with certainty about stochastic events. For example, take Mendel's cross. Although a 3:1 ratio is expected on the basis of Mendel's hypothesis, chance could mean that the data yield a 1:1 ratio (532:532), yet the mechanism could be the one that Mendel suggested. In other words, we could flip an honest coin and get ten heads in a row. Conversely, Mendel could have gotten exactly a 3:1 ratio (798:266) in his F_2 generation, yet his hypothesis of segregation could have been wrong. The point is that any time we deal with probabilistic events there is some chance that the data will lead us to support a bad hypothesis or reject a good one. Statistics quantifies these chances. We cannot say with certainty that a 2.84:1 ratio represents a 3:1 ratio; we can say, however, that we have a certain degree of confidence in the ratio. Statistics helps us ascertain these **confidence limits.**

Statistics is a branch of probability theory that helps the experimental geneticist in three ways. First, part of statistics deals with **experimental design**. A bit of thought applied before an experiment may help the investigator design the experiment in the most efficient way. Although he did not know statistics, Mendel's experimental design was very good. The second way in which statistics is helpful is in summarizing data. Familiar terms such as *mean* and *standard deviation* are part of the body of descriptive statistics that takes large masses of data and reduces them to one or two meaningful values. We examine further some of these terms and concepts in the chapter on quantitative inheritance (chapter 18).

Hypothesis Testing

The third way that statistics is valuable to geneticists is in the **testing of hypotheses:** determining whether to support or reject a hypothesis by comparing the data to the predictions of the hypothesis. This area is the most germane to our current discussion. For example, was the ratio of 787:277 really indicative of a 3:1 ratio? Since we know now that we cannot answer with an absolute yes, how can we decide to what level the data support the predicted 3:1 ratio?

Statisticians would have us proceed as follows. To begin, we need to establish how much variation to expect. We can determine this by calculating a **sampling distribution:** the frequencies with which various possible events could occur in a particular experiment. For example, if we self-fertilized a heterozygous tall plant, we would expect a 3:1 ratio of tall to dwarf plants among the progeny. (The 3:1 ratio is our hypothesis based on the assumption that height is genetically controlled by one locus with two alleles.) If we looked at the first four offspring, what is the probability we would see three tall and one dwarf plant? We can calculate the answer using the formula for the terms of the binomial expansion:

$$P = \frac{4!}{3!1!}(3/4)^3(1/4)^1 = 108/256 = 0.42$$

Similarly, we can calculate the probability of getting all tall (81/256 = 0.32), two tall and two dwarf (54/256 = 0.21), one tall and three dwarf (12/256 = 0.05), and all dwarf (1/256 = 0.004) in this first set of four. Table 4.1 shows this distribution, as well as the distributions for samples of eight and forty progeny. Figure 4.1 shows these distributions in graph form.

As sample sizes increase (from four to eight to forty in fig. 4.1), the sampling distribution takes on the shape of a smooth curve with a peak at the true ratio of 3:1 (75% tall progeny)—that is, there is a high probability of getting very close to the true ratio. However, there is some chance the ratio will be fairly far off, and a very small part of the time our ratio will be very far off. It is important to see that any ratio could arise in a given experiment even though the true ratio is 3:1. At what point do we decide that an experimental result is not indicative of a 3:1 ratio?

Statisticians have agreed on a convention. When all the frequencies are plotted, as in figure 4.1, we can treat the area under the curve as one unit, and we can draw lines to mark 95% of this area (fig. 4.2). Any ratios in-

Table 4.1 **Sampling Distribution for Sample Sizes of Four, Eight, and Forty, Given a 3:1 Ratio of Tall and Dwarf Plants**

$n = 4$		$n = 8$		$n = 40$	
No. Tall Plants	**Probability***	**No. Tall Plants**	**Probability***	**No. Tall Plants**	**Probability***
4	$\frac{81}{256} = 0.32$	8	0.10	40	0.00001
		7	0.27	39	0.0001
3	$\frac{108}{256} = 0.42$	6	0.31	38	0.0009
		5	0.21		. . .
2	$\frac{54}{256} = 0.21$	4	0.09	30	0.14
		3	0.02		. . .
1	$\frac{12}{256} = 0.05$	2	0.004	2	0.59×10^{-20}
		1	0.0004	1	0.10×10^{-21}
0	$\frac{1}{256} = 0.004$	0	0.00002	0	0.83×10^{-24}

* Probabilities are calculated from the binomial theorem.
probability = $(n!/s!t!)p^s q^t$
where n = number of progeny observed
s = number of progeny that are tall
t = number of progeny that are dwarf
p = probability of a progeny plant being tall (3/4)
q = probability of a progeny plant being dwarf (1/4)

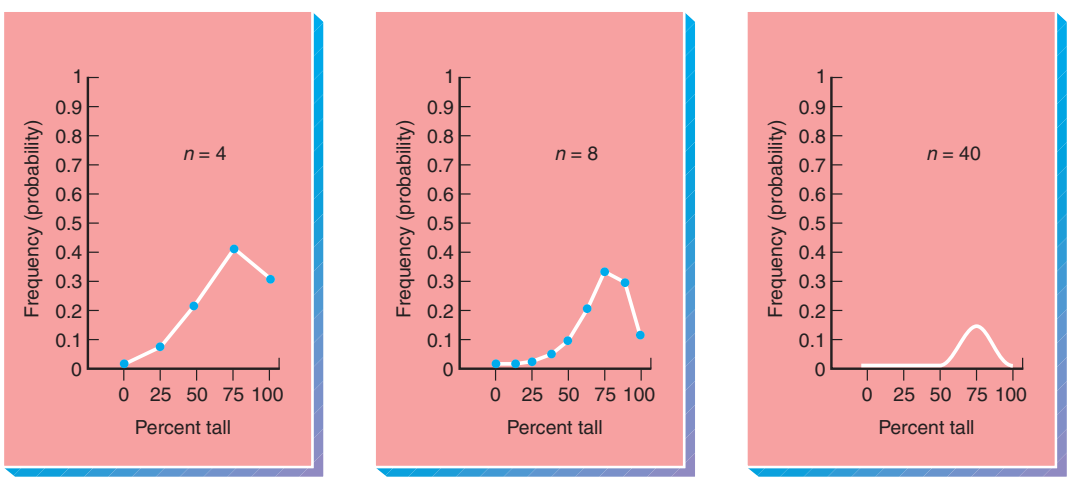

Figure 4.1 Sampling distributions from an experiment with an expected ratio of three tall to one dwarf plant. As the sample size, *n,* gets larger, the distribution curve becomes smoother. These distributions are plotted terms of the binomial expansion (see table 4.1). Note also that as *n* gets larger, the peak of the curve gets lower because as more points (possible ratios) are squeezed in along the x-axis, the probability of producing any one ratio decreases.

cluded within the 95% limits are considered supportive of (failing to reject) the hypothesis of a 3:1 ratio. Any ratio in the remaining 5% area is considered unacceptable. (Other conventions also exist, such as rejection within the outer 10% or 1% limits; we consider these at the end of the chapter.) Thus, it is possible to see whether the experimental data support our hypothesis (in this case, the hypothesis of 3:1). One in twenty times (5%) we will make a **type I error:** We will reject a true hypothesis. (A **type II error** is failing to reject a false hypothesis.)

To determine whether to reject a hypothesis, we must derive a frequency distribution for each type of experiment. Mendel could have used the distribution shown in figure 4.1 for seed coat or seed color, as long as he was expecting a 3:1 ratio and had a similar sample size. What about independent assortment, which predicts a 9:3:3:1 ratio? A geneticist would have to calculate a new sampling distribution based on a 9:3:3:1 ratio and a particular sample size. Statisticians have devised shortcut methods by using standardized probability distributions. Many are in use, such as the *t*-distribution, binomial distribution, and chi-square distribution. Each is useful for particular kinds of data; geneticists usually use the chi-square distribution to test hypotheses regarding breeding data.

Chi-Square

When sample subjects are distributed among discrete categories such as tall and dwarf plants, geneticists frequently use the **chi-square distribution** to evaluate data. The formula for converting categorical experimental data to a chi-square value is

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

where χ is the Greek letter chi, O is the observed number for a category, E is the expected number for that category, and Σ means to sum the calculations for all categories.

A chi-square (χ^2) value of 0.60 is calculated in table 4.2 for Mendel's data on the basis of a 3:1 ratio. If Mendel had originally expected a 1:1 ratio, he would have calculated a chi-square of 244.45 (table 4.3). However, these χ^2 values have little meaning in themselves: they are not probabilities. We can convert them to probabilities by determining where the chi-square value falls in relation to the area under the chi-square distribution curve. We usually use a chi-square table that contains probabilities that have already been calculated (table 4.4). Before we can use this table, however, we must define the concept of **degrees of freedom.**

Reexamination of the chi-square formula and tables 4.2 and 4.3 reveals that each category of data contributes to the total chi-square value, because chi-square is a summed value. We therefore expect the chi-square value to increase as the total number of categories increases. That is, the more categories involved, the larger the chi-square value, even if the sample fits relatively well against the hypothesized ratio. Hence, we need some way of keeping track of categories. We can do this with degrees of freedom, which is basically a count of independent categories. With Mendel's data, the total number of offspring is 1,064, of which 787 had tall stems. Therefore, the short-stem group had to consist of 277 plants (1,064 − 787) and isn't an independent category. For our purposes here, degrees of freedom equal the number of categories minus one. Thus, with two phenotypic categories, there is only one degree of freedom.

Table 4.4, the table of chi-square probabilities, is read as follows. Degrees of freedom appear in the left column. We are interested in the first row, where there is one degree of freedom. The numbers across the top of the table are the probabilities. We are interested in the next-to-the-last column, headed by the 0.05. We thus gain the following information from the table: *The probability is 0.05 of getting a chi-square value of 3.841 or larger by chance alone, given that the hypothesis is correct.* This statement formalizes the information in our discussion of frequency distributions. Hence, we are interested in how large a chi-square value will be found in the 5% unacceptable area of the curve. For Mendel's plant experiment, the **critical chi-square** (at $p = 0.05$, one degree of freedom) is 3.841. This is the value to which we compare the calculated χ^2 values (0.60 and 244.45). Since the chi-square value for the 3:1 ratio is 0.60 (table 4.2), which is less than the critical value of 3.841, we do not reject the hypothesis of a 3:1 ratio. But since χ^2 for the 1:1 ratio (table 4.3) is 244.45, which is greater than the critical value, we reject the hypothesis of a 1:1 ratio. Notice that once we did the chi-square test for the 3:1 ratio and failed to reject the hypothesis, no other statistical tests were needed: Mendel's data are consistent with a 3:1 ratio.

A word of warning when using the chi-square: If the expected number in any category is less than five, the conclusions are not reliable. In that case, you can repeat the experiment to obtain a larger sample size, or you can

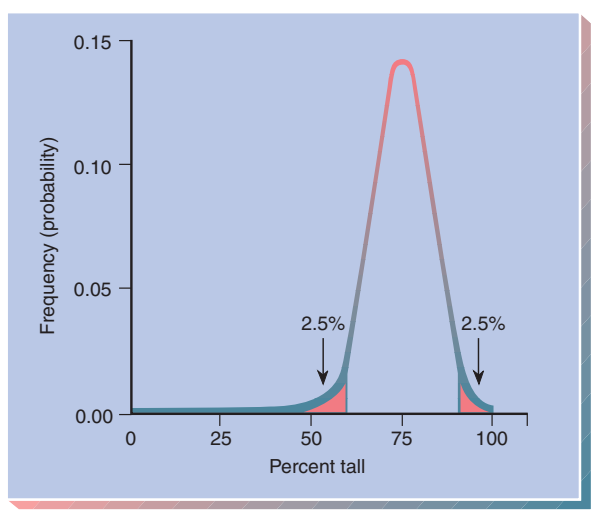

Figure 4.2 Sampling distribution of figure 4.1, $n = 40$. By convention, 5% of the area is marked off (2.5% at each end).

combine categories. Note also that chi-square tests are always done on whole numbers, not on ratios or percentages.

Failing to Reject Hypotheses

Hypothesis testing, in general, involves testing the assumption that there is no difference between the observed and the expected samples. Therefore, the hypothesis against which the data are tested is referred to as the **null hypothesis.** If the null hypothesis is not rejected, then we say that the data are consistent with it, not that the hypothesis has been proved. (As previously discussed, it is always possible we are not rejecting a false hypothesis or are rejecting the true one.) If, however, the hypothesis is rejected, as we rejected a 1:1 ratio for Mendel's data, we fail to reject the alternative hypothesis: that there is a difference between the observed and the expected values. We may then retest the data against some other hypothesis. (We don't say "accept the hypothesis" but rather "fail to reject the hypothesis," because supportive numbers could arise for many reasons. Our failure to reject is tentative acceptance of a hypothesis. However, we are on stronger ground when we reject a hypothesis.)

The use of the 0.05 probability level as a cutoff for rejecting a hypothesis is a convention called the **level of significance.** When a hypothesis is rejected at that level, statisticians say that the data depart *significantly* from the expected ratio. Other levels of significance are also

Table 4.2 **Chi-Square Analysis of One of Mendel's Experiments, Assuming a 3:1 Ratio**

	Tall Plants	Dwarf Plants	Total
Observed numbers (O)	787	277	1,064
Expected ratio	3/4	1/4	
Expected numbers (E)	798	266	1,064
$O - E$	−11	11	
$(O - E)^2$	121	121	
$(O - E)^2/E$	0.15	0.45	$0.60 = \chi^2$

Table 4.3 **Chi-Square Analysis of One of Mendel's Experiments, Assuming a 1:1 Ratio**

	Tall Plants	Dwarf Plants	Total
Observed numbers (O)	787	277	1,064
Expected ratio	1/2	1/2	
Expected numbers (E)	532	532	1,064
$O - E$	255	−255	
$(O - E)^2$	65,025	65,025	
$(O - E)^2/E$	122.23	122.23	$244.45 = \chi^2$

Table 4.4 **Chi-Square Values**

Degrees of Freedom	Probabilities						
	0.99	0.95	0.80	0.50	0.20	0.05	0.01
1	0.000	0.004	0.064	0.455	1.642	3.841	6.635
2	0.020	0.103	0.446	1.386	3.219	5.991	9.210
3	0.115	0.352	1.005	2.366	4.642	7.815	11.345
4	0.297	0.711	1.649	3.357	5.989	9.488	13.277
5	0.554	1.145	2.343	4.351	7.289	11.070	15.086
6	0.872	1.635	3.070	5.348	8.558	12.592	16.812
7	1.239	2.167	3.822	6.346	9.803	14.067	18.475
8	1.646	2.733	4.594	7.344	11.030	15.507	20.090
9	2.088	3.325	5.380	8.343	12.242	16.919	21.666
10	2.558	3.940	6.179	9.342	13.442	18.307	23.209
15	5.229	7.261	10.307	14.339	19.311	24.996	30.578
20	8.260	10.851	14.578	19.337	25.038	31.410	37.566
25	11.524	14.611	18.940	24.337	30.675	37.652	44.314
30	14.953	18.493	23.364	29.336	36.250	43.773	50.892

used, such as 0.01. If a calculated chi-square is greater than the critical value in the table at the 0.01 level, we say that the data depart in a *highly significant* manner from the null hypothesis. Since the chi-square value at the 0.01 level is larger than the value at the 0.05 level, it is more difficult to reject a hypothesis at this level and hence more convincing when it is rejected. Other levels of rejection are also set. In clinical trials of medication, for example, experimenters attempt to make it very easy to reject the null hypothesis: a level of significance of 0.10 or higher is set. The rationale is that it is not desirable to discard a drug or treatment that may well be beneficial. Since the null hypothesis states that the drug has no effect—that is, the control and drug groups show the same response—clinicians would rather be overly conservative. Not rejecting the hypothesis means concluding that the drug has no effect. Rejecting the hypothesis means that the drug has some effect and should be tested further. It is much better to have to retest some drugs that are actually worthless than to discard drugs that have potential value.

SUMMARY

STUDY OBJECTIVE 1: To understand the rules of probability and how they apply to genetics 71–74

We have examined the rules of probability theory relevant to genetic experiments. Probability theory allows us to predict the outcomes of experiments. The probability (P) of independent events occurring is calculated by multiplying their separate probabilities. The probability of mutually exclusive events occurring is calculated by adding their individual probabilities. And the probability of unordered events is defined by the polynomial expansion $(p + q + r + \ldots)^n$:

$$P = \frac{n!}{s!t!u!\ldots} p^s q^t r^u \ldots$$

STUDY OBJECTIVE 2: To understand the use of the chi-square statistical test in genetics 74–78

To assess whether data gathered during an experiment actually support a particular hypothesis, it is necessary to determine what the probability is of getting a particular data set when the null hypothesis is correct. One way to do this is through the chi-square test:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

This test gives us a method of quantifying the confidence we can place in the results obtained from typical genetic experiments. The rules of probability and statistics allow us to devise hypotheses about inheritance and to test these hypotheses with experimental data.

SOLVED PROBLEMS

PROBLEM 1: Mendel self-fertilized a dihybrid plant that had round, yellow peas. In the offspring generation: What is the probability that a pea picked at random will be round and yellow? What is the probability that five peas picked at random will be round and yellow? What is the probability that of five peas picked at random, four will be round and yellow, and one will be wrinkled and green?

Answer: The offspring peas will be round and yellow, round and green, wrinkled and yellow, and wrinkled and green in a ratio of 9:3:3:1. Thus, the probabilities that a pea picked at random will be one of these four categories are 9/16, 3/16, 3/16, and 1/16, respectively. Thus, the probability that a pea picked at random will be round and yellow is 9/16, or 0.563. The probability of picking five of these peas in a row is $(9/16)^5$ or 0.056. The probability that of five peas picked at random, four will be round and yellow, and one will be wrinkled and green, is (substituting into the binomial equation): $(5!/4!1!)(9/16)^4(1/16)^1 = 5(9^4)/(16^5) = 5(0.006) = 0.031$.

PROBLEM 2: On a chicken farm, walnut-combed fowl that were crossed with each other produced the following offspring: walnut-combed, 87; rose-combed, 31; pea-combed, 30; and single-combed, 12. What hypothesis might you have (based on chapter 2) about the control of comb shape in fowl? Do the data support that hypothesis?

Answer: The numbers 87, 31, 30, and 12 are very similar to 90, 30, 30, and 10, which would be a perfect fit to a 9:3:3:1 ratio. We might expect that ratio, having previously learned something about how comb type is inherited in fowl. Thus, we hypothesize that inheritance of comb type is by two loci, and that dominant alleles at both result in walnut combs, a dominant allele at one locus and recessives at the other result in rose or pea combs, and the recessive alleles at both loci result in a single comb. The results of the cross of dihybrids should produce fowl with the four comb types in a 9:3:3:1 ratio of walnut-, rose-, pea-, and single-combed fowl, respectively. Therefore, our observed numbers are 87, 31, 30, and 10 (sum = 160). Our expected ratio is 9:3:3:1, or 90, 30, 30, and 10 fowl, which are 9/16, 3/16, 3/16, and 1/16, respectively, of the sum of 160. We therefore set up the following chi-square table:

	Comb Type				
	Walnut	**Rose**	**Pea**	**Single**	**Total**
Observed Numbers (O)	87	31	30	12	160
Expected Ratio	9/16	3/16	3/16	1/16	
Expected Numbers (E)	90	30	30	10	160
$O - E$	−3	1	0	2	
$(O - E)^2$	9	1	0	4	
$(O - E)^2/E$	0.1	0.033	0	0.4	$0.533 = \chi^2$

There are three degrees of freedom since there are four categories of combs. (4 − 1 = 3). The critical chi-square value with three degrees of freedom and probability of 0.05 = 7.815 (table 4.4). Since our calculated chi-square value (0.533) is less than this critical value, we cannot reject our hypothesis. In other words, our data are consistent with the hypothesis of a 9:3:3:1 phenotypic ratio, indicative of a two-locus genetic model with dominance at each locus.

EXERCISES AND PROBLEMS*

PROBABILITY

1. Assuming a 1:1 sex ratio, what is the probability that five children produced by the same parents will consist of
- **a.** three daughters and two sons?
- **b.** alternating sexes, starting with a son?
- **c.** alternating sexes?
- **d.** all daughters?
- **e.** all the same sex?
- **f.** at least four daughters?
- **g.** a daughter as the eldest child and a son as the youngest?

(*See also* USE OF RULES)

2. Phenylthiocarbamide (PTC) tasting is dominant (T) to nontasting (t). If a taster woman with a nontaster father produces children with a taster man, and the man previously had a nontaster daughter, what would be the probability that
- **a.** their first child would be a nontaster?
- **b.** their first child would be a nontaster girl?
- **c.** if they had six children, they would have two nontaster sons, two nontaster daughters, and two taster sons?
- **d.** their fourth child would be a taster daughter?

(*See also* USE OF RULES)

3. Albinism is recessive; assume for this problem that blue eyes are also recessive (albinos have blue eyes). What is the probability that two brown-eyed persons, heterozygous for both traits, would produce (remembering epistasis)
- **a.** five albino children?
- **b.** five albino sons?
- **c.** four blue-eyed daughters and a brown-eyed son?
- **d.** two sons genotypically like their father and two daughters genotypically like their mother?

(*See also* USE OF RULES)

4. On the average, about one child in every ten thousand live births in the United States has phenylketonuria (PKU). What is the probability that
- **a.** the next child born in a Boston hospital will have PKU?
- **b.** after that child with PKU is born, the next child born will have PKU?
- **c.** two children born in a row will have PKU?

5. In fruit flies, the diploid chromosome number is eight.
- **a.** What is the probability that a male gamete will contain only paternal centromeres or only maternal centromeres?

*Answers to selected exercises and problems are on page A-4.

b. What is the probability that a zygote will contain only centromeres from male grandparents? (Disregard the problems that the sex chromosomes may introduce.)

6. How many seeds should Mendel have tested to determine with complete certainty that a plant with a dominant phenotype was heterozygous? With 99% certainty? With 95% certainty? With "pretty reliable" certainty?

7. What chance do a man and a woman have of producing one son and one daughter?

8. PKU and albinism are two autosomal recessive disorders, unlinked in human beings. If two people, each heterozygous for both traits, produce a child, what is the chance of their having a child with
a. PKU?
b. either PKU or albinism?
c. both traits?

9. In human beings, the absence of molars is inherited as a dominant trait. If two heterozygotes have four children, what is the probability that
a. all will have no molars?
b. three will have no molars and one will have molars?
c. the first two will have molars and the second two will have no molars?

(*See also* USE OF RULES)

10. Galactosemia is inherited as a recessive trait. If two normal heterozygotes produce children, what is the chance that
a. one of four children will be affected?
b. three children will be born in this order: normal boy, affected girl, affected boy?

(*See also* USE OF RULES)

11. A normal man (A) whose grandfather had galactosemia and a normal woman (B) whose mother was galactosemic want to produce a child. What is the probability that their first child will be galactosemic?

12. A city had nine hundred deaths during the year, and of these, three hundred were from cancer and two hundred from heart disease. What is the probability that the next death will be from
a. cancer?
b. either cancer or heart disease?

13. A plant that has the genotype *AA bb cc DD EE* is mated with one that is *aa BB CC dd ee.* F_1 individuals are selfed. What is the chance of getting an F_2 plant whose genotype exactly matches the genotype of one of the parents?

USE OF RULES

14. The ability to taste phenylthiocarbamide is dominant in human beings. If a heterozygous taster mates with a nontaster, what is the probability that of their five children, only one will be a taster?

15. In mice, coat color is determined by two independent genes, A and C, as indicated here: *A-C-,* agouti; *aaC-,* black; $A\text{-}c^a c^a$ and $aac^a c^a$, albino. If the following two mice are crossed $AaCc^a \times Aac^a c^a$, what is the probability that among the first six offspring, two will be agouti, two will be black, and two will be albino?

STATISTICS

16. The following data are from Mendel's original experiments. Suggest a hypothesis for each set and test this hypothesis with the chi-square test. Do you reach different conclusions with different levels of significance?
a. Self-fertilization of round-seeded hybrids produced 5,474 round seeds and 1,850 wrinkled ones.
b. One particular plant from *a* yielded 45 round seeds and 12 wrinkled ones.
c. Of the 565 plants raised from F_2 round-seeded plants, 372, when self-fertilized, gave both round and wrinkled seeds in a 3:1 proportion, whereas 193 yielded only round seeds.
d. A violet-flowered, long-stemmed plant was crossed with a white-flowered, short-stemmed plant, producing the following offspring:

47 violet, long-stemmed plants
40 white, long-stemmed plants
38 violet, short-stemmed plants
41 white, short-stemmed plants

17. Mendel self-fertilized pea plants with round and yellow peas. In the next generation he recovered the following numbers of peas:

315 round and yellow peas
108 round and green peas
101 wrinkled and yellow peas
32 wrinkled and green peas

What is your hypothesis about the genetic control of the phenotype? Do the data support this hypothesis?

18. Two agouti mice are crossed, and over a period of a year they produce 48 offspring with the following phenotypes:

28 agouti mice
7 black mice
13 albino mice

What is your hypothesis about the genetic control of coat color in these mice? Do the data support that hypothesis?

19. Two curly-winged flies, when mated, produce sixty-one curly and thirty-five straight-winged progeny. Use a chi-square test to determine whether these numbers fit a 3:1 ratio.

20. A short-winged, dark-bodied fly is crossed with a long-winged, tan-bodied fly. All the F_1 progeny are long-winged and tan-bodied. F_1 flies are crossed among themselves to yield 84 long-winged, tan-bodied flies; 27 long-winged dark-bodied flies; 35 short-winged, tan-bodied flies; and 14 short-winged, dark-bodied flies.

a. What ratio do you expect in the progeny?

b. Use the chi-square test to evaluate your hypothesis. Is the observed ratio within the expected range?

CRITICAL THINKING QUESTIONS

1. A friend shows you three closed boxes, one of which contains a prize, and asks you to choose one. Your friend then opens one of the two remaining boxes, a box she knows is empty. At that point, she gives you the opportunity to change your choice to the last remaining box. Should you?

2. If all couples wanted at least one child of each sex, approximately what would the average family size be?

Suggested Readings for chapter 4 are on page B-2.

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5

SEX DETERMINATION, SEX LINKAGE, AND PEDIGREE ANALYSIS

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4. To use pedigrees to infer inheritance patterns 97

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Three generations of a family.

(© Frank Siteman/Tony Stone Images.)

We ended chapter 3 with a discussion of the chromosomal theory of heredity, stated lucidly in 1903 by Walter Sutton, that genes are located on chromosomes. In 1910, T. H. Morgan, a 1933 Nobel laureate, published a paper on the inheritance of white eyes in fruit flies. The mode of inheritance for this trait, discussed later in this chapter, led inevitably to the conclusion that the locus for this gene is on a chromosome that determines the sex of the flies: when a white-eyed male was mated with a red-eyed female, half of the F_2 sons were white-eyed and half were red-eyed; all F_2 daughters were red-eyed. Not only was this the first evidence that localized a particular gene to a particular chromosome, but this study also laid the foundation for our understanding of the genetic control of sex determination.

SEX DETERMINATION

Patterns

At the outset, we should note that the sex of an organism usually depends on a very complicated series of developmental changes under genetic and hormonal control. However, often one or a few genes can determine which pathway of development an organism takes. Those switch genes are located on the **sex chromosomes,** a heteromorphic pair of chromosomes, when those chromosomes exist.

However, sex chromosomes are not the only determinants of an organism's sex. The ploidy of an individual, as in many hymenoptera (bees, ants, wasps), can determine sex; males are haploid and females are diploid. Allelic mechanisms may determine sex by a single allele or multiple alleles not associated with heteromorphic chromosomes; even environmental factors may control sex. For example, temperature determines the sex of some geckos, and the sex of some marine worms and gastropods depends on the substrate on which they land. In this chapter, however, we concentrate on chromosomal sex-determining mechanisms.

Sex Chromosomes

Basically, four types of chromosomal sex-determining mechanisms exist: the XY, ZW, X0, and compound chromosomal mechanisms. In the XY case, as in human beings or fruit flies, the females have a homomorphic pair of chromosomes (XX) and males are heteromorphic (XY). In the ZW case, males are homomorphic (ZZ), and females are heteromorphic (ZW). (XY and ZW are chromosome notations and imply nothing about the sizes or shapes of these chromosomes.) In the X0 case, the organism has only one sex chromosome, as in some grasshoppers and beetles; females are usually XX and males X0. And in the compound chromosome case, several X and Y chromosomes combine to determine sex, as in bedbugs and some beetles. We need to emphasize that the chromosomes themselves do not determine sex, but the genes they carry do. In general, the genotype determines the type of gonad, which then determines the phenotype of the organism through male or female hormonal production.

The XY System

The XY situation occurs in human beings, in which females have forty-six chromosomes arranged in twenty-three homologous, homomorphic pairs. Males, with the same number of chromosomes, have twenty-two homomorphic pairs and one heteromorphic pair, the XY pair (fig. 5.1). During meiosis, females produce gametes that contain only the X chromosome, whereas males produce two kinds of gametes, X- and Y-bearing (fig. 5.2). For this reason, females are referred to as **homogametic** and males as **heterogametic.** As you can see from figure 5.2, in people, fertilization has an equal chance of producing either male or female offspring. In *Drosophila,* the system is the same, but the Y chromosome is almost 20% larger than the X chromosome (fig. 5.3).

Since both human and *Drosophila* females normally have two X chromosomes, and males have an X and a Y chromosome, it seems impossible to know whether maleness is determined by the presence of a Y chromosome or the absence of a second X chromosome. One way to resolve this problem would be to isolate individuals with odd numbers of chromosomes. In chapter 8, we examine the causes and outcomes of anomalous chromosome numbers. Here, we consider two facts from that chapter. First, in rare instances, individuals form, although they are not necessarily viable, with extra sets of chromosomes. These individuals are referred to as **polyploids** (*triploids* with 3*n, tetraploids* with 4*n,* etc.). Second, also infrequently, individuals form that have more or fewer than the normal number of any one chromosome. These **aneuploids** usually come about when a pair of chromosomes fails to separate properly during meiosis, an occurrence called **nondisjunction.** The existence of polyploid and aneuploid individuals makes it possible to test whether the Y chromosome is male determining. For example, a person or a fruit fly that has all the proper nonsex chromosomes, or **autosomes** (forty-four in human beings, six in *Drosophila*), but only a single X without a Y would answer our question. If the Y were absolutely male determining, then this X0 individual should be female. However, if the sex-determining mechanism is a result of the number of X chromosomes, this individual should be a male. As it turns out, an X0 individual is a *Drosophila* male and a human female.

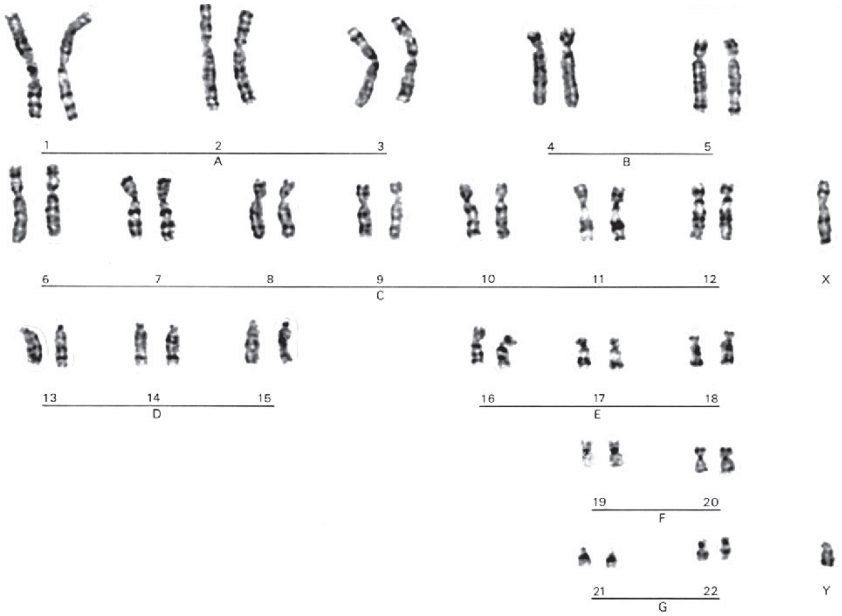
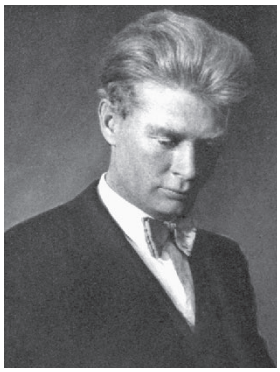


Figure 5.1 Human male karyotype. Note the X and Y chromosomes. A female would have a second X chromosome in place of the Y. (Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

Genic Balance in Drosophila

When geneticist Calvin Bridges, working with *Drosophila,* crossed a triploid (3*n*) female with a normal male, he observed many combinations of autosomes and sex chromosomes in the offspring. From his results, Bridges suggested in 1921 that sex in *Drosophila* is determined by the balance between (ratio of) autosomal alleles that favor maleness and alleles on the X chromosomes that favor femaleness. He calculated a ratio of X chromosomes to autosomal sets to see if this ratio would predict the sex of a fly. An **autosomal set** (A) in *Drosophila* consists of one chromosome from each autosomal pair, or three chromosomes. (An autosomal set in human beings consists of twenty-two chromosomes.) Table 5.1, which presents his results, shows that Bridges's **genic balance**



Calvin B. Bridges (1889–1938). (From *Genetics* 25 (1940): frontispiece. Courtesy of the Genetics Society of America.)

	Sperm One autosomal set plus X	**Sperm** One autosomal set plus Y
Ovum One autosomal set plus X	Two autosomal sets plus XX Daughter	Two autosomal sets plus XY Son

Figure 5.2 Segregation of human sex chromosomes during meiosis, with subsequent zygote formation.

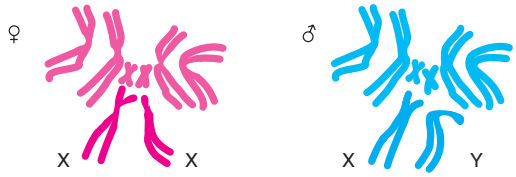


Figure 5.3 Chromosomes of *Drosophila melanogaster.*

Table 5.1 **Data Supporting Bridges's Theory of Sex Determination by Genic Balance in *Drosophila***

Number of X Chromosomes	Number of Autosomal Sets (A)	Total Number of Chromosomes	$\frac{X}{A}$ Ratio	Sex
3	2	9	1.50	Metafemale
4	3	13	1.33	Female
4	4	16	1.00	Female
3	3	12	1.00	Female
2	2	8	1.00	Female
1	1	4	1.00	Female
2	3	11	0.67	Intersex
1	2	7	0.50	Male
1	3	10	0.33	Metamale

theory of sex determination was essentially correct. When the X:A ratio is 1.00, as in a normal female, or greater than 1.00, the organism is a female. When this ratio is 0.50, as in a normal male, or less than 0.50, the organism is a male. At 0.67, the organism is an **intersex. Metamales** (X/A = 0.33) and **metafemales** (X/A = 1.50) are usually very weak and sterile. The metafemales usually do not even emerge from their pupal cases.

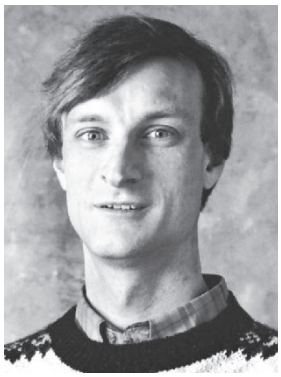
A **sex-switch** gene has been discovered that directs female development. This gene, **Sex-lethal** (*Sxl*), is located on the X chromosome. (It was originally called *female-lethal* because mutations of this gene killed female embryos.) Apparently, *Sxl* has two states of activity. When it is "on," it directs female development; when it is "off," maleness ensues. Other genes located on the X chromosome and the autosomes regulate this sex-switch gene. Genes on the X chromosome that act to regulate *Sxl* into the on state (female development) are called **numerator elements** because they act on the numerator of the X/A genic balance equation. Genes on the autosomes that act to regulate *Sxl* into the off state (male development) are called **denominator elements.** Geneticists have discovered four numerator elements—genes named *sisterless-a, sisterless-b, sisterless-c,* and *runt*. *Sxl* "counts" the number of X chromosomes; it turns on when two are present. It counts by measuring the level of the numerator genes' protein product. If the level is high, *Sxl* turns on, and the organism develops as a female. If the level is relatively low, *Sxl* does not turn on, and development proceeds as a male.

Sex Determination in Human Beings

Since the X0 genotype in human beings is a female (having Turner syndrome), it seems reasonable to conclude that the Y chromosome is male determining in human beings. The fact that persons with Klinefelter syndrome (XXY, XXXY, XXXXY) are all male, and XXX, XXXX, and other multiple-X karyotypes are all female, verifies this idea. (More details on these anomalies are presented in chapter 8.) For a long time, researchers have sought a single gene, a **testis-determining factor (TDF),** located on the Y chromosome that acts as a sex switch to initiate male development. Human embryologists had discovered that during the first month of embryonic development, the gonads that develop are neither testes nor ovaries, but instead are indeterminate. At about six or seven weeks of development, the indeterminate gonads become either ovaries or testes.

In the 1950s, Ernst Eichwald found that males had a protein on their cell surfaces not found in females; he discovered that female mice rejected skin grafts from genetically identical brothers, whereas the brothers accepted grafts from sisters. This implies that an antigen exists on the surface of male cells that is not found on female cells. This protein was called the *histocompatibility Y antigen* **(H-Y antigen).** The gene for this protein was found on the Y chromosome, near the centromere. At first, scientists believed it to be the sex switch: if the gene were present, the gonads would begin development as testes. Further male development, as in male secondary sexual characteristics, came about through the testosterone the functional testes produced. If the gene were absent, the gonads would develop into ovaries. Recently, however, by studying "sex-reversed" individuals, biologists refuted this theory.

Sex-reversed individuals are XX males or XY females. David Page, at the Whitehead Institute for Biomedical Research, found twenty XX males who had a small piece of the short arm of the Y chromosome attached to one of their X chromosomes. He found six XY females in whom the Y chromosome was missing the same small piece at the end of its short arm. This region, which did not contain the *HYA* gene, must carry the testis-determining factor. The first candidate gene from this region believed to code for the testis-determining factor was named the



David Page (1956–).
(Courtesy of Dr. David Page.)

***ZFY* gene,** for zinc finger on the Y chromosome. Zinc fingers are protein configurations known to interact with DNA (discussed in detail in chapter 16). Thus, researchers believed that the *ZFY* gene, coding for the testis-determining factor, worked by directly interacting with DNA. (Later in the book we look at the way regulatory genes, whose proteins interact with DNA, work.) However, men who lack the *ZFY* gene have been found, suggesting that the testis-determining factor is very close to, but not, the *ZFY* gene. From work in mice, it has been suggested that the *ZFY* gene controls the initiation of sperm cell development, but not maleness.

In 1991, Robin Lovell-Badge and Peter Goodfellow and their colleagues in England isolated a gene called **Sex-determining region Y (*SRY*)**—*Sry* in mice—adjacent to the *ZFY* gene. *Sry* has been positively identified as the testis-determining factor because, when injected into normal (XX) female mice, it caused them to develop as males (fig. 5.4). Although these XX males are sterile, they appear as normal males in every other way. (We discuss in chapter 13 how scientists introduce new genes into an organism.) Note also that the mouse and human systems are very similar genetically, and the homologous genes have been isolated from both. However, at present, the human *SRY* gene does not convert XX female mice into males. Like the *ZFY* gene product, Sry protein (the protein the *SRY* gene produces) also binds to DNA.

Robin Lovell-Badge (1953–).
(Courtesy of Robin Lovell-Badge.)

Peter Goodfellow (1951–).
(Courtesy of Peter Goodfellow.)

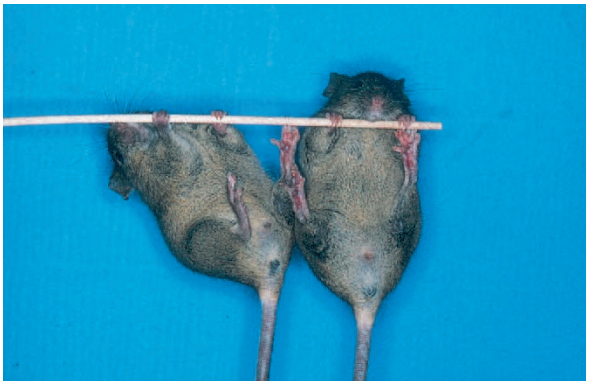


Figure 5.4 Normal male mouse (*left*) and female littermate given the *Sry* gene (*right*). Both mice are indistinguishably male.
(Courtesy of Robin Lovell-Badge.)

The Sry protein appears to bind to at least two genes. One, the p450 aromatase gene, has a protein product that converts the male hormone testosterone to the female hormone estradiol; the Sry protein inhibits production of p450 aromatase. The second gene the Sry protein affects is the gene for the Müllerian-inhibiting substance, which induces testicular development and the digression of female reproductive ducts; the Sry protein enhances this gene's activity. Thus, the Sry protein points an indifferent embryo toward maleness and the maintenance of testosterone production. The sex switch initiates a developmental sequence involving numerous genes. Eva Eicher and Linda Washburn have developed a model in which two pathways of coordinated gene action help determine sex, one pathway for each sex. The first gene in the ovary-determining pathway is termed *ovary determining* (*Od*). The first gene in the testis-determining pathway must function before the *Od* gene begins, in order to allow XY individuals to develop as males. Once the steps of a pathway are initiated, the other pathway is inhibited (fig. 5.5).

Other Chromosomal Systems

The X0 system, sometimes referred to as an X0-XX system, occurs in many species of insects. It functions just as the XY chromosomal mechanism does, except that instead of a Y chromosome, the heterogametic sex (male) has only one X chromosome. Males produce gametes that contain either an X chromosome or no sex chromosome, whereas all the gametes from a female contain the X chromosome. The result of this arrangement is that females have an even number of chromosomes (all in homomorphic pairs) and males have an odd number of chromosomes.

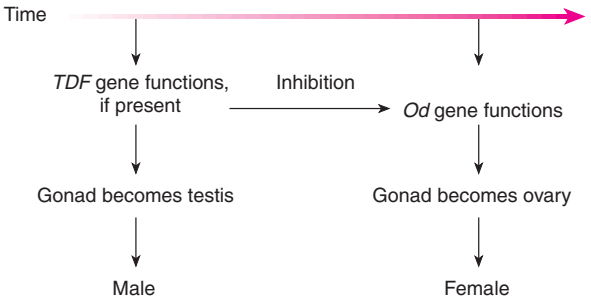


Figure 5.5 A model for the initiation of gonad determination in mammals.

The ZW system is identical to the XY system except that males are homogametic and females are heterogametic. This situation occurs in birds, some fishes, and moths.

Compound chromosomal systems tend to be complex. For example, *Ascaris incurva,* a nematode, has eight X chromosomes and one Y. The species has twenty-six autosomes. Males have thirty-five chromosomes (26A + 8X + Y), and females have forty-two chromosomes (26A + 16X). During meiosis, the X chromosomes unite end to end and so behave as one unit.

The Y Chromosome

In both human beings and fruit flies, the Y chromosome has very few functioning genes. In human beings, two homologous regions exist, one at either end of the X and Y chromosomes, allowing the chromosomes to pair during meiosis. These regions are termed **pseudoautosomal.** Mapping the Y chromosome (see chapters 6 and 13) has shown us the existence of about thirty-five genes (fig. 5.6). Other, nonfunctioning genes are present, too, remnants of a time in the evolutionary past when those genes were probably active (box 5.1). The *Drosophila* Y chromosome is known to carry genes for at least six fertility factors, two on the short arm (*ks-1* and *ks-2*) and four on the long arm (*kl-1, kl-2, kl-3,* and *kl-5*). The Y chromosome carries two other known genes: *bobbed,* which is a locus of ribosomal RNA genes (the nucleolar organizer), and *Suppressor of Stellate* or *Su(Ste),* a gene required for RNA splicing (see chapter 10). The fertility factors code for proteins needed during spermatogenesis. For example, *kl-5* codes for part of the dynein motor needed for sperm flagellar movement.

Sex Determination in Flowering Plants

Flowering plant species (angiosperms) generally have three kinds of flowers: hermaphroditic, male, and female. Hermaphroditic flowers have both male and female parts. The male parts are the anthers and filaments, making up the *stamen,* and the female parts are the stigma, style, and ovary, making up the *pistil* (see fig. 2.2). Ninety percent of angiosperms have hermaphroditic flowers. Of the 10% of the species that have unisexual flowers, some are *monoecious* (Greek, one house), bearing both male and female flowers on the same plant (e.g., walnut); and some are *dioecious* (Greek, two houses), having plants with just male or just female flowers (e.g., date palm).

Within the group of plant species with unisexual flowers, sex-determining mechanisms vary. Some species have a single locus determining sex, some have two or more loci involved in sex determination, and some have X and Y chromosomes. In most of the species with X and Y chromosomes, the sex chromosomes are indistinguishable. Among these species, most have heterogametic males, although in some species, such as the strawberry, females are heterogametic. In the very few species that have distinguishable X and Y chromosomes—only thirteen are known—two sex-determination mechanisms are found. One is similar to the system in mammals, in which the Y chromosome has a gene or genes present

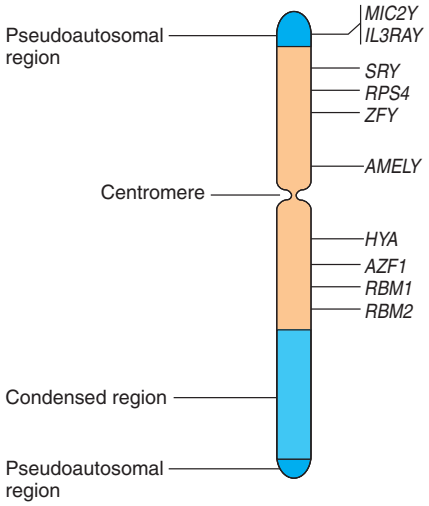


Figure 5.6 The human Y chromosome. In addition to the genes shown, the Y chromosome carries other genes, homologous to X chromosome genes, that do not function because of accumulated mutations. Some of these are in multiple copies. Note the two pseudoautosomal regions that allow synapsis between the Y and X chromosomes. The gene symbols shown include *MIC2Y,* T cell adhesion antigen; *IL3RAY,* interleukin-3 receptor; *RPS4,* a ribosomal protein; *AMELY,* amelogenin; *HYA,* histocompatibility Y antigen; *AZF1,* azoospermia factor 1 (mutants result in tailless sperm); and *RBM1, RBM2,* RNA binding proteins 1 and 2. (Adapted from Online Mendelian Inheritance in Man website. http://www3.ncbi.nlm.nih.gov/omim/. Reprinted with permission.)

BOX 5.1

Experimental Methods

Why Sex and Why Y?

Evolutionary biologists have asked, Why does sex exist? A haploid, asexual way of life seems like a very efficient form of existence. Haploid fungi can produce thousands of haploid spores, each of which can grow into a new colony. What evolutionary benefit do organisms gain by developing diploidy and sexual processes? Although this may not seem like a serious question, evolutionary biologists look for compelling answers.

In chapter 21, we discuss evolutionary thinking in some detail. For the moment, accept that evolutionary biologists look for an adaptive advantage in most evolutionary outcomes. Thus they ask, What is better about the combining of gametes to produce a new generation of offspring? Why would a diploid organism take a random sample of its genome and combine it with a random sample of someone else's genome to produce offspring? Why not simply produce offspring by mitosis? If offspring are produced by mitosis, all of an individual's genes pass into the next generation with every offspring. Not only does just half the genome of an individual pass into the next generation with every offspring produced sexually, but that half is a random jumble of what might be a very highly adapted genome. In addition, males are doubly expensive to produce because males themselves do not produce offspring: males fertilize females who produce offspring. Thus, on the surface, evolutionary biologists need to find very strong reasons for an organism to turn to sexual reproduction when an individual might be at an advantage evolutionarily to reproduce asexually.

There have been numerous suggestions as to the advantage of sex, nicely summarized in a 1994 article by James Crow, of the University of Wisconsin, in *Developmental Genetics,* and more recently in a special section of the 25 September 1998 issue of *Science* magazine. We aren't really sure what the true evolutionary reasons for sex are, but at least three explanations seem reasonable to evolutionary biologists:

- *Adjusting to a changing environment.* Sexual reproduction allows for much more variation in organisms. A haploid, asexual organism collects variation over time by mutation. A sexual organism, on the other hand, can achieve a tremendous amount of variation by recombination and fertilization. Remember that a human being can produce potentially $2^{100,000}$ different gametes. In a changing environment, a sexually reproduced organism is much more likely than an asexual organism to produce offspring that will be adapted to the changes.
- *Combining beneficial mutations.* As mentioned, a haploid, asexual organism accrues mutations as they happen over time in a given individual. A sexual organism can combine beneficial mutations each generation by recombination and fertilization. Thus, sexually reproducing organisms can adapt at a much more rapid rate than asexual organisms.
- *Removing deleterious mutations.* Mutation is more likely to produce deleterious changes than beneficial ones. An asexual organism gathers more and more deleterious mutations as time goes by (a process referred to as *Muller's ratchet,* in honor of Nobel Prize-winning geneticist H. J. Muller and referring to a ratchet wheel that can only go forward). Sexually reproducing organisms can eliminate deleterious mutations each generation by forming recombined offspring that are relatively free of mutation.

Hence, this list provides three of the generally assumed advantages of sexual reproduction that offset its disadvantages.

Another subtle question about sexual reproduction that evolutionary biologists ask is, Why is there a Y chromosome? In other words, why do we have, in some species (e.g., people), a heteromorphic pair of chromosomes involved in sex determination, with one of the chromosomes having the gene for that sex and very few other loci? In people, the Y chromosome is basically a degenerate chromosome with very few loci. This morphological difference between the members of the sex chromosome pair is puzzling. After all, chromosome pairs that do not carry sex-determining loci do not tend to be morphologically heterogeneous. Consider the following possible scenario that Virginia Morell presented in the 14 January 1994 issue of *Science.*

In a particular species in the past—evolutionarily speaking—a sex-determining gene arises on a particular chromosome. One allele at this locus confers maleness on its bearer. The absence of this allele causes the carrier to be female. At this point, millions of years ago, the sex chromosomes are not morphologically heterogeneous: the X and Y chromosomes are identical. In time,

however, the Y chromosome comes to carry a gene that is beneficial to the male but not the female. For example, there might be a gene with an allele for a colorful marking; this allele confers a reproductive advantage for the male but also confers a predatory risk on the bearer, whether male or female. Males have a reproductive advantage to outweigh the predation risk, whereas females have none. Thus, the allele is favored in males and selected against in females.

An evolutionary solution to this situation is to isolate the gene for this marking on the Y chromosome and keep it off the X chromosome so that males have it but females do not. This can take place if the two chromosomes do not recombine over most of their lengths. Assume then, that some mechanism evolves to prevent recombination of the X and Y chromosomes. Thereafter, the Y chromosome degenerates, losing most of its genes but retaining the sex-determining locus and the loci conferring an advantage on males but a disadvantage on females.

What evidence do we have that any of these links in this complex line of logic are true? To begin with, when we look at evolutionary lineages, we usually see a spectrum of species with sex chromosomes in all stages of differentiation. Evolutionary biologists generally accept the notion that the similar sex chromosomes are the original condition and the morphologically heterogeneous sex chromosomes are the more evolved condition. In addition, as reported in the same issue of *Science,* William Rice of the University of California at Santa Cruz has shown experimentally with fruit flies that if recombination is prevented between sex chromosomes, the Y chromosome degenerates; it loses the function of many loci that are also found on the X chromosome. Rice showed this with an ingenious set of experiments that successfully prevented a nascent Y chromosome from recombining with the X. The results confirmed the prediction that the Y chromosome degenerates (fig. 1).

More recently, in an October 1999 article in *Science,* Bruce Lahn and David Page, at the Massachusetts Institute of Technology, reported research findings indicating that degeneration of the human Y chromosome has taken place in four stages, starting as long as 320 million years ago in our mammalian ancestors. Using DNA sequence data and methods discussed in chapter 21, they showed that the 19 genes known from both the X and Y chromosomes are arranged as if the Y chromosome has undergone four rearrangements, each preventing further recombination of the X and Y. According to their calculations, this process began shortly after the mammals split from the birds, which themselves went on to evolve a ZW pair of sex chromosomes.

Clearly, much more work is needed to validate all the steps in this logical, evolutionary argument. However, at this point, enough empirical support exists to make the idea attractive to evolutionary biologists.

Although we have gotten a bit ahead of ourselves by talking about subtle evolutionary arguments before reaching that material in the book, it is a good idea to keep an evolutionary perspective on processes and structures. Presumably, evolution has shaped us and the biological world in which we live. If that is so, we should be able to figure out how evolution was working. That thinking should hold from the level of the molecule (e.g., enzymes and DNA) to that of the whole organism. Behind every process and structure should be a hint of the evolutionary pressures that caused that structure or process to evolve.

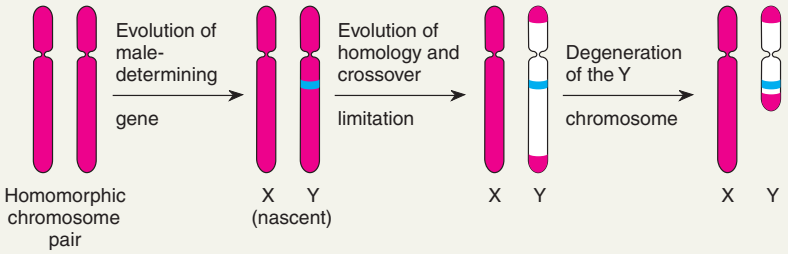


Figure 1 Evolution of a hypothetical Y chromosome. *Red* represents homologous regions, *blue* shows the male-determining gene, and *white* marks evolved areas of the Y chromosome that no longer recombine with the X chromosome.

that actively determine male-flowering plants. The other system is similar to that found in fruit flies, in which the X:A ratio determines sex.

In the mammalian-type system, the Y chromosome carries genes needed for the development of male flower parts while suppressing the development of female parts. An example of this is in the white campion *(Silene latifolia).* In the *Drosophila*-type system, found in the sorrel *(Rumex acetosa),* the ratios determine sex exactly as in the flies. That is, an X:A ratio of 0.5 or lower results in a male; a ratio of 1.0 or higher results in a female; and an intermediate ratio results in a plant with hermaphroditic flowers. It seems that all flowers have the potential to be hermaphroditic. That is, flower primordia for hermaphroditic, male, and female flowers look identical during early development. The simplest mechanism of sex determination would involve repressing the development of the female flower parts in male flowers and repressing the male flower parts in female flowers. Current research indicates that this repression of one component or another is probably involved in most flower sex determination and is under genetic and hormonal control. (We discuss further the genetic control of flower development in chapter 16.)

DOSAGE COMPENSATION

In the XY chromosomal system of sex determination, males have only one X chromosome, whereas females have two. Thus, disregarding pseudoautosomal regions, males have half the number of X-linked alleles as females for genes that are not primarily related to gender. A question arises: How does the organism compensate for this dosage difference between the sexes, given the potential for serious abnormality? In general, an incorrect number of autosomes is usually highly deleterious to an organism (see chapter 8). In human beings and other mammals, the necessary **dosage compensation** is accomplished by the inactivation of one of the X chromosomes in females so that both males and females have only one functional X chromosome per cell.

In 1949, M. Barr and E. Bertram first observed a condensed body in the nucleus that was not the nucleolus. Noting that normal female cats show a single condensed body, while males show none, these researchers referred to the body as sex chromatin, since known as a **Barr body** (fig 5.7). Mary Lyon then suggested that this Barr body represented an inactive X chromosome, which in females becomes tightly coiled into heterochromatin, a condensed, and therefore visible, form of chromatin.

Various lines of evidence support the **Lyon hypothesis** that only one X chromosome is active in any cell. First, XXY males have a Barr body, whereas X0 females have none. Second, persons with abnormal numbers of X chromosomes have one fewer Barr body than they have X chromosomes per cell: XXX females have two Barr bodies and XXXX females have three.

Mary F. Lyon (1925–).
(Courtesy of Dr. Mary F. Lyon.)

Proof of the Lyon Hypothesis

Direct proof of the Lyon hypothesis came when cytologists identified the Barr body in normal females as an X chromosome. Genetic evidence also supports the Lyon hypothesis: Females heterozygous for a locus on the X chromosome show a unique pattern of phenotypic expression. We now know that in human females, an X chromosome is inactivated in each cell on about the twelfth day of embryonic life; we also know that the inactivated X is randomly determined in a given cell. From that point on, the same X remains a Barr body for future cell generations. Thus, heterozygous females show **mosaicism** at the cellular level for X-linked traits. Instead of being typically heterozygous, they express only one or the other of the X chromosomal alleles in each cell.

Glucose-6-phosphate dehydrogenase (G-6-PD) is an enzyme that a locus on the X chromosome controls. The

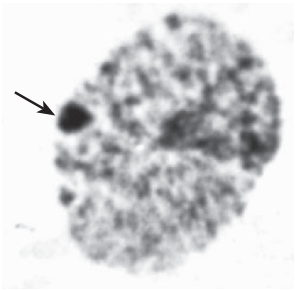


Figure 5.7 Barr body (*arrow*) in the nucleus of a cheek mucosal cell of a normal woman. This visible mass of heterochromatin is an inactivated X chromosome. (Thomas G. Brewster and Park S. Gerald, "Chromosome disorders associated with mental retardation," *Pediatric Annals,* 7, no. 2, 1978. Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

enzyme occurs in several different allelic forms that differ by single amino acids. Thus, both forms (A and B) will dehydrogenate glucose-6-phosphate—both are fully functional enzymes—but because they differ by an amino acid, they can be distinguished by their rate of migration in an electrical field (one form moves faster than another). This electrical separation, termed **electrophoresis,** is carried out by placing samples of the enzymes in a supporting gel, usually starch, polyacrylamide, agarose, or cellulose acetate (fig. 5.8 and box 5.2). After the electric current is applied for several hours, the enzymes move in the gel as bands, revealing the distance each enzyme traveled. Since blood serum is a conglomerate of proteins from many cells, the serum of a female heterozygote (fig. 5.8, lane 3) has both A and B forms (bands), whereas any single cell (lanes 4–10) has only one or the other. Since the gene for glucose-6-phosphate dehydrogenase is carried on the X chromosome, this electrophoretic display indicates that only one X is active in any particular cell.

Another aspect of the glucose-6-phosphate dehydrogenase system provides further proof of the Lyon hypothesis. If a cell has both alleles functioning, both A and B proteins should be present. Since the functioning glucose-6-phosphate dehydrogenase enzyme is a dimer (made up of two protein subunits), 50% of the enzymes should be heterodimers (AB). These would form a third, intermediate band between the A form (AA dimer) and the B form (BB dimer; fig. 5.9). The lack of heterodimers in the blood of heterozygotes is further proof that both G-6-PD alleles are not active within the same cells. That is, in any one cell, only AA or BB dimers can form, because no single cell has both the A and B forms.

The Lyon hypothesis has been demonstrated with many X-linked loci, but the most striking examples are those for color phenotypes in some mammals. For example, the tortoiseshell pattern of cats is due to the inactivation of X chromosomes (fig. 5.10). Tortoiseshell cats are normally females heterozygous for the *yellow* and *black* alleles of the X-linked color locus. They exhibit patches of these two colors, indicating that at a certain stage in development, one or the other of the X chromosomes was inactivated and all of the ensuing daughter cells in that line kept the same X chromosome inactive. The result is patches of coat color.

The X chromosome is inactivated starting at a point called the **X inactivation center (XIC).** That region contains a gene called *XIST* (for *X inactive-specific transcripts*, referring to the transcriptional activity of this gene in the inactivated X chromosome). The *XIST* gene has been putatively identified as the gene that initiates the inactivation of the X chromosome. This gene is known to be active only in the inactive X chromosome in a normal XX female. Another aspect of "Lyonization" is that several other loci are known to be active on the inactivated X chromosome; they are active in both X chromosomes, even though one is heterochromatic (inactivated). Although several of these loci are in the pseudoautosomal region of the short arm of the X chromosome, several other of the thirty or more genes known to be active are on other places on the mammalian X chromosome. Active genes on the inactive X include the gene for the enzyme steroid sulphatase; the red-cell antigen Xg^a; *MIC2;* a *ZFY*-like gene termed *ZFX;* the gene for Kallmann syndrome; and several others.

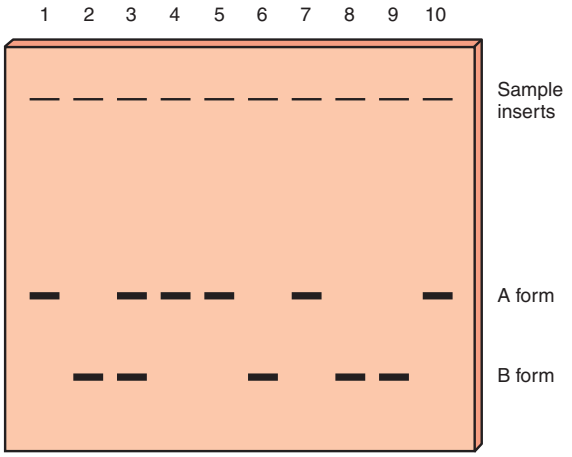


Figure 5.8 Electrophoretic gel stained for glucose-6-phosphate dehydrogenase. Lanes *1–3* contain blood from an *AA* homozygote, a *BB* homozygote, and an *AB* heterozygote, respectively. Lanes *4–10* contain homogenates of individual cells of an *AB* heterozygote.

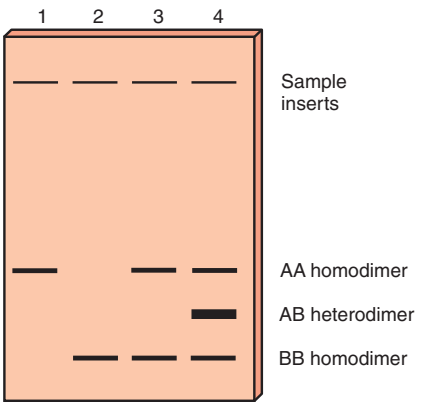


Figure 5.9 Electrophoretic gel stained for glucose-6-phosphate dehydrogenase. Lanes *1* and *2* contain blood serum from *AA* and *BB* women, respectively, and lane *3* contains serum from an *AB* heterozygote. Lane *4* shows the pattern expected if both the *A* and *B* alleles were active within the same cell.

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BOX 5.2

Experimental Methods

Electrophoresis

Electrophoresis, a technique for separating relatively similar types of molecules (for example, proteins and nucleic acids), has opened up new and exciting areas of research in population, biochemical, and molecular genetics. It has allowed us to see variations in large numbers of loci, previously difficult or impossible to sample. In biochemical genetics, electrophoretic techniques can be used to study enzyme pathways. In molecular genetics, electrophoresis is used to sequence nucleotides (see chapter 13) and to assign various loci to particular chromosomes. In population genetics (see chapter 21), electrophoresis has made it possible to estimate the amount of variability that occurs in natural populations.

Here we discuss protein electrophoresis, a process that entails placing a sample—often blood serum or a cell homogenate—at the top of a gel prepared from a suitable substrate (e.g., hydrolyzed starch, polyacrylamide, or cellulose acetate) and a buffer. An electrical current is

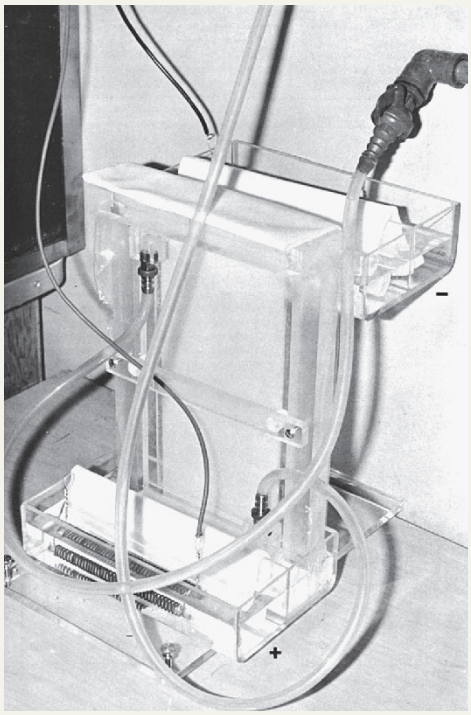


Figure 1 Vertical starch gel apparatus. Current flows from the upper buffer chamber to the lower one by way of the paper wicks and the starch gel. Cooling water flows around the system. (R. P. Canham, "Serum protein variations and selection in fluctuating populations of cricetid rodents," Ph.D. thesis, University of Alberta, 1969. Reproduced by permission.)

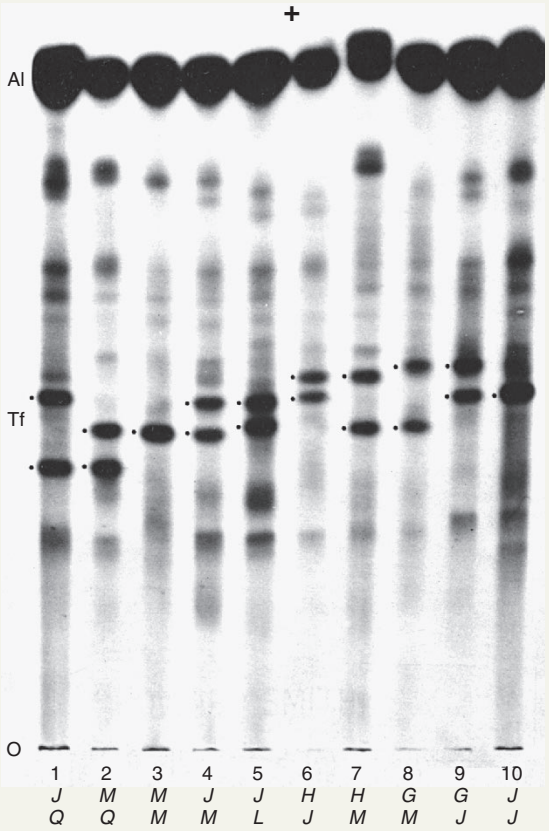


Figure 2 Ten samples of deer mouse *(Peromyscus maniculatus)* blood studied for general protein. *Al* is albumin and *Tf* is transferrin, the two most abundant proteins in mammalian blood. The six *Tf* allozymes are labeled G, H, J, L, M, and Q. (R. P. Canham, "Serum protein variations and selection in fluctuating populations of cricetid rodents," Ph.D. thesis, University of Alberta, 1969. Reproduced by permission.)

passed through the gel to cause charged molecules to move (fig. 1), and the gel is then treated with a dye that stains the protein. In the simplest case, if a protein is homogeneous (usually the product of a homozygote), it forms a single band on the gel. If it is heterogeneous (usually the product of a heterozygote), it forms two bands. This is because the two allelic protein products differ by an amino acid; they have different electrical charges and therefore travel through the gel at different rates (see fig. 5.8). The term **allozyme** refers to different electrophoretic forms of an enzyme controlled by alleles at the same locus.

Figure 2 shows samples of mouse blood serum that have been stained for protein. Most of the staining reveals albumins and β-globulins (transferrin). Because they are present in very small concentrations, many enzymes present in the serum are not visible, but a stain that is specific for a particular enzyme can make that enzyme visible on the gel. For example, lactate dehydrogenase (LDH) can be located because it catalyzes this reaction:

$$\text{lactic acid} + \text{NAD}^+ \xleftrightarrow{\text{LDH}} \text{pyruvic acid} + \text{NADH}$$

Thus, we can stain specifically for the lactate dehydrogenase enzyme by adding the substrates of the enzyme (lactic acid and nicotinamide adenine dinucleotide, NAD^+) and a suitable stain specific for a product of the enzyme reaction (pyruvic acid or nicotinamide adenine dinucleotide, reduced form, NADH). That is, if lactic acid and NAD^+ are poured on the gel, only lactate dehydrogenase converts them to pyruvic acid and NADH. We can then test for the presence of NADH by having it reduce the dye, nitro blue tetrazolium, to the blue precipitate, formazan, an electron carrier. We then add all the preceding reagents and look for blue bands on the gel (fig. 3).

In addition to its uses in population genetics and chromosome mapping, electrophoresis has been extremely useful in determining the structure of many proteins and for studying developmental pathways. As we can see from the lactate dehydrogenase gel in figure 3, five bands can occur. In some tissues of a homozygote, these bands occur roughly in a ratio of 1:4:6:4:1. This pattern can come about if the enzyme is a tetramer whose four subunits are random mixtures of two gene products (from the *A* and *B* loci). Thus we would get

AAAA (1/16)
AAAB (4/16)
AABB (6/16)
ABBB (4/16)
BBBB (1/16)

(Note that the ratio 1:4:6:4:1 is the expansion of $[A + B]^4$, and the relative "intensity" of each band—the number of protein doses—is calculated from the rule of unordered events described in chapter 4.)

continued

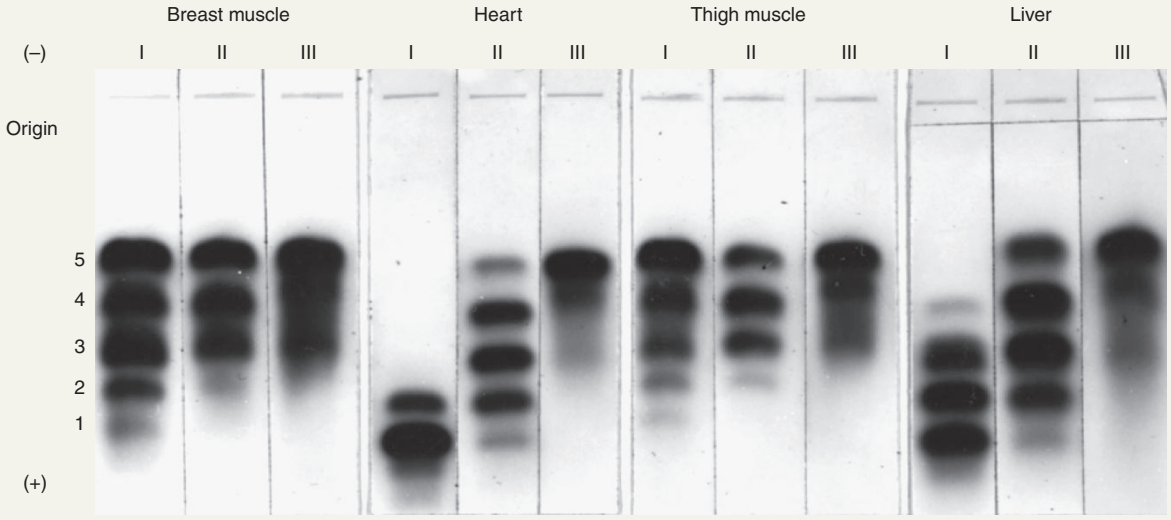


Figure 3 Lactate dehydrogenase isozyme patterns in pigeons. Note the five bands for some individual samples. Lanes *I, II,* and *III* under each tissue type indicate the range of individual variation. (W. H. Zinkham, et al., "A Variant of Lactate Dehydrogenase in Somatic Tissues of Pigeons" in *Journal of Experimental Zoology* 162, no. 1 (June):45–46, 1966. Reproduced by permission of the Wistar Institute.)

BOX 5.2 (CONTINUED)

Protein chemists have verified this tetramer model. In this way, electrophoresis has helped us determine the structure of several enzymes. (The term **isozymes** refers to multiple electrophoretic forms of an enzyme due to subunit interaction rather than allelic differences.) Chemists have also discovered that the five forms differ in concentration in different tissues of the body (fig. 4). This has led to various hypotheses as to how the production of enzymes is controlled developmentally.

Electrophoresis is also valuable in clinical diagnosis. In various diseases, cell destruction causes the release of proteins into the bloodstream. Thus, the lactate dehydrogenase pattern is found in the blood in certain disease states (fig. 5). This is why examination of the blood LDH is often a diagnostic test used to pick up early signs of heart and liver disease (among others).

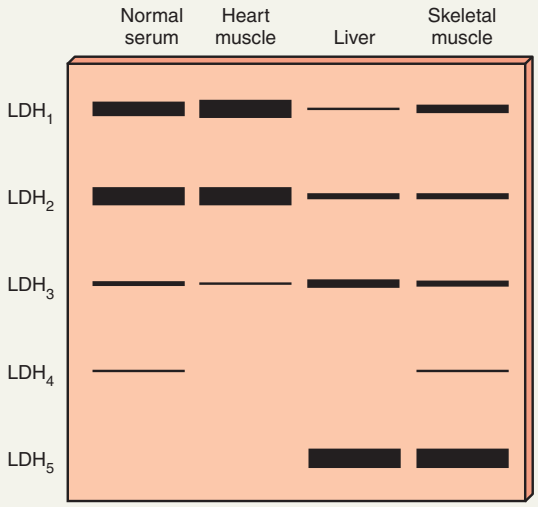


Figure 4 LDH patterns found in different tissues in human beings.

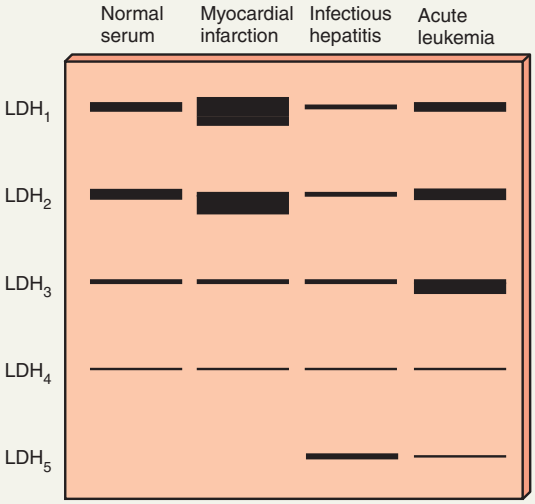


Figure 5 LDH patterns from normal human serum and from serum affected by various disease states.

The gene product of *XIST* is an RNA that does not seem to be translated into a protein. Rather, using localization techniques, geneticists have found this RNA is associated with Barr bodies, coating the inactive chromosome. Current research is aimed at determining the details of this interaction.

Dosage Compensation for Drosophila

Dosage compensation also occurs in fruit flies, and it appears that the gene activity of X chromosome loci is also about equal in males and females. The mechanism is different from that in mammals since no Barr bodies are found in fruit flies. Instead, the male's single X chromosome is hyperactive, approaching the level of transcriptional activity of both of the female's X chromosomes combined. Researchers have discovered a multisubunit protein complex called MSL (for male-specific lethal) that binds to hundreds of sites on the single X chromosome in males. Presumably, the binding mediates the hyperactivity of the genes on the X chromosome. (We discuss control of transcription later in the book.) At least five genes contribute products to this protein complex: *msl1, msl2, msl3, mle,* and *mof.* (*Mle* comes from *m*ale*le*ss, and *mof* comes from *m*ales absent *o*n the *f*irst.) Along with this protein complex are RNAs that also bind to the male X chromosome. These RNAs, also implicated in dosage compensation, are the products of the *rox1* and *rox2* genes (for *R*NA *o*n the *X*). Together, the MSL protein complex and the RNAs comprise a **compensasome.**

Mutant alleles of the male-specific lethal (*msl*) genes disrupt dosage compensation in males and are, as their names imply, lethal. However, they appear to have no effect in females. Expression of at least one of these genes, *msl2,* is repressed by the protein product of the *Sxl* gene. Thus, sex determination and dosage compensation are

Figure 5.10 Tortoiseshell cat. A female heterozygous for the X-linked *yellow* and *black* alleles. (Courtesy of Donna Bass.)

ultimately under the control of the same master switch gene, *Sxl*. This should not be surprising since the ability of *Sxl* to count the number of X chromosomes in a cell makes it the most efficient initiator of both sexual development and dosage compensation.

SEX LINKAGE

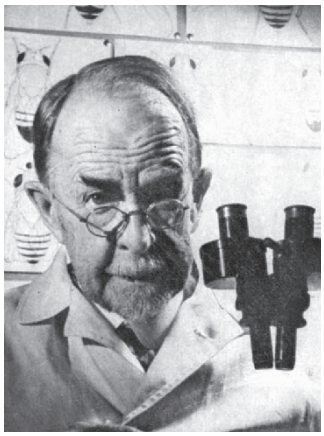
In an XY chromosomal system of sex determination, the pattern of inheritance for loci on the heteromorphic sex chromosomes differs from the pattern for loci on the homomorphic autosomal chromosomes because alleles of the sex chromosome are inherited in association with the sex of the offspring. Alleles on a male's X chromosome go to his daughters but not to his sons, because the presence of his X chromosome normally determines that his offspring is a daughter. For example, the inheritance pattern of hemophilia (failure of blood to clot), the common form of which is caused by an allele located on the X chromosome, has been known since the end of the eighteenth century. It was known that mostly men had the disease, whereas women could pass on the disease without actually having it. (In fact, the general nature of the inheritance of this trait was known in biblical times. The Talmud—the Jewish book of laws and traditions—specified exemptions to circumcision on the basis of hemophilia among relatives consistent with an understanding of who was at risk.)

Before we continue, we need to make a small distinction. Since both X and Y are sex chromosomes, three different patterns of inheritance are possible, all sex linked (for loci found only on the X chromosome, only on the Y chromosome, or on both). However, the term **sex-linked** usually refers to loci found only on the X chromosome; the term **Y-linked** is used to refer to loci found only on the Y chromosome, which control **holandric traits** (traits found only in males). Loci found on both the X and Y chromosomes are called *pseudoautosomal.* In human beings, at least four hundred loci are known to be on the X chromosome; only a few are known to be on the Y chromosome.

X Linkage in Drosophila

T. H. Morgan demonstrated the **X-linked** pattern of inheritance in *Drosophila* in 1910, when a white-eyed male appeared in a culture of wild-type (red-eyed) flies (fig. 5.11). This male was crossed with a wild-type female. All of the offspring were wild-type. However, when these F_1 individuals were crossed with each other, their offspring fell into two categories (fig. 5.12). All the females and half the males were wild-type, whereas the remaining half of the males were white-eyed. Ultimately, Morgan and others interpreted this to mean that the white-eye locus was on the X chromosome. We can redraw figure 5.12 to include the sex chromosomes of Morgan's flies (fig. 5.13). We denote the X chromosome with the white-eye allele as X^w. Similarly X^+ is the X chromosome with the wild-type allele, and Y is the Y chromosome, which does not have this locus.

Another property of sex linkage appears in figure 5.13. Since females have two X chromosomes, they can have normal homozygous and heterozygous allelic combinations. But males, with only one copy of the X chromosome, can be neither homozygous nor heterozygous.



Thomas Hunt Morgan (1866–1945). (From *Genetics* 32 (1947): frontispiece. Courtesy of the Genetics Society of America.)

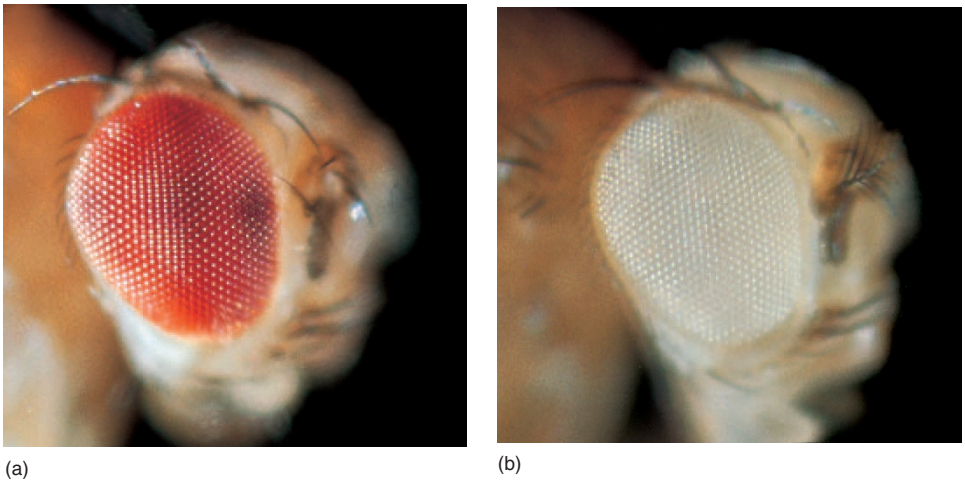


Figure 5.11 (*a*) Wild-type (red-eyed) and (*b*) white-eyed fruit flies. (Carolina Biological Supply Company.)

Instead, the term **hemizygous** describes the presence of X-linked genes (and other genes present in only one copy) in males. Since only one allele is present, a single copy of a recessive allele determines the phenotype in a phenomenon called **pseudodominance.** Thus, a male with one *w* allele is white-eyed, the allele acting in a dominant fashion. This is the same way one copy of a dominant autosomal allele would determine the phenotype of a normal diploid organism. Hence the term *pseudodominance.*

Nonreciprocity

The X-linked pattern has long been known as the **crisscross pattern of inheritance** because the father passes a trait to his daughters, who pass it to their sons. Figure 5.14 shows why this analysis is correct and the inheritance pattern is not reciprocal through a cross between a white-eyed female and a wild-type male. Here the F_1 males are white-eyed, the F_1 females are wild-type, and 50% of each sex in the F_2 generation are white-eyed. Such nonreciprocity and different ratios in the two sexes suggest sex linkage, which the crisscross pattern confirms.

Figure 5.15 shows the inheritance pattern of a sex-linked trait in chickens, in which the male is the homogametic sex (ZZ). The gene for barred plumage is Z linked, and barred plumage is dominant to nonbarred plumage. If we substitute white-eyed for nonbarred and male for female, we get the same pattern as in fruit flies (fig. 5.13)—in which, of course, females are homogametic.

The Y chromosome in fruit flies carries the pseudoautosomal *bobbed* locus (*bb*), the nucleolar organizer. In the homozygous recessive state, it causes bristles to shorten. Figures 5.16 and 5.17 show the results of reciprocal crosses involving *bobbed.* In both cases, one quarter of the F_2 individuals are *bobbed.* In one cross it is males, and in the other it is females.

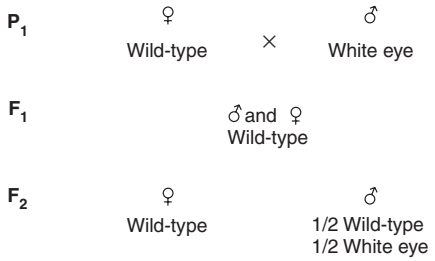


Figure 5.12 Pattern of inheritance of the white-eye trait in *Drosophila.*

Sex-Limited and Sex-Influenced Traits

Aside from X-linked, holandric, and pseudoautosomal inheritance, two inheritance patterns show nonreciprocity without necessarily being under the control of loci on the sex chromosomes. **Sex-limited traits** are traits expressed in only one sex, although the genes are present in both. In women, breast and ovary formation are sex-limited traits, as are facial hair distribution and sperm production in men. Nonhuman examples are plumage patterns in birds—in many species, the male is brightly colored—and horns found only in males of certain sheep species. Milk yield in mammals is expressed phenotypically only in females. **Sex-influenced,** or **sex-conditioned, traits** appear in both sexes but occur in one sex more than the other. Pattern, or premature, baldness in human beings is an example of a sex-influenced trait. In women, it is usually expressed as a thinning of hair rather than as balding. Apparently testosterone, the male hormone, is required for the full expression of the allele.

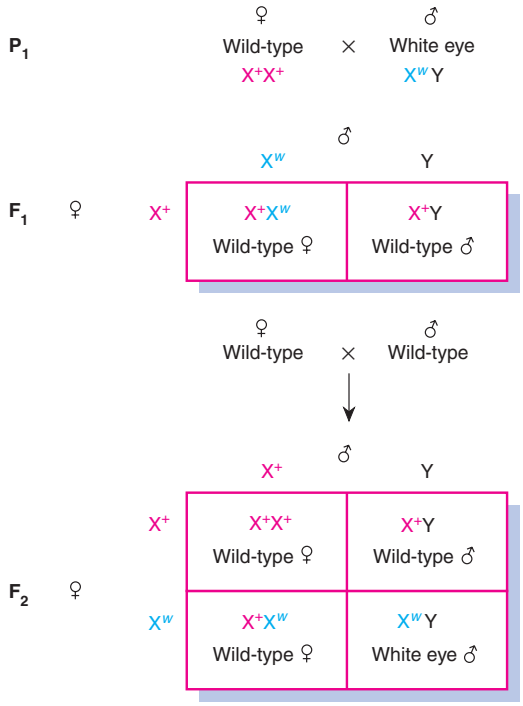


Figure 5.13 Crosses of figure 5.12 redrawn to include the sex chromosomes.

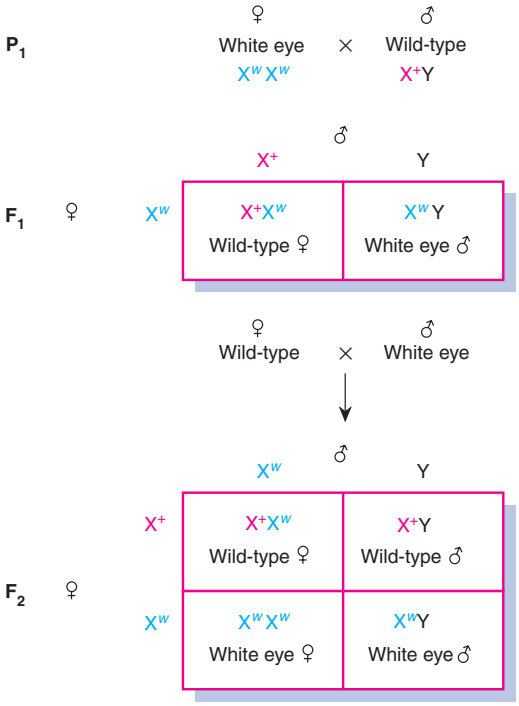


Figure 5.14 Reciprocal cross to that in figure 5.13.

PEDIGREE ANALYSIS

Inheritance patterns in many organisms are relatively easy to determine, because crucial crosses can test hypotheses about the genetic control of a particular trait. Many of these same organisms produce an abundance of offspring so that investigators can gather numbers large enough to compute ratios. Recall Mendel's work with garden peas; his 3:1 ratio in the F_2 generation led him to suggest the rule of segregation. If Mendel's sample sizes had been smaller, he might not have seen the ratio. Think of the difficulties Mendel would have faced had he decided to work with human beings instead of pea plants. Human geneticists face the same problems today. The occurrence of a trait in one of four children does not necessarily indicate a true 3:1 ratio.

To determine the inheritance pattern of many human traits, human geneticists often have little more to go on than a **pedigree** that many times does not include critical mating combinations. Frequently uncertainties and ambiguities plague pedigree analysis, a procedure whereby conclusions are often a product of the process of elimination. Other difficulties human geneticists encounter are the lack of **penetrance** and different degrees of **expressivity** in many traits. Both are aspects of the expression of a phenotype.

Penetrance and Expressivity

Penetrance refers to the appearance in the phenotype of genotypically determined traits. Unfortunately for geneticists, not all genotypes "penetrate" the phenotype. For example, a person could have the genotype that specifies vitamin-D-resistant rickets and yet not have rickets (a bone disease). This disease is caused by a sex-linked dominant allele and is distinguished from normal vitamin D deficiency by its failure to respond to low levels of vitamin D. It does, however, respond to very high levels of vitamin D and is thus treatable. In any case, in some family trees, affected children are born to unaffected parents. This would violate the rules of dominant inheritance because one of the parents must have had the allele yet did not express it. The fact that the parent actually had the allele is demonstrated by the occurrence of low levels of phosphorus in the blood, a pleiotropic effect of the same allele. The low-phosphorus aspect of the phenotype is always fully penetrant.

Thus, certain genotypes, often those for developmental traits, are not always fully penetrant. Most genotypes, however, are fully penetrant. For example, no known cases exist of individuals homozygous for albinism who do not actually lack pigment. Vitamin-D-resistant rickets illustrates another case in which a phenotype that is not genetically determined mimics a phenotype that is. This

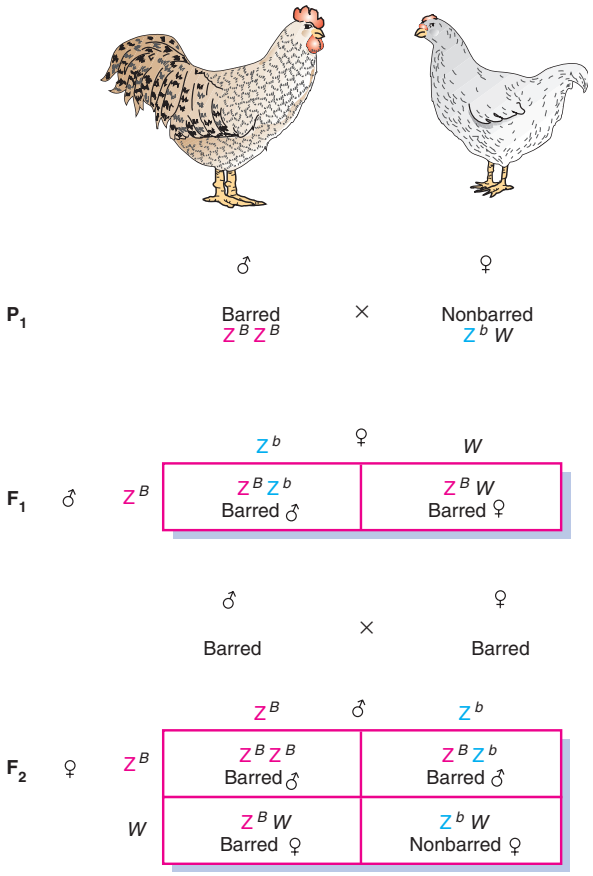


Figure 5.15 Inheritance pattern of barred plumage in chickens in which males are homogametic (ZZ) and females are heterogametic (ZW).

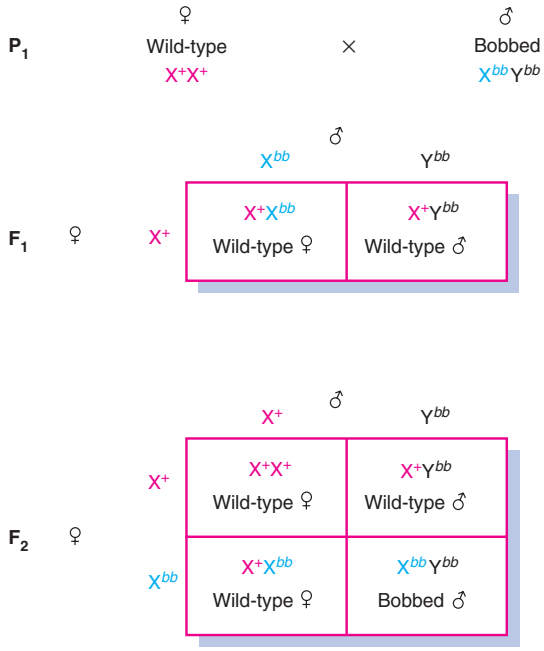


Figure 5.16 Inheritance pattern of the *bobbed* locus in *Drosophila*.

phenocopy is the result of dietary deficiency or environmental trauma. A dietary deficiency of vitamin D, for example, produces rickets that is virtually indistinguishable from genetically caused rickets.

Many developmental traits not only sometimes fail to penetrate, but also show a variable pattern of expression, from very mild to very extreme, when they do. For example, cleft palate is a trait that shows both variable penetrance and variable expressivity. Once the genotype penetrates, the severity of the impairment varies considerably, from a very mild external cleft to a very severe clefting of the hard and soft palates. Failure to penetrate and variable expressivity are not unique to human traits but are characteristic of developmental traits in many organisms.

Family Tree

One way to examine a pattern of inheritance is to draw a family tree. Figure 5.18 defines the symbols used in constructing a family tree, or pedigree. The circles represent females, and the squares represent males. Symbols that are filled in represent individuals who have the trait under study; these individuals are said to be **affected.** The open symbols represent those who do not have the trait. Direct horizontal lines between two individuals (one male, one female) are called marriage lines. Children are attached to a marriage line by a vertical line. All the brothers and sisters **(siblings** or **sibs)** from the same parents are connected by a horizontal line above their symbols. Siblings are numbered below their symbols according to birth order (fig. 5.19), and generations are numbered on the right in Roman numerals. When the sex of a child is unknown, the symbol is diamond-shaped (e.g., the children of III-1 and III-2 in fig. 5.19). A number within a symbol represents the number of siblings not separately listed. Individuals IV-7 and IV-8 in figure 5.19 are fraternal (dizygotic or nonidentical) twins: they originate from the same point. Individuals III-3 and III-4 are identical (monozygotic) twins: they originate from the same short vertical line.

When other symbols occur in a pedigree, they are usually defined in the legend. Individual V-5 in figure 5.19 is called a **proband** or **propositus** (female, **proposita**). The arrow pointing to individual V-5 indicates that the pedigree was ascertained through this individual, usually by a physician or clinical investigator.

On the basis of the information in a pedigree, geneticists attempt to determine the mode of inheritance

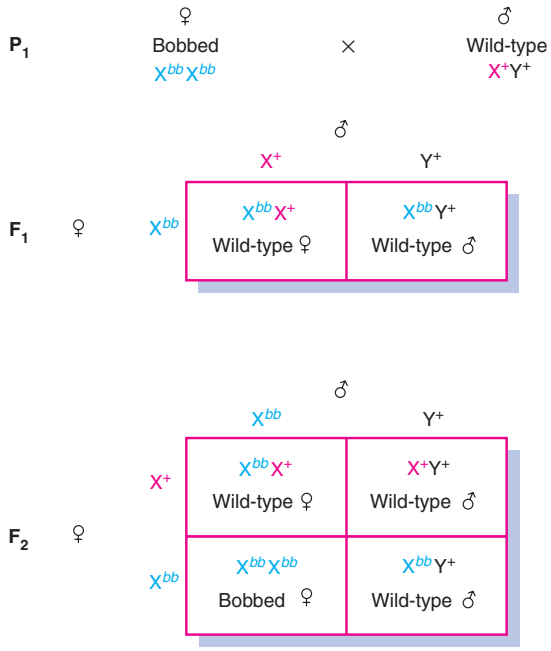


Figure 5.17 Reciprocal cross to that in figure 5.16.

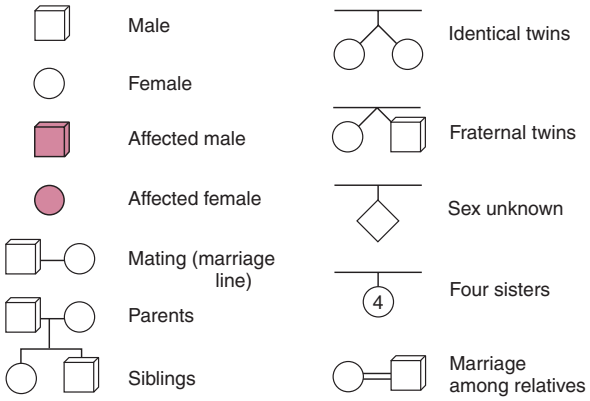


Figure 5.18 Symbols used in a pedigree.

of a trait. There are two types of questions the pedigree might be used to answer. First, are there patterns within the pedigree that are consistent with a particular mode of inheritance? Second, are there patterns within the pedigree that are inconsistent with a particular mode of inheritance? Often, it is not possible to determine the mode of inheritance of a particular trait with certainty. McKusick has reported that, as of 2001, the mode of inheritance of over nine thousand loci in human beings was known with some confidence, including autosomal dominant, autosomal recessive, and sex-linked genes.

Dominant Inheritance

If we look again at the pedigree in figure 5.19, several points emerge. First, polydactyly (fig. 5.20) occurs in every generation. Every affected child has an affected parent—no generations are skipped. This suggests dominant inheritance. Second, the trait occurs about equally in both sexes; there are seven affected males and six affected females in the pedigree. This indicates autosomal rather than sex-linked inheritance. Thus, so far, we would categorize polydactyly as an autosomal dominant trait. Note also that individual IV-11, a male, passed on the trait to two of his three sons. This would rule out sex linkage. (Remember that a male gives his X chromosome to all of his daughters but none of his sons. His sons receive his Y chromosome.) Consistency in many such pedigrees, has confirmed that an autosomal dominant gene causes polydactyly.

Polydactyly shows variable penetrance and expressivity. The most extreme manifestation of the trait is an extra digit on each hand (fig. 5.20) and one or two extra toes on each foot. However, some individuals have only extra toes, some have extra fingers, and some have an asymmetrical distribution of digits such as six toes on one foot and seven on the other.

Recessive Inheritance

Figure 5.21 is a pedigree with a different pattern of inheritance. Here affected individuals are not found in each generation. The affected daughters, identical triplets, come from unaffected parents. They represent, in fact, the first appearance of the trait in the pedigree. A telling point is that the parents of the triplets are first cousins; a mating between relatives is referred to as **consanguineous.** If the degree of relatedness is closer than law permits, the union is called **incestuous.** In all states, brother-sister and mother-son marriages are forbidden; and in all states except Georgia, father-daughter marriages are forbidden. Georgia did not intend to permit father-daughter marriages. However, the law was drafted using biblical terminology that inadvertently did not prohibit a man from marrying his daughter or his grandmother. Thirty states prohibit the marriage of first cousins.

Consanguineous matings often produce offspring that have rare recessive, and often deleterious, traits. The reason is that through common ancestry (e.g., when first cousins have a pair of grandparents in common), a rare allele can be passed on both sides of the pedigree and become homozygous in a child. The occurrence of a trait in a pedigree with common ancestry is often good evidence

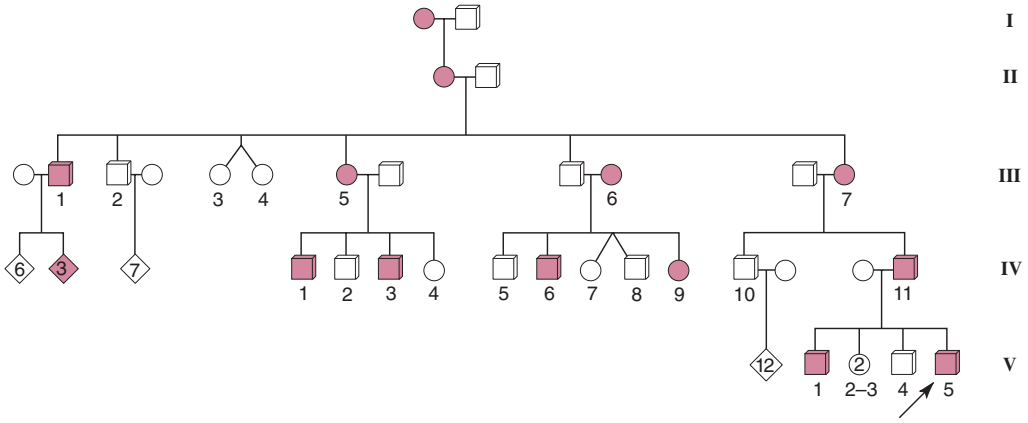


Figure 5.19 Part of a pedigree for polydactyly.

for an autosomal recessive mode of inheritance. Consanguinity by itself does not guarantee that a trait has an autosomal recessive mode of inheritance; all modes of inheritance appear in consanguineous pedigrees. Conversely, recessive inheritance is not confined to consanguineous pedigrees. Hundreds of recessive traits are known from pedigrees lacking consanguinity.

Sex-Linked Inheritance

Figure 5.22 is the pedigree of Queen Victoria of England. Through her children, hemophilia was passed on to many of the royal houses of Europe. Several interesting aspects of this pedigree help to confirm the method of inheritance. First, generations are skipped. Although Alexis (1904–18) was a hemophiliac, neither his parents nor his grandparents were. This pattern occurs in several other places in the pedigree and indicates a recessive mode of inheritance. From other pedigrees and from the biochemical nature of the defect, scientists have determined that hemophilia is a recessive trait.

Further inspection of the pedigree in figure 5.22 reveals that all the affected individuals are sons, strongly suggesting sex linkage. Since males are hemizygous for the X chromosome, more males than females should have the phenotype of a sex-linked recessive trait because males do not have a second X chromosome that might carry the normal allele. If this is correct, we can make several predictions. First, since all males get their X chromosomes from their mothers, affected males should be the offspring of carrier (heterozygous) females. A female is automatically a carrier if her father had the disease. She has a 50% chance of being a carrier if her brother, but not her father, has the disease. In that case, her mother was a carrier. The pedigree in figure 5.22 is consistent with these predictions.

Figure 5.20 Hands of a person with polydactyly. Manifestations range in severity from one extra finger or toe to one or more extra digits on each hand and foot. (© L.V. Bergman/The Bergman Collection.)

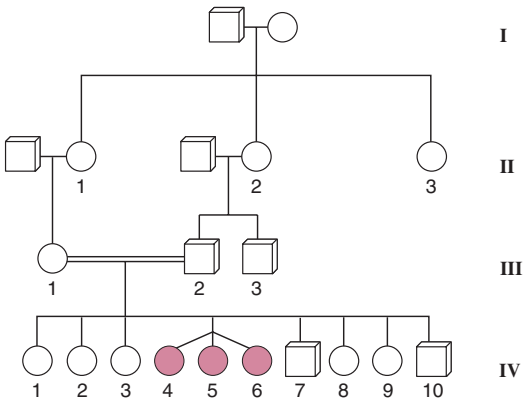
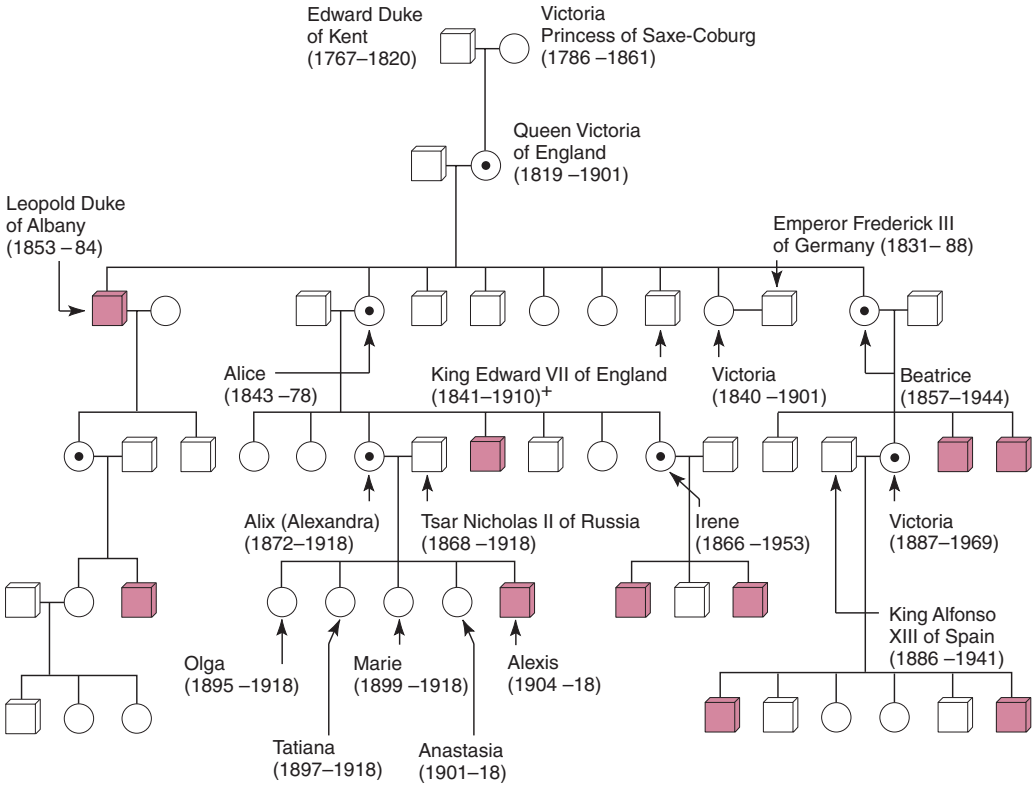


Figure 5.21 Part of a pedigree of hypotrichosis (early hair loss).



Normal female

Normal, but known carrier (heterozygous) female

Normal male

Affected male

+ Descendants include present British royal family

Figure 5.22 Hemophilia in the pedigree of Queen Victoria of England. In the photograph of the Queen and some of her descendants, three carriers—Queen Victoria (*center*), Princess Irene of Prussia (*right*), and Princess Alix (Alexandra) of Hesse (*left*)—are indicated. (Photo © Mary Evans Picture Library/Photo Researchers, Inc.)

In no place in the pedigree is the trait passed from father to son. This would defy the route of an affected X chromosome. We can conclude from the pedigree that hemophilia is a sex-linked recessive trait. (Several different inherited forms of hemophilia are known, each deficient in one of the steps in the pathway that forms fibrinogen, the blood clot protein. Two of these forms, "classic" hemophilia A and hemophilia B, also called Christmas disease, are sex linked. Other hemophilias are autosomal.)

One other interesting point about this pedigree is that there is no evidence of the disease in Queen Victoria's ancestors, yet she was obviously a heterozygote, having one affected son and two daughters who were known carriers. Thus, though she was born to what appears to be a homozygous normal mother and a hemizygous normal father, one of Queen Victoria's X chromosomes had the hemophilia allele. This could have happened if a change (mutation) had occurred in one of the gametes that formed Queen Victoria. (We explore the mechanisms of mutation in chapter 12.)

Figure 5.23 is another pedigree that points to dominant inheritance because the trait skips no generations. The pedigree shows the distribution of low blood-phosphorus levels, the fully penetrant aspect of vitamin-D-resistant rickets, among the sexes. Affected males pass on the trait to their daughters but not their sons. This pattern follows that of the X chromosome: a male passes it on to all of his daughters but to none of his sons. Although this pedigree accords with a sex-linked dominant mode of inheritance, it does not rule out autosomal inheritance. The pedigree shown is a small part of one involving hundreds of people, all with phenotypes consistent with the hypothesis of sex-linked dominant inheritance.

In figure 5.23, there is the slight possibility that the trait is recessive. This could be true if the male in generation I and the mates of II-5 and II-7 were all heterozygotes. Since this is a rare trait, the possibility that all these conditions occurred is small. For example, if one person in fifty (0.02) is a heterozygote, then the probability of three heterozygotes mating within the same pedigree is $(0.02)^3$, or eight in one million. The rareness of this event further supports the hypothesis of dominant inheritance. The expected patterns for the various types of inheritance in pedigrees can be summarized in the following four categories:

Autosomal Recessive Inheritance

1. Trait often skips generations.
2. An almost equal number of affected males and females occur.
3. Traits are often found in pedigrees with consanguineous matings.
4. If both parents are affected, all children should be affected.
5. In most cases when unaffected people mate with affected individuals, all children are unaffected. When at least one child is affected (indicating that the unaffected parent is heterozygous), approximately half the children should be affected.
6. Most affected individuals have unaffected parents.

Autosomal Dominant Inheritance

1. Trait should not skip generations (unless trait lacks full penetrance).
2. When an affected person mates with an unaffected person, approximately 50% of their offspring should be affected (indicating also that the affected individual is heterozygous).
3. The trait should appear in almost equal numbers among the sexes.

Sex-Linked Recessive Inheritance

1. Most affected individuals are male.
2. Affected males result from mothers who are affected or who are known to be carriers (heterozygotes) because they have affected brothers, fathers, or maternal uncles.
3. Affected females come from affected fathers and affected or carrier mothers.
4. The sons of affected females should be affected.
5. Approximately half the sons of carrier females should be affected.

Sex-Linked Dominant Inheritance

1. The trait does not skip generations.
2. Affected males must come from affected mothers.
3. Approximately half the children of an affected heterozygous female are affected.
4. Affected females come from affected mothers or fathers.
5. All the daughters, but none of the sons, of an affected father are affected.

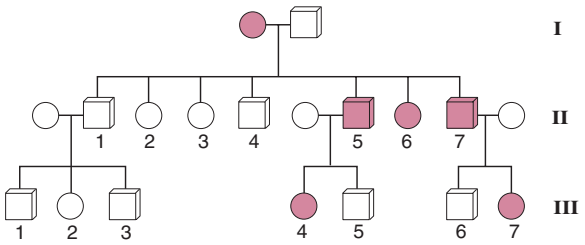


Figure 5.23 Part of a pedigree of vitamin-D-resistant rickets. Affected individuals have low blood-phosphorus levels. Although the sample is too small for certainty, dominance is indicated because every generation was affected, and sex linkage is suggested by the distribution of affected individuals.

SUMMARY

This chapter begins a four-chapter sequence that analyzes the relationship of genes to chromosomes. We begin with the study of sex determination.

STUDY OBJECTIVE 1: To analyze the causes of sex determination in various organisms 83–90

Sex determination in animals is often based on chromosomal differences. In human beings and fruit flies, females are homogametic (XX) and males are heterogametic (XY). In human beings, a locus on the Y chromosome, *SRY,* determines maleness; in *Drosophila,* sex is determined by the balance between genes on the X chromosome and genes on the autosomes that regulate the state of the sex-switch gene, *Sxl.*

STUDY OBJECTIVE 2: To understand methods of dosage compensation 90–95

Different organisms have different ways of solving problems of dosage compensation for loci on the X chromosome. In human beings, one of the X chromosomes in cells in a woman is Lyonized, or inactivated. Lyonization in women leads to cellular mosaicism for most loci on the X chromosome. In *Drosophila,* the X chromosome in males is hyperactive.

STUDY OBJECTIVE 3: To analyze the inheritance patterns of traits that loci on the sex chromosomes control 95–97

Since different chromosomes are normally associated with each sex, inheritance of loci located on these chromosomes shows specific, nonreciprocal patterns. The white-eye locus in *Drosophila* was the first case when a locus was assigned to the X chromosome. Over four hundred sex-linked loci are now known in human beings.

STUDY OBJECTIVE 4: To use pedigrees to infer inheritance patterns 97–102

Human genetic studies use pedigree analysis to determine inheritance patterns because it is impossible to carry out large-scale, controlled human crosses. However, not all traits determined by genotype are apparent in the phenotype, and this lack of penetrance can pose problems in genetic analysis.

SOLVED PROBLEMS

PROBLEM 1: A Female fruit fly with a yellow body is discovered in a wild-type culture. The female is crossed with a wild-type male. In the F_1 generation, the males are yellow-bodied and the females are wild-type. When these flies are crossed among themselves, the F_2 produced are both yellow-bodied and wild-type, equally split among males and females (see fig. 1). Explain the genetic control of this trait.

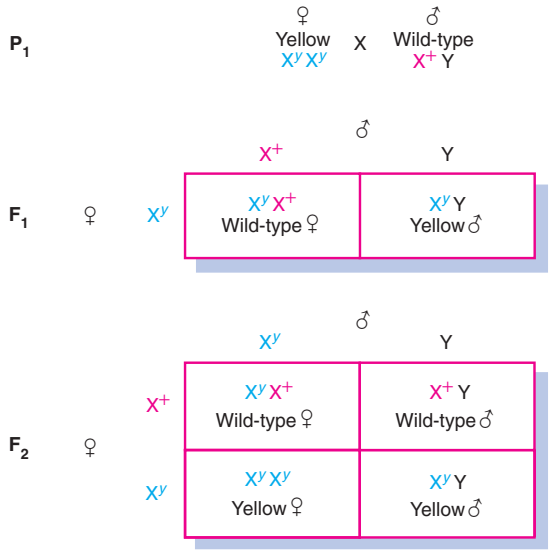


Figure 1 Cross between yellow-bodied and wild-type fruit flies.

Answer: Since the results in the F_1 generation differ between the two sexes, we suspect that a sex-linked locus is responsible for the control of body color. If we assume that it is a recessive trait, then the female parent must have been a recessive homozygote, and the male must have been a wild-type hemizygote. If we assign the wild-type allele as X^+, the yellow-body allele as X^y, and the Y chromosome as Y, then figure 1, showing the crosses into the F_2 generation, is consistent with the data. Thus, a recessive X-linked gene controls yellow body color in fruit flies.

PROBLEM 2: The affected individuals in the pedigree in figure 2 are chronic alcoholics (data from the National Institute of Alcohol Abuse and Alcoholism). What can you say about the inheritance of this trait?

Answer: We begin by assuming 100% penetrance. If that is the case, then we can rule out either sex-linked or autosomal recessive inheritance because both parents had the trait, yet they produced some unaffected children.

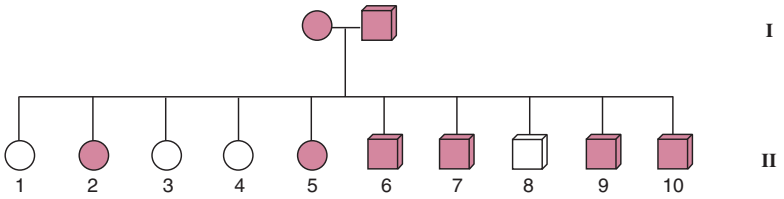


Figure 2 A pedigree for alcoholism.

Nor can the mode of inheritance be by a sex-linked dominant gene because an affected male would have only affected daughters, since his daughters get copies of his single X chromosome. We are thus left with autosomal dominance as the mode of inheritance. If that is the case, then both parents must be heterozygotes; otherwise, all the children would be affected. If both parents are heterozygotes, we expect a 3:1 ratio of affected to unaffected offspring (a cross of $Aa \times Aa$ produces offspring of A-:aa in a 3:1 ratio); here, the ratio is 6:4. If we did a chi-square test, the expected numbers would be 7.5:2.5 (3/4 and 1/4, respectively, of 10). Although the expected value of 2.5 makes it inappropriate to do a chi-square test (the expected value is too small), we can see that the observed and expected numbers are very close. Thus, from the pedigree we would conclude that an autosomal dominant allele controls chronic alcoholism. (Although the analysis is consistent, we actually cannot draw that conclusion about alcoholism because other pedigrees are not consistent with 100% penetrance, a one-gene model, or the lack of environmental influences. In fact, scientists are currently debating whether alcoholism is inherited at all. These types of problems related to complex human traits are discussed in chapter 18.)

PROBLEM 3: A female fly with orange eyes is crossed with a male fly with short wings. The F_1 females have wild-type (red) eyes and long wings; the F_1 males have orange eyes and long wings. The F_1 flies are crossed to yield

47 long wings, red eyes
45 long wings, orange eyes
17 short wings, red eyes
14 short wings, orange eyes

with no differences between the sexes. What is the genetic basis of each trait?

Answer: In the F_1 flies, we see a difference in eye color between the sexes, indicating some type of sex linkage. Since the females are wild-type, wild-type is probably dominant to orange. We can thus diagram the cross for eye color as

(female) X^oX^o orange		X^+Y (male) red		P_1
		↓		
X^+X^o red		X^oY orange		F_1
		↓		
X^+X^o red	X^oX^o orange	X^+Y red	X^oY orange	F_2

We would thus expect to see equal numbers of red-eyed and orange-eyed males and females, which is what we observe. Now look at long versus short wings. If we disregard eye color, wing length seems to be under autosomal control with short wings being recessive. Thus, the parents are homozygotes (ss and s^+s^+), the F_1 offspring are heterozygotes (s^+s), and the F_2 progeny have a phenotypic ratio of 3:1, wild-type (long) to short wings.

EXERCISES AND PROBLEMS*

SEX DETERMINATION

1. What is the difference between an X and a Z chromosome?
2. Transformer (*tra*) is an autosomal recessive gene that converts chromosomal females into sterile males. A female *Drosophila* heterozygous for the transformer allele (*tra*) is mated with a normal male homozygous for transformer. What is the sex ratio of their offspring? What is the sex ratio of their offspring's offspring?
3. The autosomal recessive doublesex (*dsx*) gene converts males and females into developmental intersexes. Two fruit flies, both heterozygous for the *doublesex* (*dsx*) allele, are mated. What are the sexes of their offspring?
4. What is a sex switch? What genes serve as sex switches in human beings and *Drosophila?*

*Answers to selected exercises and problems are on page A-4.

DOSAGE COMPENSATION

5. The diagram of an electrophoretic gel in figure 3 shows activity for a particular enzyme. Lane 1 is a sample from a "fast" homozygote. Lane 2 is a sample from a "slow" homozygote. In lane 3, the blood from the first two was mixed. Lane 4 comes from one of the children of the two homozygotes.

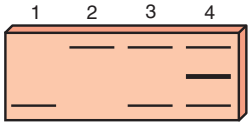


Figure 3 The activity of a particular enzyme as revealed on an electrophoretic gel.

Can you guess the structure of the enzyme? If this were an X-linked trait, what pattern would you expect from a heterozygous female's

a. whole blood?
b. individual cells?

6. How many different zones of activity (bands) would you see on a gel stained for lactate dehydrogenase (LDH) activity from a person homozygous for the A protein gene but heterozygous for the B protein gene? Are the bands due to the activity of allozymes or isozymes?

7. How many Barr bodies would you see in the nuclei of persons with the following sex chromosomes?

a. X0
b. XX
c. XY
d. XXY
e. XXX
f. XXXXX
g. XX/XY mosaic

What would the sex of each of these persons be? If these were the sex chromosomes of individual *Drosophila* that were diploid for all other chromosomes, what would their sexes be?

8. Calico cats have large patches of colored fur. What does this indicate about the age of onset of Lyonization (is it early or late)? Tortoiseshell cats have very small color patches. Explain the difference between the two phenotypes.

SEX LINKAGE

9. In *Drosophila,* the lozenge phenotype, caused by a sex-linked recessive allele (*lz*), is narrow eyes. Diagram to the F_2 generation a cross of a lozenge male and a homozygous normal female. Diagram the reciprocal cross.

10. Sex linkage was originally detected in 1906 in moths with a ZW sex-determining mechanism. In the currant moth, a pale color (*p*) is recessive to the wild-type and located on the Z chromosome. Diagram reciprocal crosses to the F_2 generation in these moths.

11. What family history of hemophilia would indicate to you that a newborn male baby should be exempted from circumcision?

12. What is the difference between *pseudodominance* and *phenocopy?*

13. In *Drosophila,* cut wings are controlled by a recessive sex-linked allele (*ct*), and fuzzy body is controlled by a recessive autosomal allele (*fy*). When a fuzzy female is mated with a cut male, all the members of the F_1 generation are wild-type. What are the proportions of F_2 phenotypes, by sex?

14. Consider the following crosses in canaries:

Parents	Progeny
a. pink-eyed female × pink-eyed male	all pink-eyed
b. pink-eyed female × black-eyed male	all black-eyed
c. black-eyed female × pink-eyed male	all females pink-eyed, all males black-eyed

Explain these results by determining which allele is dominant and how eye color is inherited.

15. Consider the following crosses involving yellow and gray true-breeding *Drosophila:*

Cross	F_1	F_2
gray female × yellow male	all males gray, all females gray	97 gray females, 42 yellow males, 48 gray males
yellow female × gray male	all females gray, all males yellow	?

a. Is color controlled by an autosomal or an X-linked gene?
b. Which allele, gray or yellow, is dominant?
c. Assume 100 F_2 offspring are produced in the second cross. What kinds and what numbers of progeny do you expect? List males and females separately.

16. A man with brown teeth mates with a woman with normal white teeth. They have four daughters, all with brown teeth, and three sons, all with white teeth. The sons all mate with women with white teeth, and all their children have white teeth. One of the daughters (A) mates with a man with white teeth (B), and they have two brown-toothed daughters, one white-toothed daughter, one brown-toothed son, and one white-toothed son.

a. Explain these observations.
b. Based on your answer to *a,* what is the chance that the next child of the A-B couple will have brown teeth?

17. In human beings, red-green color blindness is inherited as an X-linked recessive trait. A woman with normal vision whose father was color-blind marries a man with normal vision whose father was also color-

blind. This couple has a color-blind daughter with a normal complement of chromosomes. Is infidelity suspected? Explain.

18. A white-eyed male fly is mated with a pink-eyed female. All the F_1 offspring have wild-type red eyes. F_1 individuals are mated among themselves to yield:

Females		**Males**	
red-eyed	450	red-eyed	231
pink-eyed	155	white-eyed	301
		pink-eyed	70

Provide a genetic explanation for the results.

19. In *Drosophila,* white eye is an X-linked recessive trait, and ebony body is an autosomal recessive trait. A homozygous white-eyed female is crossed with a homozygous ebony male.

a. What phenotypic ratio do you expect in the F_1 generation?

b. What phenotypic ratio do you expect in the F_2 generation?

c. Suppose the initial cross was reversed: ebony female × white-eyed male. What phenotypic ratio would you expect in the F_2 generation?

20. In *Drosophila,* abnormal eyes can result from mutations in many different genes. A true-breeding wild-type male is mated with three different females, each with abnormal eyes. The results of these crosses are as follows:

		Females	**Males**
male × abnormal-1	→	all normal	all normal
male × abnormal-2	→	1/2 normal, 1/2 abnormal	1/2 normal, 1/2 abnormal
male × abnormal-3	→	all abnormal	all abnormal

Explain the results by determining the mode of inheritance for each abnormal trait.

21. A black and orange female cat is crossed with a black male, and the progeny are as follows:

females: two black, three orange and black

males: two black, two orange

Explain the results.

22. Based on the following *Drosophila* crosses, explain the genetic basis for each trait and determine the genotypes of all individuals:

white-eyed, dark-bodied female × red-eyed, tan-bodied male

F_1: females are all red-eyed, tan-bodied; males are all white-eyed, tan-bodied

F_2: 27 red-eyed, tan-bodied
24 white-eyed, tan-bodied
9 red-eyed, dark-bodied
7 white-eyed, dark-bodied

(No differences between males and females in the F_2 generation.)

PEDIGREE ANALYSIS

23. What is the difference between penetrance and expressivity?

24. What are the possible modes of inheritance in pedigrees *a–c* in figure 4? What modes of inheritance are not possible for a given pedigree?

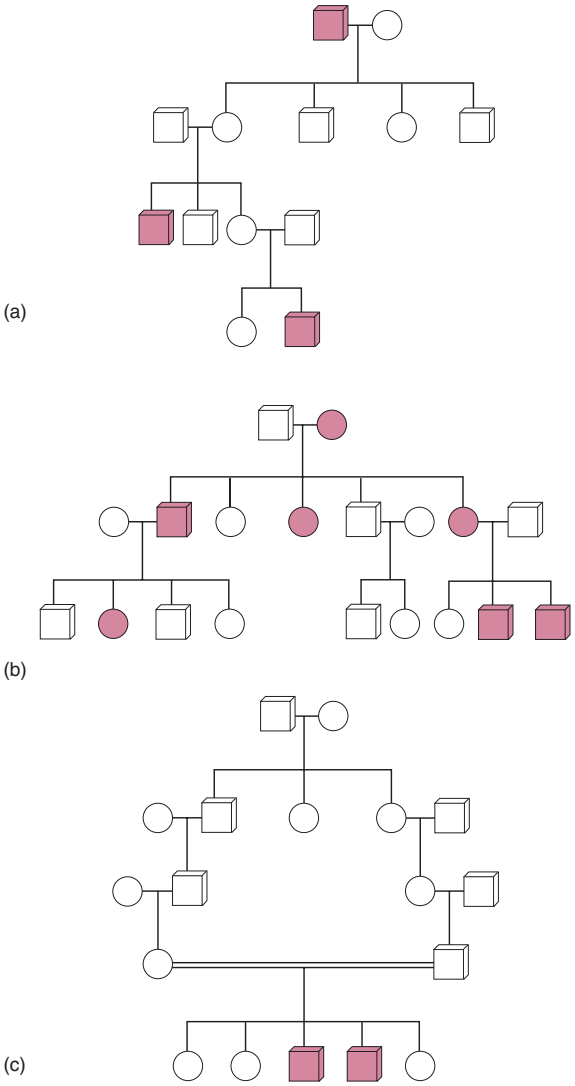


Figure 4 Three pedigrees showing different modes of inheritance.

25. In pedigrees *a–d* in figure 5, which show the inheritance of rare human traits, including twin production, determine which modes of inheritance are most probable, possible, or impossible.

26. Hairy ears, a human trait expressed as excessive hair on the rims of ears in men, shows reduced penetrance (less than 100% penetrant). Mechanisms proposed include Y linkage, autosomal dominance, and autosomal recessiveness. Construct a pedigree consistent with each of these mechanisms.

27. Construct pedigrees for traits that *could not be*
a. autosomal recessive.
b. autosomal dominant.
c. sex-linked recessive.
d. sex-linked dominant.

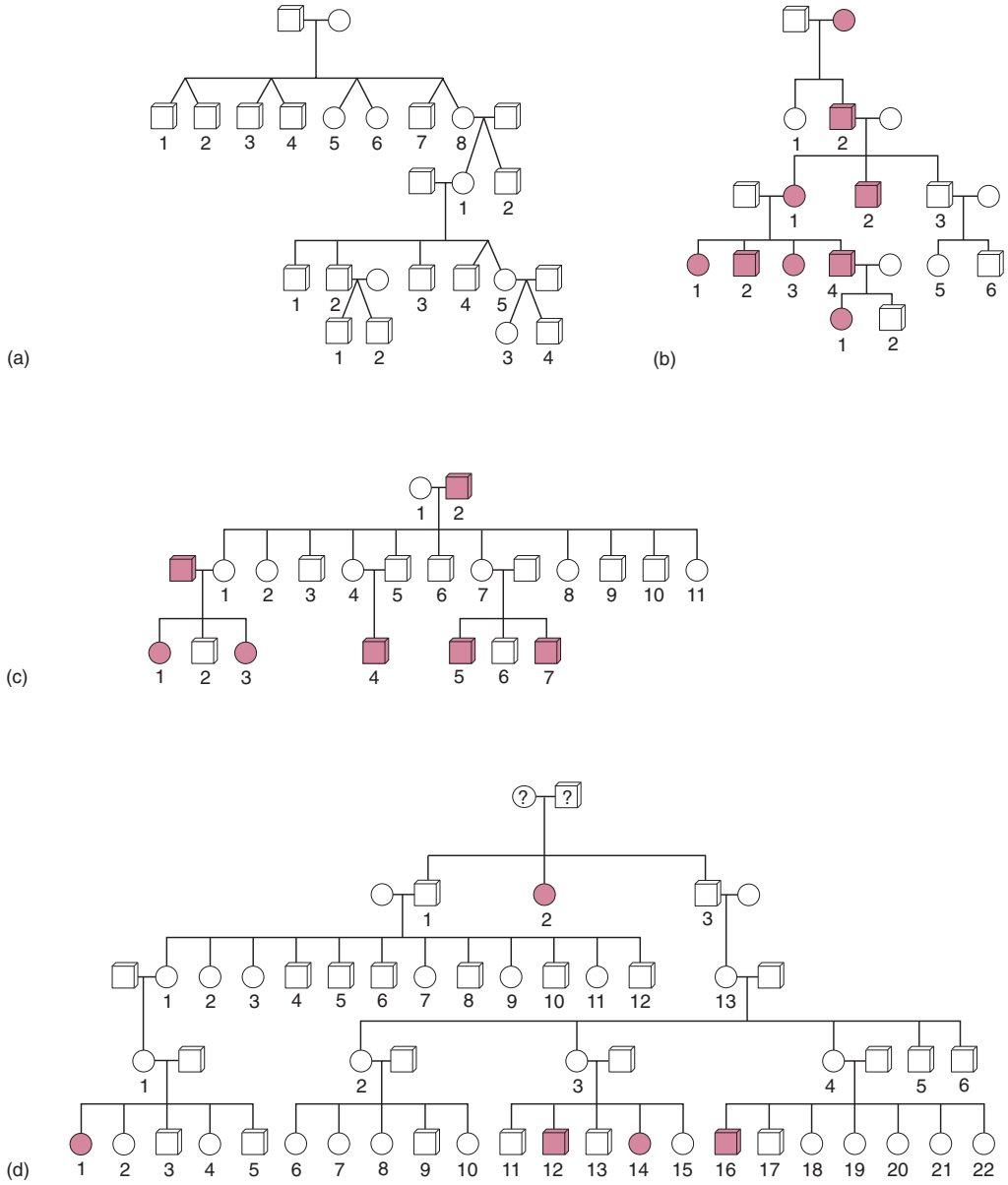


Figure 5 Pedigrees of rare human traits, including twin production (*a*).

28. Determine the possible modes of inheritance for each trait in pedigrees *a–c* in figure 6.

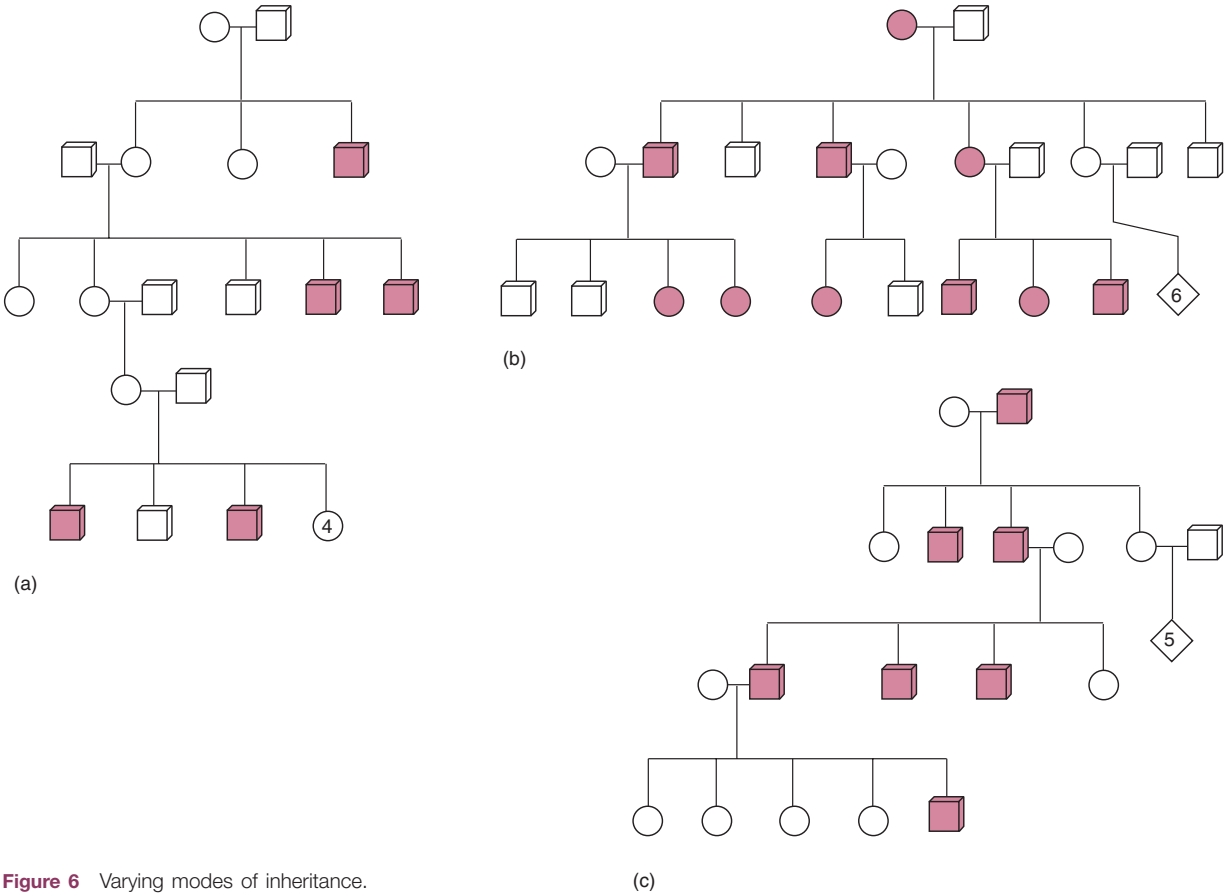


Figure 6 Varying modes of inheritance.

CRITICAL THINKING QUESTIONS

1. What effects do null alleles, alleles that produce no protein product, have in electrophoretic systems? How could you tell if a null allele were present?
2. In 1918, the Bolsheviks killed Tsar Nicholas II of Russia and his family (fig. 5.22). However, the remains of one daughter, Princess Anastasia, were never recovered. At one point, a woman appeared who claimed to be Anastasia. How could you validate her claim genetically?

Suggested Readings for chapter 5 are on page B-2.

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6

LINKAGE AND MAPPING IN EUKARYOTES

STUDY OBJECTIVES

1. To learn about analytical techniques for locating the relative positions of genes on chromosomes in diploid eukaryotic organisms 110
2. To learn about analytical techniques for locating the relative positions of genes on chromosomes in ascomycete fungi 122
3. To learn about analytical techniques for locating the relative positions of genes on human chromosomes 132

STUDY OUTLINE

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Scanning electron micrograph (false color) of a fruit fly, *Drosophila melanogaster.*

(© Dr. Jeremy Burgess/SPL/Photo Researchers.)

After Sutton suggested the chromosomal theory of inheritance in 1903, evidence accumulated that genes were located on chromosomes. For example, Morgan showed by an analysis of inheritance patterns that the white-eye locus in Drosophila is located on the X chromosome. Given that any organism has many more genes than chromosomes, it follows that each chromosome has many loci. Since chromosomes in eukaryotes are linear, it also follows that genes are arranged in a linear fashion on chromosomes, like beads on a string. Sturtevant first demonstrated this in 1913. In this chapter, we look at analytical techniques for mapping chromosomes—techniques for determining the relationship between different genes on the same chromosome. These techniques are powerful tools that allow us to find out about the physical relationships of genes on chromosomes without ever having to see a gene or a chromosome. We determine that genes are on the same chromosome when the genes fail to undergo independent assortment, and then we use recombination frequencies to determine the distance between genes.

If loci were locked together permanently on a chromosome, allelic combinations would always be the same. However, at meiosis, crossing over allows the alleles of associated loci to show some measure of independence. A geneticist can use crossing over between loci to determine how close one locus actually is to another on a chromosome and thus to map an entire chromosome and eventually the entire **genome** (genetic complement) of an organism.

Loci carried on the same chromosome are said to be linked to each other. There are as many **linkage groups** (l) as there are autosomes in the haploid set plus sex chromosomes. *Drosophila* has five linkage groups ($2n = 8$; $l = 3$ autosomes + X + Y), whereas human beings have twenty-four linkage groups ($2n = 46$; $l = 22$ autosomes + X + Y). Prokaryotes and viruses, which usually have a single chromosome, are discussed in chapter 7.

Historically, classical mapping techniques, as described in this chapter and the next, gave researchers their only tools to determine the relationships of particular genes and their chromosomes. When geneticists know the locations of specific genes, they can study them in relation to each other and begin to develop a comprehensive catalogue of the genome of an organism. Knowing the location of a gene also helps in isolating the gene and studying its function and structure. And mapping the genes of different types of organisms (diploid, haploid, eukaryotic, prokaryotic) gives geneticists insight into genetic processes. More recently, recombinant DNA technology has allowed researchers to sequence whole genomes, including the human and fruit fly genomes; this means they now know the exact locations of all the genes on all the chromosomes of these organisms (see chapter 13). Geneticists are now creating massive databases containing this information, much of it available for free or by subscription on the World Wide Web. Until investigators mine all this information for all organisms of interest, they will still use analytical techniques in the laboratory and field to locate genes on chromosomes.

DIPLOID MAPPING

Two-Point Cross

In *Drosophila,* the recessive band gene (*bn*) causes a dark transverse band on the thorax, and the detached gene (*det*) causes the crossveins of the wings to be either detached or absent (fig. 6.1). A banded fly was crossed with a detached fly to produce wild-type, dihybrid offspring in the F_1 generation. F_1 females were then testcrossed to banded, detached males (fig. 6.2). (There is no crossing over in male fruit flies; in experiments designed to detect linkage, heterozygous females—in which crossing over occurs—are usually crossed with homozygous recessive males.) If the loci were assorting independently, we would expect a 1:1:1:1 ratio of the four possible phenotypes. However, of the first one thousand offspring examined, experimenters recorded a ratio of 2:483: 512:3.

Several points emerge from the data in figure 6.2. First, no simple ratio is apparent. If we divide by two, we get a ratio of 1:241:256:1.5. Although the first and last categories seem about equal, as do the middle two, no simple numerical relation seems to exist between the middle and end categories. Second, the two cate-

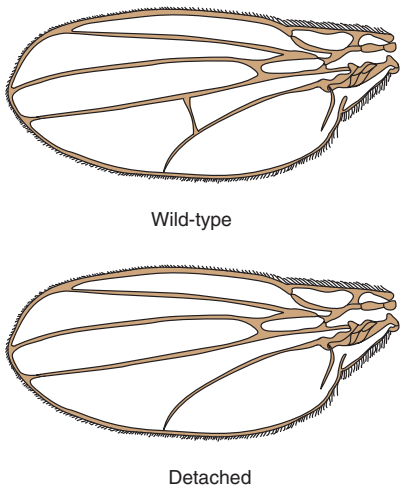

Figure 6.1 Wild-type (det^+) and detached (*det*) crossveins in *Drosophila*.

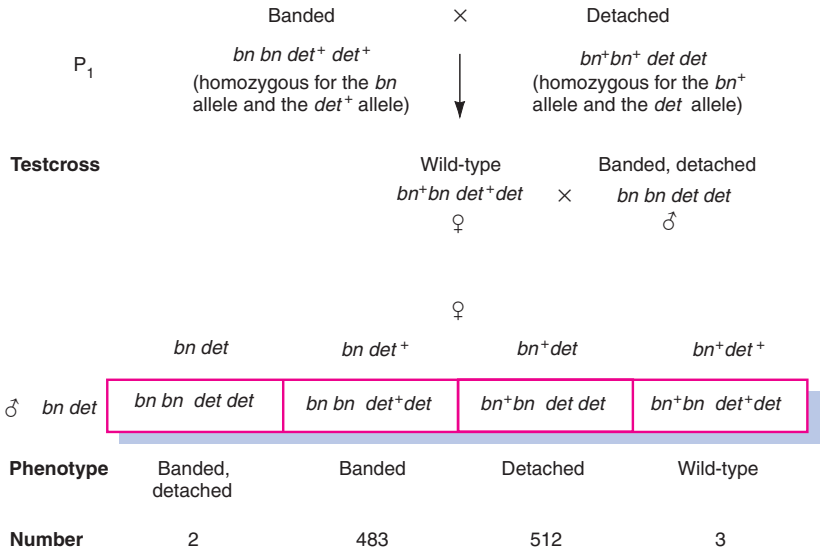

Figure 6.2 Testcrossing a dihybrid *Drosophila.*

gories in very high frequency have the same phenotypes as the original parents in the cross (P_1 of fig. 6.2). That is, banded flies and detached flies were the original parents as well as the great majority of the testcross offspring. We call these phenotypic categories **parentals,** or **nonrecombinants.** On the other hand, the testcross offspring in low frequency combine the phenotypes of the two original parents (P_1). These two categories are referred to as **nonparentals,** or **recombinants.** The simplest explanation for these results is that the banded and detached loci are located near each other on the same chromosome (they are a linkage group), and therefore they move together as associated alleles during meiosis.

We can analyze the original cross by drawing the loci as points on a chromosome (fig. 6.3). This shows that 99.5% of the testcross offspring (the nonrecombinants) come about through the simple linkage of the two loci. The remaining 0.5% (the recombinants) must have arisen through a crossover of homologues, from a chiasma at meiosis, between the two loci (fig. 6.4). Note that since it is not possible to tell from these crosses which chromosome the loci are actually on or where the centromere is in relation to the loci, the centromeres are not included in the figures. The crossover event is viewed as a breakage and reunion of two chromatids lying adjacent to each other during prophase I of meiosis. Later in this chapter, we find cytological proof for this; in chapter 12, we explore the molecular mechanisms of this breakage and reunion process.

From the testcross in figure 6.3, we see that 99.5% of the gametes produced by the dihybrid are nonrecombinant, whereas only 0.5% are recombinant. This very small frequency of recombinant offspring indicates that the two loci lie very close to each other on their particular chromosome. In fact, we can use the recombination percentages of gametes, and therefore of testcross offspring, as estimates of distance between loci on a chromosome: 1% recombinant offspring is referred to as one **map unit** (or one **centimorgan,** in honor of geneticist T. H. Morgan, the first geneticist to win the Nobel Prize; box 6.1). Although a map unit is not a physical distance, it is a relative distance that makes it possible to know the order of and relative separation between loci on a chromosome. In this case, the two loci are 0.5 map units apart. (From sequencing various chromosomal segments—see chapter 13—we have learned that the relationship between centimorgans and DNA base pairs is highly variable, depending on species, sex, and region of the chromosome. For example, in human beings, 1 centimorgan can vary between 100,000 and 10,000,000 base pairs. In the fission yeast, *Schizosaccharomyces pombe,* 1 centimorgan is only about 6,000 base pairs.)

The arrangement of the *bn* and *det* alleles in the dihybrid of figure 6.3 is termed the **trans** configuration, meaning "across," because the two mutants are across from each other, as are the two wild-type alleles. The alternative arrangement, in which one chromosome carries both mutants and the other chromosome carries both wild-type alleles (fig. 6.5), is referred to as the **cis** configuration. (Two other terms, **repulsion** and **coupling,** have the same meanings as *trans* and *cis,* respectively.)

A cross involving two loci is usually referred to as a **two-point cross;** it gives us a powerful tool for dissecting the makeup of a chromosome. The next step in our

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BOX 6.1

Historical Perspectives

The Nobel Prize

On 10 December each year, the king of Sweden awards the Nobel Prizes at the Stockholm Concert Hall. The date is the anniversary of Alfred Nobel's death. Awards are given annually in physics, chemistry, medicine and physiology, literature, economics, and peace. In 2000, each award was worth $900,000, although an award sometimes goes to two or three recipients. The prestige is priceless.

Winners of the Nobel Prize are chosen according to the will of Alfred Nobel, a wealthy Swedish inventor and industrialist, who held over three hundred patents when he died in 1896 at the age of sixty-three. Nobel developed a detonator and processes for detonation of nitroglycerine, a substance invented by Italian chemist Ascanio Sobrero in 1847. In the form Nobel developed, the explosive was patented as dynamite. Nobel also invented several other forms of explosives. He was a benefactor of Sobrero, hiring him as a consultant and paying his wife a pension after Sobrero died.

Nobel believed that dynamite would be so destructive that it would serve as a deterrent to war. Later, realizing that this would not come to pass, he instructed that his fortune be invested and the interest used to fund the awards. The first prizes were awarded in 1901. Each award consists of a diploma, medal, and check.

American, British, German, French, and Swedish citizens have earned the most prizes (table 1). Table 2 features some highlights of Nobel laureate achievements in genetics.

The Nobel medal. The medal is half a pound of 23-karat gold, measures about 2 1/2 inches across, and has Nobel's face and the dates of his birth and death on the front. The diplomas that accompany the awards are individually designed. (Reproduced by permission of the Nobel Foundation.)

Table 1 Distribution of Nobel Awards to the Top Five Recipient Nations (Including 2000 Winners)

	Physics	Chemistry	Medicine and Physiology	Peace	Literature	Economics	Total
United States	77	46	88	20	9	30	270
Britain	20	24	25	9	8	5	91
Germany	18	27	15	4	6	1	71
France	12	7	7	8	12	1	47
Sweden	4	4	7	5	7	2	29

Table 2 Some Nobel Laureates in Genetics (Medicine and Physiology; Chemistry)

Name	Year	Nationality	Cited for
Thomas Hunt Morgan	1933	USA	Discovery of how chromosomes govern heredity
Hermann J. Muller	1946	USA	X-ray inducement of mutations
George W. Beadle	1958	USA	Genetic regulation of biosynthetic pathways
Edward L. Tatum	1958	USA	
Joshua Lederberg	1958	USA	Bacterial genetics
Severo Ochoa	1959	USA	Discovery of enzymes that synthesize nucleic acids
Arthur Kornberg	1959	USA	
Francis H. C. Crick	1962	British	Discovery of the structure of DNA
James D. Watson	1962	USA	
Maurice Wilkins	1962	British	
François Jacob	1965	French	Regulation of enzyme biosynthesis
André Lwoff	1965	French	
Jacques Monod	1965	French	
Peyton Rous	1966	USA	Tumor viruses
Robert W. Holley	1968	USA	Unraveling of the genetic code
H. Gobind Khorana	1968	USA	
Marshall W. Nirenberg	1968	USA	
Max Delbrück	1969	USA	Viral genetics
Alfred Hershey	1969	USA	
Salvador Luria	1969	USA	
Renato Dulbecco	1975	USA	Tumor viruses
Howard Temin	1975	USA	Discovery of reverse transcriptase
David Baltimore	1975	USA	
Werner Arber	1978	Swiss	Discovery and use of restriction endonucleases
Hamilton Smith	1978	USA	
Daniel Nathans	1978	USA	
Walter Gilbert	1980	USA	Techniques of sequencing DNA
Frederick Sanger	1980	British	
Paul Berg	1980	USA	Pioneer work in recombinant DNA
Baruj Benacerraf	1980	USA	Genetics of immune reactions
Jean Dausset	1980	French	
George Snell	1980	USA	
Aaron Klug	1982	British	Crystallographic work on protein-nucleic acid complexes
Barbara McClintock	1983	USA	Transposable genetic elements
Cesar Milstein	1984	British/Argentine	Immunogenetics
Georges Koehler	1984	German	
Niels K. Jerne	1984	British/Danish	
Susumu Tonegawa	1987	Japanese	Antibody diversity
J. Michael Bishop	1989	USA	Proto-oncogenes
Harold E. Varmus	1989	USA	
Thomas R. Cech	1989	USA	Enzymatic properties of RNA
Sidney Altman	1989	Canada	
Kary Mullis	1993	USA	Polymerase chain reaction
Michael Smith	1993	Canada	Site-directed mutagenesis
Richard Roberts	1993	British	Discovery of intervening sequences in RNA
Phillip Sharp	1993	USA	
E. B. Lewis	1995	USA	Genes control development

continued

BOX 6.1 CONTINUED

Table 2 ***continued***

Name	Year	Nationality	Cited for
Christiane Nüsslein-Volhard	1995	German	
Eric Wieschaus	1995	USA	
Stanley B. Prusiner	1997	USA	Discovery of prions
Günter Blobel	1999	German	Signal recognition during protein synthesis

analysis is to look at three loci simultaneously so that we can determine their relative order on the chromosome. More important, we can also analyze the effects of multiple crossovers, which cannot be detected in a two-point cross, on map distances. Two crossovers between two loci can cause the chromosome to look as if no crossovers took place, causing us to underestimate map distances. Thus we need a third locus, between the first two, to detect multiple crossover events.

Three-Point Cross

Analysis of three loci, each segregating two alleles, is referred to as a **three-point cross.** We will examine wing morphology, body color, and eye color in *Drosophila.* Black body (*b*), purple eyes (*pr*), and curved wings (*c*) are all recessive genes. Since the most efficient way to study linkage is through the testcross of a multihybrid, we will study these three loci by means of the crosses shown in

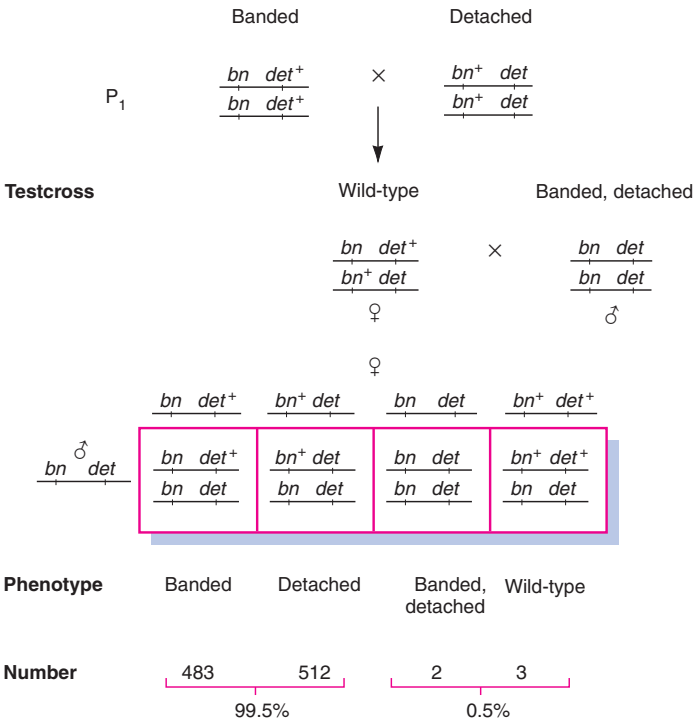


Figure 6.3 Chromosomal arrangement of the two loci in the crosses of figure 6.2. A line arbitrarily represents the chromosomes on which these loci are actually situated.

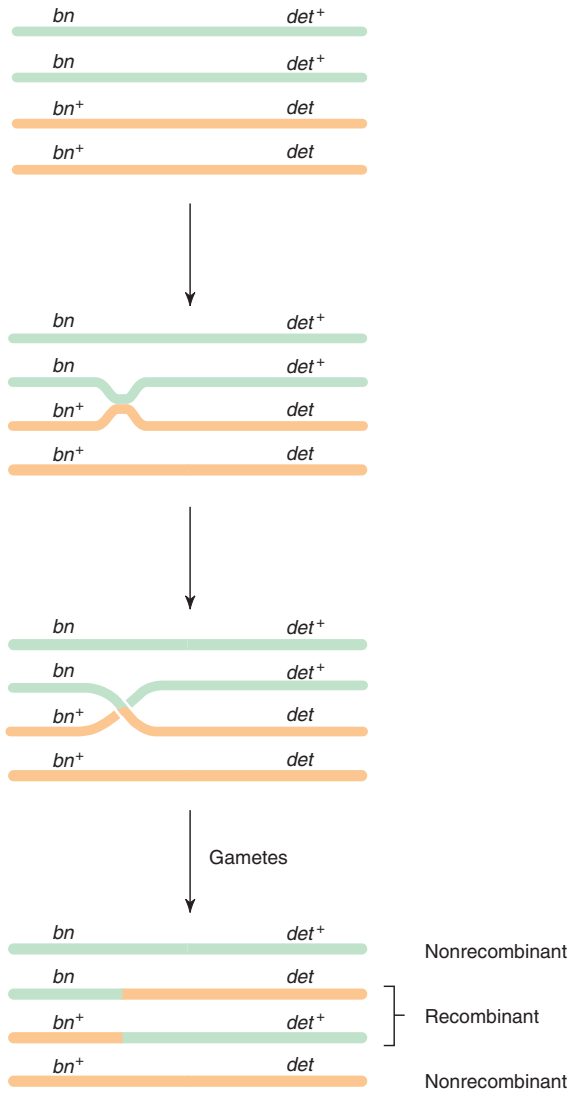


Figure 6.4 Crossover of homologues during meiosis between the *bn* and *det* loci in the tetrad of the dihybrid female.

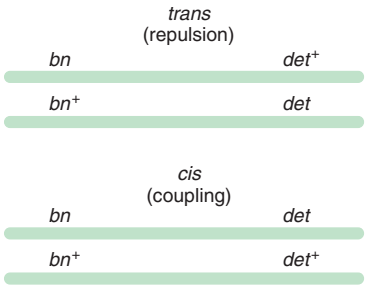


Figure 6.5 *Trans* (repulsion) and *cis* (coupling) arrangements of dihybrid chromosomes.

figure 6.6. One point in this figure should be clarified. Since the organisms are diploid, they have two alleles at each locus. Geneticists use various means to present this situation. For example, the recessive homozygote can be pictured as

1. *bb prpr cc*
2. *b/b pr/pr c/c* or $\frac{b}{b}\ \frac{pr}{pr}\ \frac{c}{c}$
3. *b pr c/b pr c* or $\frac{b\ \ pr\ \ c}{b\ \ pr\ \ c}$

A slash (also called a rule line) is used to separate alleles on homologous chromosomes. Thus (*1*) is used tentatively, when we do not know the linkage arrangement of the loci, (*2*) is used to indicate that the three loci are on different chromosomes, and (*3*) indicates that all three loci are on the same chromosome.

In figure 6.6, the trihybrid organism is testcrossed. If independent assortment is at work, the eight types of resulting gametes should appear with equal frequencies, and thus the eight phenotypic classes would each make up one-eighth of the offspring. However, if there were *complete linkage,* so that the loci are so close together on the same chromosome that virtually no crossing over takes place, we would expect the trihybrid to produce only two gamete types in equal frequency and to yield two phenotypic classes identical to the original parents. This would occur because, under complete linkage, the trihybrid would produce only two chromosomal types in gametes: the *b pr c* type from one parent and the b^+ pr^+ c^+ type from the other. Crossing over between linked loci would produce eight phenotypic classes in various proportions depending on the distances between loci. The actual data appear in table 6.1.

The data in the table are arranged in reciprocal classes. Two classes are reciprocal if between them they contain each mutant phenotype just once. Wild-type and black, purple, curved classes are thus reciprocal, as are the purple, curved and the black classes. Reciprocal classes occur in roughly equal numbers: 5,701 and 5,617; 388 and 367; 1,412 and 1,383; and 60 and 72. As we shall see, a single meiotic recombinational event produces reciprocal classes. Wild-type and black, purple, curved are the two nonrecombinant classes. The purple, curved class of 388 is grouped with the black class of 367. These two would be the products of a crossover between the *b* and the *pr* loci if we assume that the three loci are linked and that the gene order is *b pr c* (fig. 6.7). The next two classes, of 1,412 and 1,383 flies, would result from a crossover between *pr* and *c,* and the last set, 60 and 72, would result from two crossovers, one between *b* and *pr* and the other between

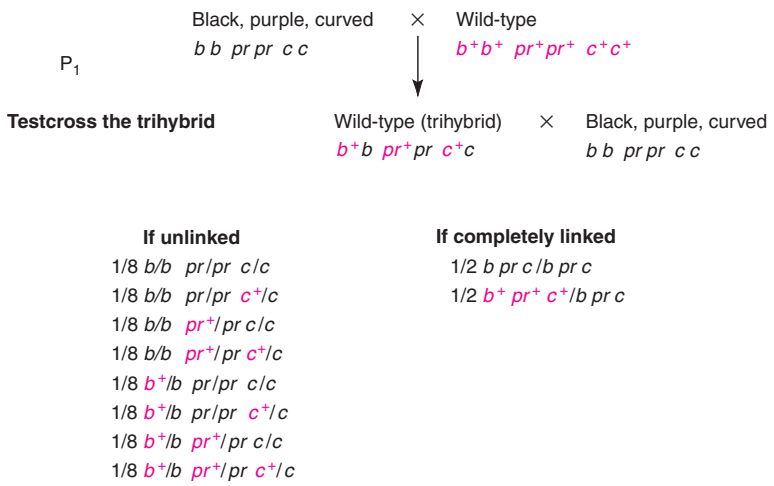


Figure 6.6 Possible results in the testcross progeny of the *b pr c* trihybrid.

pr and *c* (fig. 6.8). Groupings according to these recombinant events are shown at the right in table 6.1.

In the final column of table 6.1, recombination between *b* and *c* is scored. Only those recombinant classes that have a new arrangement of *b* and *c* alleles, as compared with the parentals, are counted. This last column shows us what a *b–c,* two-point cross would have revealed had we been unaware of the *pr* locus in the middle.

Map Distances

The percent row in table 6.1 reveals that 5.9% (887/15,000) of the offspring in the *Drosophila* trihybrid testcross resulted from recombination between *b* and *pr,* 19.5% between *pr* and *c,* and 23.7% between *b* and *c.* These numbers allow us to form a tentative map of the loci (fig. 6.9). There is, however, a discrepancy. The distance between *b* and *c* can be calculated in two ways. By adding the two distances, *b–pr* and *pr–c,* we get 5.9 + 19.5 = 25.4 map units; yet by directly counting the recombinants (the last column of table 6.1), we get a distance of only 23.7 map units. What causes this discrepancy of 1.7 map units?

Returning to the last column of table 6.1, we observe that the double crossovers (60 and 72) are not counted, yet each actually represents two crossovers in this re-

Table 6.1 **Results of Testcrossing Female *Drosophila* Heterozygous for Black Body Color, Purple Eye Color, and Curved Wings ($b^+b\ pr^+pr\ c^+c \times bb\ prpr\ cc$)**

Phenotype	Genotype	Number	Alleles from Trihybrid Female	Number Recombinant Between		
				b and *pr*	*pr* and *c*	*b* and *c*
Wild-type	$b^+b\ pr^+pr\ c^+\ c$	5,701	$b^+\ pr^+\ c^+$			
Black, purple, curved	*bb prpr cc*	5,617	*b pr c*			
Purple, curved	$b^+b\ prpr\ cc$	388	$b^+\ pr\ c$	388		388
Black	$bb\ pr^+pr\ c^+c$	367	$b\ pr^+\ c^+$	367		367
Curved	$b^+b\ pr^+pr\ cc$	1,412	$b^+\ pr^+\ c$		1,412	1,412
Black, purple	$bb\ prpr\ c^+c$	1,383	$b\ pr\ c^+$		1,383	1,383
Purple	$b^+b\ prpr\ c^+c$	60	$b^+pr\ c^+$	60	60	
Black, curved	$bb\ pr^+pr\ cc$	72	$b\ pr^+\ c$	72	72	
Total		15,000		887	2,927	3,550
Percent				5.9	19.5	23.7

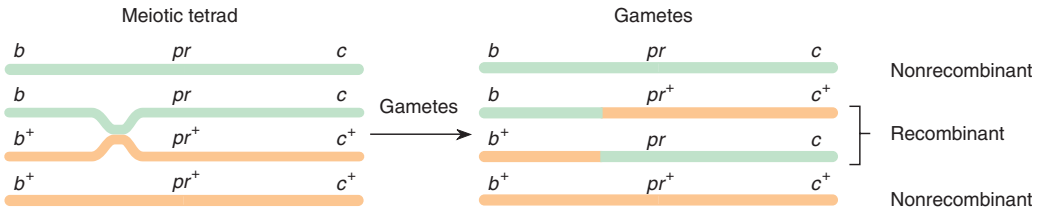


Figure 6.7 Results of a crossover between the black and purple loci in *Drosophila.*

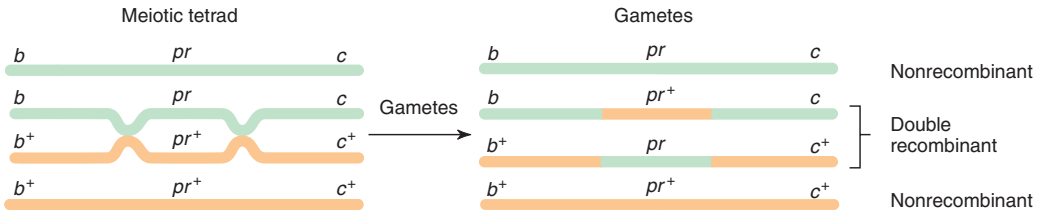


Figure 6.8 Results of a double crossover in the *b pr c region of the Drosophila* chromosome.

gion. The reason they are not counted is simply that if we observed only the end loci of this chromosomal segment, we would not detect the double crossovers; the first one of the two crossovers causes a recombination between the two end loci, whereas the second one returns these outer loci to their original configuration (see fig. 6.8). If we took the 3,550 recombinants between *b* and *c* and added in twice the total of the double recombinants, 264, we would get a total of 3,814. This is 25.4 map units, which is the more precise figure we calculated before. The farther two loci are apart on a chromosome, the more double crossovers occur between them. Double crossovers tend to mask recombinants, as in our example, so that distantly linked loci usually appear closer than they really are. Thus, the most accurate map distances are those established on very closely linked loci. In other words, summed short distances are more accurate than directly measured larger distances.

The results of the previous experiment show that we can obtain at least two map distances between any two loci: measured and actual. Measured map distance between two loci is the value obtained from a two-point cross. Actual map distance is an idealized, more accurate value obtained from summing short distances between many intervening loci. We obtain the short distances from crosses involving other loci between the original two. When we plot measured map distance against actual map distance, we obtain the curve in figure 6.10. This curve is called a **mapping function.** This graph is of both practical and theoretical value. Pragmatically, it allows

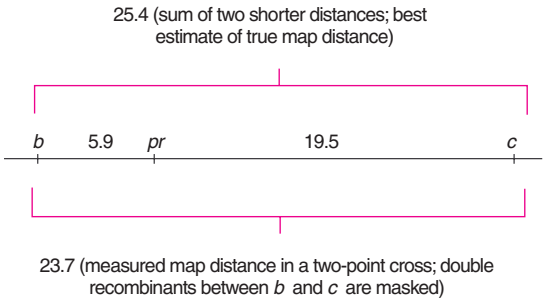


Figure 6.9 Tentative map of the black, purple, and curved chromosome in *Drosophila.* Numbers are map units (centimorgans).

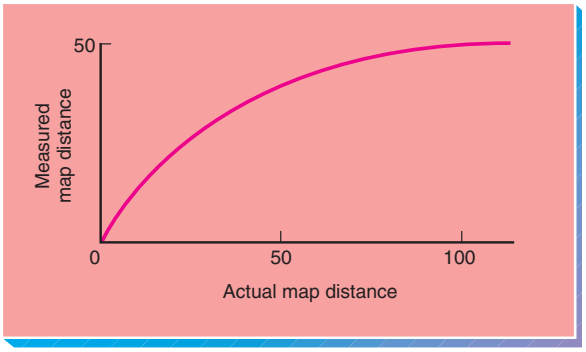


Figure 6.10 Mapping function.

us to convert a measured map distance into a more accurate one. Theoretically, it shows that measured map distance never exceeds 50 map units in any one cross. Multiple crossovers reduce the apparent distance between two loci to a maximum of 50 map units, the value that independent assortment produces (50% parentals, 50% recombinants).

Gene Order

Although we performed the previous analysis merely assuming that *pr* was in the middle, the data in table 6.1 confirm our original assumption that the gene order is *b pr c.* Of the four pairs of reciprocal phenotypic classes in table 6.1, one pair has the highest frequency (5,701 and 5,617) and one pair has the lowest (60 and 72). The pair with highest frequency is the nonrecombinant group. The one with the lowest frequency is the double recombinant group, the one in which only the middle locus has been changed from the parental arrangement. A comparison of either of the double recombinant classes with either of the nonrecombinant classes shows the gene that is in the middle and, therefore, the gene order. In other words, the data allow us to determine gene order. Since b^+ pr^+ c^+ was one of the nonrecombinant gametes, and b^+ *pr* c^+ was one of the double recombinant gametes, *pr* stands out as the changed locus, or the one in the middle. In a similar manner, comparing *b* pr^+ *c* with *b pr c* would also point to *pr* as the inside locus (or **inside marker**). So would comparing b^+ *pr* c^+ with *b pr c* or *b* pr^+ *c* with b^+ *pr* c^+. In each case, the middle locus, *pr,* displays the different pattern, whereas the allelic arrangements of the outside markers, *b* and *c,* behave in concert.

If this seems confusing, simply compare the double crossovers and nonrecombinants to find one of each in which two alleles are identical. For example, the double recombinant b^+ *pr* c^+ and the nonrecombinant b^+ pr^+ c^+ share the b^+ and c^+ alleles. The *pr* locus is mutant in one case and wild-type in the other. Hence, *pr* is the locus in the middle.

From the data in table 6.1, we can confirm the association of alleles in the trihybrid parent. That is, since the data came from testcrossing a trihybrid, the allelic configuration in that trihybrid is reflected in the nonrecombinant classes of offspring. In this case, one is the result of a b^+ pr^+ c^+ gamete, the other, of a *b pr c* gamete. Thus, the trihybrid had the genotype *b pr c*/b^+ pr^+ c^+: all alleles were in the *cis* configuration.

Coefficient of Coincidence

The next question in our analysis of this three-point cross is, are crossovers occurring independently of each other? That is, does the observed number of double recombinants equal the expected number? In the example, there were 132/15,000 double crossovers, or 0.88%. The expected number is based on the independent occurrence of crossing over in the two regions measured. That is, 5.9% of the time there is a crossover in the *b–pr* region, which we can express as a probability of occurrence of 0.059. Similarly, 19.5% of the time there is a crossover in the *pr–c* region, or a probability of occurrence of 0.195. A double crossover should occur as a product of the two probabilities: $0.059 \times 0.195 = 0.0115$. This means that 1.15% of the gametes (1.15% of 15,000 = 172.5) should be double recombinants. In our example, the observed number of double recombinant offspring is lower than expected (132 observed, 172.5 expected). This implies a **positive interference,** in which the occurrence of the first crossover reduces the chance of the second. We can express this as a **coefficient of coincidence,** defined as

$$\frac{\text{observed number of double recombinants}}{\text{expected number of double recombinants}}$$

In the example, the coefficient of coincidence is 132/172.5 = 0.77. In other words, only 77% of the expected double crossovers occurred. Sometimes we express this reduced quantity of double crossovers as the *degree of interference,* defined as

$$\text{interference} = 1 - \text{coefficient of coincidence}$$

In our example, the interference is 23%.

It is also possible to have **negative interference,** in which we observe more double recombinants than expected. In this situation, the occurrence of one crossover seems to enhance the probability that crossovers will occur in adjacent regions.

Another Example

Let us work out one more three-point cross, in which neither the middle gene nor the *cis-trans* relationship of the alleles in the trihybrid F_1 parent is given. On the third chromosome of *Drosophila,* hairy (*h*) causes extra bristles on the body, thread (*th*) causes a thread-shaped arista (antenna tip), and rosy (*ry*) causes the eyes to be reddish brown. All three traits are recessive. Trihybrid females were testcrossed; the phenotypes from one thousand offspring are listed in table 6.2. At this point, it is possible to use the data to determine the parental genotypes (the P_1 generation, assuming that they were homozygotes), the gene order, the map distances, and the coefficient of coincidence. The table presents the data in no particular order, as an experimenter might have recorded them. Phenotypes are tabulated and, from these, the genotypes can be reconstructed. Notice that the data can be put into the form found in table 6.1;

Table 6.2 **Offspring from a Trihybrid ($h^+h\ ry^+ry\ th^+th$) Testcross ($x\ hh\ ryry\ thth$) in *Drosophila***

Phenotype	Genotype (order unknown)	Number
Thread	$h^+ry^+th/h\ ry\ th$	359
Rosy, thread	$h^+ry\ th/h\ ry\ th$	47
Hairy, rosy, thread	$h\ ry\ th/h\ ry\ th$	4
Hairy, thread	$h\ ry^+th/h\ ry\ th$	98
Rosy	$h^+ry\ th^+/h\ ry\ th$	92
Hairy, rosy	$h\ ry\ th^+/h\ ry\ th$	351
Wild-type	$h^+ry^+th^+/h\ ry\ th$	6
Hairy	$h\ ry^+th^+/h\ ry\ th$	43

Table 6.3 **Data from Table 6.2 Arranged to Show Recombinant Regions**

Trihybrid's Gamete	Number	*h–th*	*th–ry*	*h–ry*
$h^+th\ ry^+$	359			
$h\ th^+ry$	351			
$h\ th\ ry^+$	98	98		98
h^+th^+ry	92	92		92
$h^+th\ ry$	47		47	47
$h\ th^+ry^+$	43		43	43
$h\ th\ ry$	4	4	4	
$h^+th^+ry^+$	6	6	6	
Total	1,000	200	100	280

we see a large reciprocal set (359 and 351), a small reciprocal set (4 and 6), and large and small intermediate sets (98 and 92, 47 and 43).

From the data presented, is it obvious that the three loci are linked? The pattern, as just mentioned, is identical to that of the previous example, in which the three loci were linked. (What pattern would appear if two of the loci were linked and one assorted independently? See problem 6 at the end of the chapter.) Next, what is the allelic arrangement in the trihybrid parent? The offspring with the parental, or nonrecombinant, arrangements are the reciprocal pair in highest frequency. Table 6.2 shows that thread and hairy, rosy offspring are the nonrecombinants. Thus, the nonrecombinant gametes of the trihybrid F_1 parent were $h\ ry\ th^+$ and $h^+\ ry^+\ th$, which is the allelic arrangement of the trihybrid with the actual order still unknown—$h\ ry\ th^+/h^+\ ry^+\ th$. (What were the genotypes of the parents of this trihybrid, assuming they were homozygotes?) Continuing, which gene is in the middle? From table 6.2, we know that $h\ ry\ th$ and $h^+\ ry^+\ th^+$ are the double recombinant gametes of the trihybrid parent because they occur in such low numbers. Comparison of these chromosomes with either of the nonrecombinant chromosomes ($h^+\ ry^+\ th$ or $h\ ry\ th^+$) shows that the thread (*th*) locus is in the middle. We now know that the original trihybrid had the following chromosomal composition: $h\ th^+\ ry/h^+\ th\ ry^+$. The *h* and *ry* alleles are in the *cis* configuration, with *th* in the *trans* configuration.

We can now compare the chromosome from the trihybrid in each of the eight offspring categories with the parental arrangement and determine the regions that had crossovers. Table 6.3 does this. We can see that the *h–th* distance is 20 map units, the *th–ry* distance is 10 map units, and the apparent *h–ry* distance is 28 map units (fig. 6.11). As in the earlier example, the *h–ry* discrepancy is from not counting the double crossovers twice each: 280 + 2(10) = 300, which is 30 map units and the more accurate figure. Last, we wish to know what the coefficient of coincidence is. The expected occurrence of double recombinants is 0.200 × 0.100 = 0.020, or 2%. Two percent of 1,000 = 20. Thus

$$\text{coefficient of coincidence} = \frac{\text{observed number of double recombinants}}{\text{expected number of double recombinants}} = 10/20 = 0.50$$

Only 50% of the expected double crossovers occurred.

Geneticists have mapped the chromosomes of many eukaryotic organisms from three-point crosses of this type—those of *Drosophila* are probably the most extensively studied. *Drosophila* and other species of flies have giant **polytene** salivary gland chromosomes, which arise as a result of **endomitosis.** In this process,

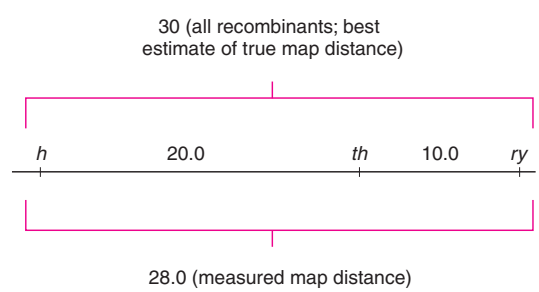


Figure 6.11 Map of the *h th ry* region of the *Drosophila* chromosome, with numerical discrepancy in distances. Numbers are map units (centimorgans).

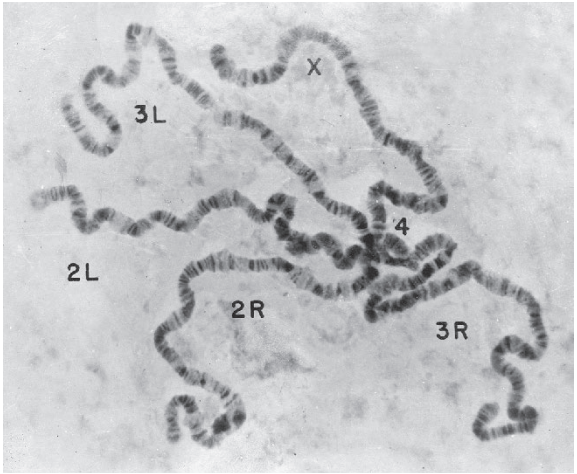


Figure 6.12 Giant salivary gland chromosomes of *Drosophila.* X, 2, 3, and 4 are the four nonhomologous chromosomes. *L* and *R* indicate the *left* and *right* arms (in relation to the centromere). The dark bands are chromomeres. (B. P. Kaufman, "Induced Chromosome Rearrangements in *Drosophila melanogaster,*" *Journal of Heredity,* 30:178–90, 1939. Reproduced by permission of Oxford University Press.)

the chromosomes replicate, but the cell does not divide. In the salivary gland of the fruit fly, homologous chromosomes synapse and then replicate to make about one thousand copies, forming very thick structures with a distinctive pattern of bands called chromomeres (fig. 6.12). Using methods chapter 8 will discuss, scientists have mapped many loci to particular bands. Part of the *Drosophila* chromosomal map is presented in figure 6.13 (see also box 6.2). Locate the loci we have mapped so far to verify the map distances.

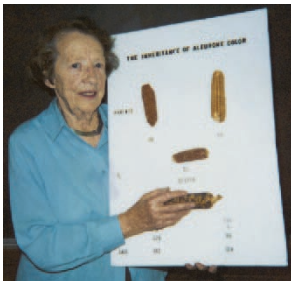
In summary, we know that two or more loci are linked if offspring do not fall into simple Mendelian ratios. Map distances are the percentage of recombinant offspring in a testcross. With three loci, determine the parental (nonrecombinant) and double recombinant groups first. Then establish the locus in the middle, and recast the data in the correct gene order. The most accurate map distances are those obtained by summing shorter distances. Determine a coefficient of coincidence by comparing observed number of double recombinants to expected number.

Cytological Demonstration of Crossing Over

If we are correct that a chiasma during meiosis is the visible result of a physical crossover, then we should be able to demonstrate that genetic crossing over is accompanied by cytological crossing over. That is, the recombination event should entail the exchange of physical parts of homologous chromosomes. This can be demonstrated if we can distinguish between two homologous chromosomes, a technique Creighton and McClintock first used in maize (corn) and Stern first applied to *Drosophila,* both in 1931. We will look at Creighton and McClintock's experiment.

Harriet Creighton and Barbara McClintock worked with chromosome 9 in maize ($n = 10$). In one strain, they found a chromosome with abnormal ends. One end had a knob, and the other had an added piece of chromatin from another chromosome (fig. 6.14). This knobbed chromosome was thus clearly different from its normal homologue. It also carried the dominant colored (*C*) allele and the recessive waxy texture (*wx*) allele. After mapping studies showed that *C* was very close to the knob and *wx* was close to the added piece of chromatin, Creighton and McClintock made the cross shown in figure 6.14. The dihybrid plant with heteromorphic chromosomes was crossed with the normal homomorphic plant (only normal chromosomes) that had the genotype of *c Wx/c wx* (colorless and nonwaxy phenotype). If a crossover occurred during meiosis in the dihybrid in the region between *C* and *wx*, a physical crossover, visible cytologically (under the microscope), should also occur, causing the knob to become associated with an otherwise normal chromosome and the extra piece of chromosome 9 to be associated with a knobless chromosome. Four types of gametes would result (fig. 6.14).

Barbara McClintock (1902–1992). (Courtesy of Cold Spring Harbor Research Library Archives. Photographer, David Miklos.)



Harriet B. Creighton (1909–). (Courtesy of Harriet B. Creighton.)

BOX 6.2

Historical Perspectives

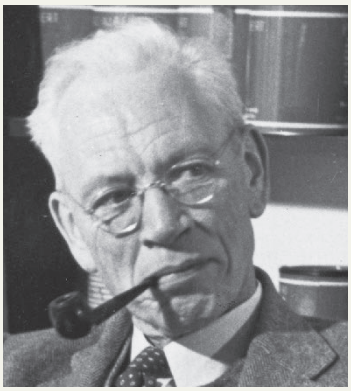
The First Chromosomal Map

The first chromosomal map ever published included just five loci on the X chromosome of *Drosophila melanogaster* (fig. 1). It was published in 1913 by Alfred H. Sturtevant, who began working in Thomas Hunt Morgan's "fly lab" while an undergraduate student at Columbia University. The fly lab included H. J. Muller, later to win a Nobel Prize, and Calvin B. Bridges, whose work on sex determination in *Drosophila* we discussed in the last chapter.

Sturtevant worked with six mutants: yellow body (*y*); white (*w*), eosin (w^e), and vermilion eyes (*v*); and miniature (*m*) and rudimentary wings (*r*). (White and eosin are actually allelic; Sturtevant found no crossing over between the two "loci.") Using crosses similar to the ones we outline in this chapter, he constructed the map shown in figure 1. The map distances we accept today are very similar to the ones he obtained.

Sturtevant's work was especially important at this point because his data supported several basic concepts, including the linear arrangement of genes, which argued for the placement of genes on chromosomes as the only linear structures in the nucleus. Sturtevant also pointed out crossover interference. His summary is clear and succinct:

> It has been found possible to arrange six sex-linked factors in *Drosophila* in a linear series, using the number of crossovers per one hundred cases as an index of the distance between any two factors. This scheme gives consistent results, in the main.
>
> A source of error in predicting the strength of association between untried factors is found in double crossing over. The occurrence of this phenomenon is demonstrated, and it is shown not to occur as often as would be expected from a purely mathematical point of view, but the conditions governing its frequency are as yet not worked out.
>
> These results . . . form a new argument in favor of the chromosome view of inheritance, since they strongly indicate that the factors investigated are arranged in a linear series, at least mathematically.



Alfred H. Sturtevant (1891–1970). (Courtesy of the Archives, California Institute of Technology.)

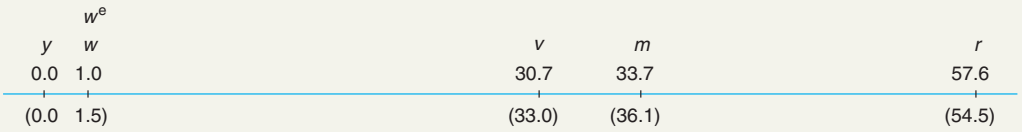


Figure 1 The first chromosomal linkage map. Five loci in *Drosophila melanogaster* are mapped to the X chromosome. The numbers in parentheses are the more accurately mapped distances recognized today. We also show today's allelic designations rather than Sturtevant's original nomenclature. (Data from Sturtevant. "The linear arrangement of six sex-linked factors in *Drosophila,* as shown by their mode of association," *Journal of Experimental Zoology,* 14:43–59, 1913.)

Of twenty-eight offspring examined, all were consistent with the predictions of the Punnett square in figure 6.14. Those of class 8 (lower right box) with the colored, waxy phenotype all had a knobbed interchange chromosome as well as a normal homologue. Those with the colorless, waxy phenotype (class 4) had a knobless interchange chromosome. All of the colored, nonwaxy phenotypes (classes 5, 6, and 7) had a knobbed, normal chromosome, which indicated that only classes 5 and 6 were in the sample. Of the two that were tested, both were *WxWx,* indicating that they were of class 5. The remaining classes (1, 2, and 3) were of the colorless, nonwaxy phenotype. All were knobless. Of those that contained only normal chromosomes, some were

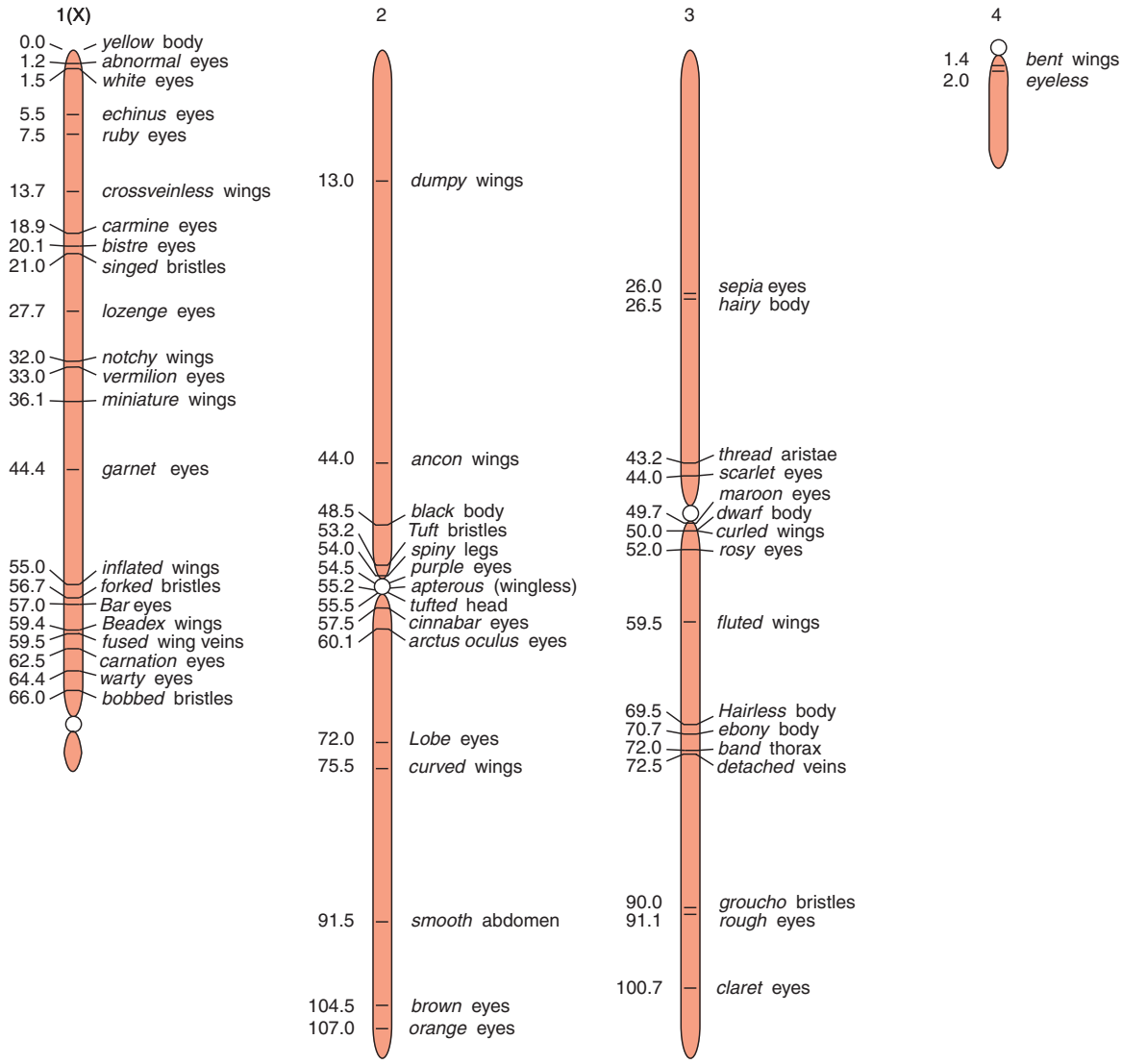


Figure 6.13 Partial map of the chromosomes of *Drosophila melanogaster.* The centromere is marked by an open circle.
(From C. Bridges, "Salavary Chromosome Maps," *Journal of Heredity,* 26:60–64, 1935. Reprinted with permission of Oxford University Press.)

WxWx (class 1) and some were heterozygotes (*Wxwx*, class 2). Of those containing interchange chromosomes, two were heterozygous, representing class 3. Two were homozygous, *WxWx*, yet interchange-normal heteromorphs. These represent a crossover in the region between the waxy locus and the extra piece of chromatin, producing a knobless-*c*-*Wx*-extra-piece chromosome. When combined with a *c*-*Wx*-normal chromosome, these would give these anomalous genotypes. The sample size was not large enough to pick up the reciprocal event. Creighton and McClintock concluded: "Pairing chromosomes, heteromorphic in two regions, have been shown to exchange parts at the same time they exchange genes assigned to these regions."

HAPLOID MAPPING (TETRAD ANALYSIS)

For *Drosophila* and other diploid eukaryotes, the genetic analysis considered earlier in this chapter is referred to as **random strand analysis.** Sperm cells, each of which carry only one chromatid of a meiotic tetrad, unite with

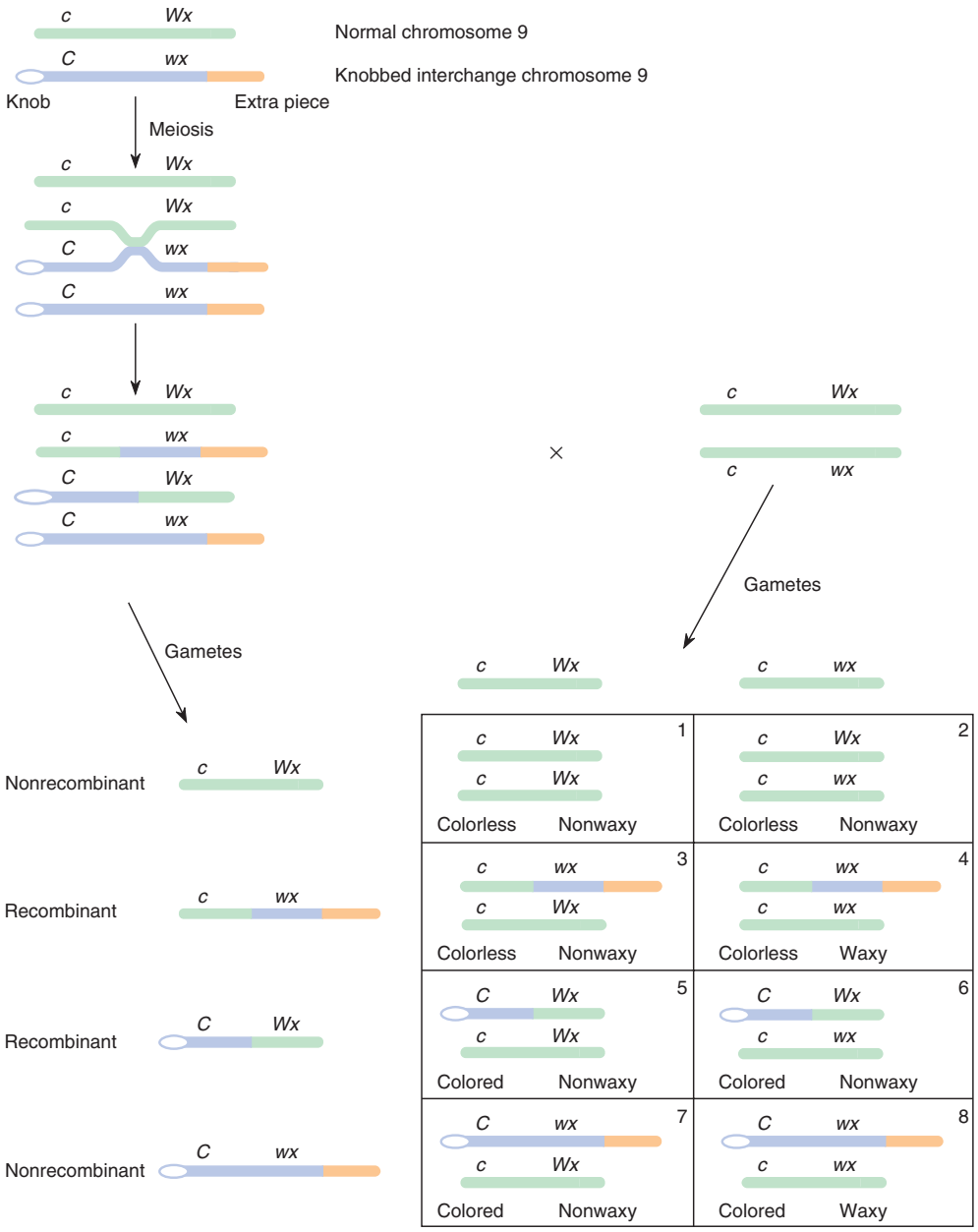


Figure 6.14 Creighton and McClintock's experiment in maize demonstrated that genetic crossover correlates with cytological crossing over.

eggs, which also carry only one chromatid from a tetrad. Thus, zygotes are the result of the random uniting of chromatids.

Fungi of the class Ascomycetes retain the four haploid products of meiosis in a sac called an **ascus.** These organisms provide a unique opportunity to look at the total products of meiosis in a tetrad. Having the four products of meiosis allowed geneticists to determine such basics as the reciprocity of crossing over and the fact that DNA replication occurs before crossing over. Different techniques are used for these analyses. We will look at two fungi, the common baker's yeast, *Saccharomyces cerevisiae,* and pink bread mold, *Neurospora crassa,* both of which retain the products of meiosis as **ascospores.**

Phenotypes of Fungi

At this point, you might wonder what phenotypes fungi such as yeast and *Neurospora* express. In general, microorganisms have phenotypes that fall into three broad categories: colony morphology, drug resistance, and nutritional requirements. Many microorganisms can be cultured in petri plates or test tubes that contain a supporting medium such as agar, to which various substances can be added (fig. 6.15). Wild-type *Neurospora,* the familiar pink bread mold, generally grows in a filamentous form, whereas yeast tends to form colonies. Various mutations exist that change colony morphology. In yeast, the *ade* gene causes the colonies to be red. In *Neurospora,* fluffy (*fl*), tuft (*tu*), dirty (*dir*), and colonial (*col4*) are all mutants of the basic growth form. In addition, wild-type *Neurospora* is sensitive to the sulfa drug sulfonamide, whereas one of its mutants (*Sfo*) actually requires sulfonamide in order to survive and grow. Yeast shows similar sensitivities to antifungal agents.

Nutritional-requirement phenotypes provide great insight not only into genetic analysis but also into the biochemical pathways of metabolism, as mentioned in chapter 2. Wild-type *Neurospora* can grow on a medium containing only sugar, a nitrogen source, some organic acids and salts, and the vitamin biotin. This is referred to as **minimal medium.** However, several different mutant types, or strains, of **Neurospora** cannot grow on this minimal medium until some essential nutrient is added. For example, one mutant strain will not grow on minimal medium, but will grow if one of the amino acids, arginine, is added (fig. 6.16). From this we can infer that the wild-type has a normal, functional enzyme in the synthetic pathway of arginine. The arginine-requiring mutant has an allele that specifies an enzyme that is incapable of converting one of the intermediates in the pathway directly into arginine or into one of the precursors to arginine. We can see that if the synthetic pathway is long, many different loci may have alleles that cause the strain to require arginine (fig. 6.17). This, in fact, happens, and the different loci are usually named arg_1, arg_2, and so on. There are numerous biosynthetic pathways in yeast and *Neurospora,* and mutants exhibit many different nutritional requirements. Mutants can be induced experimentally by radiation or by chemicals and other treatments. These, then, are the tools we use to analyze and map the chromosomes of microorganisms, including yeast and *Neurospora.* These techniques are expanded on in the next chapter.

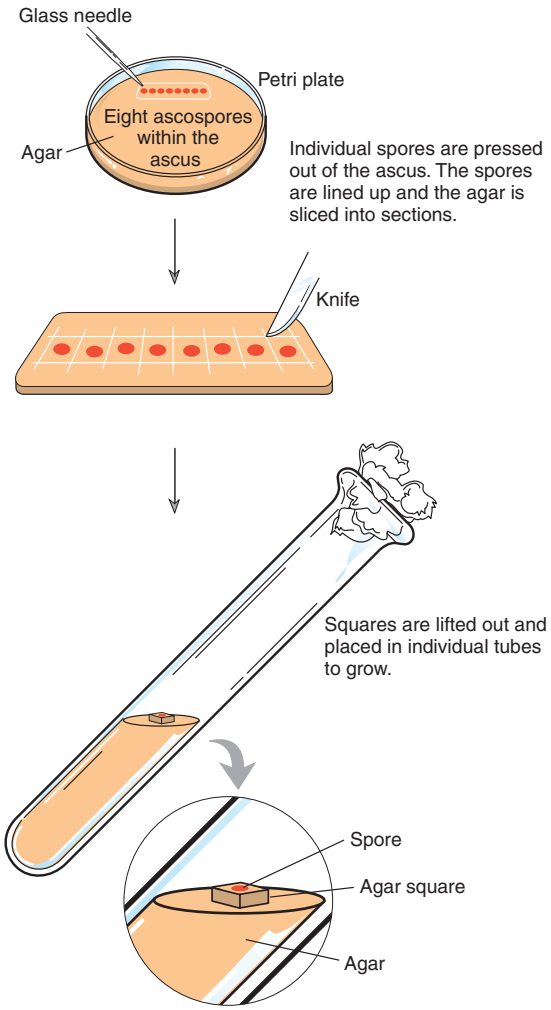


Figure 6.15 Spore isolation technique in *Neurospora.*

Unordered Spores (Yeast)

Baker's, or budding, yeast, *Saccharomyces cerevisiae,* exists in both a haploid and diploid form (fig. 6.18). The haploid form usually forms under nutritional stress (starvation). When better conditions return, haploid cells of the two sexes, called **a** and α **mating types,** fuse to form the diploid. (Mating types are generally the result of a one-locus, two-allele genetic system that determines that only opposite mating types can fuse. We discuss this system in more detail in chapter 16.) The haploid is again established by meiosis under starvation conditions. In yeast, all the products of meiosis are contained in the ascus. Let us look at a mapping problem, using the *a* and *b* loci for convenience.

When an *ab* spore (or gamete) fuses with an a^+b^+ spore (or gamete), and the diploid then undergoes meiosis, the spores can be isolated and grown as haploid colonies, which are then observed for the phenotypes the two loci control. Only three patterns can occur (table 6.4).

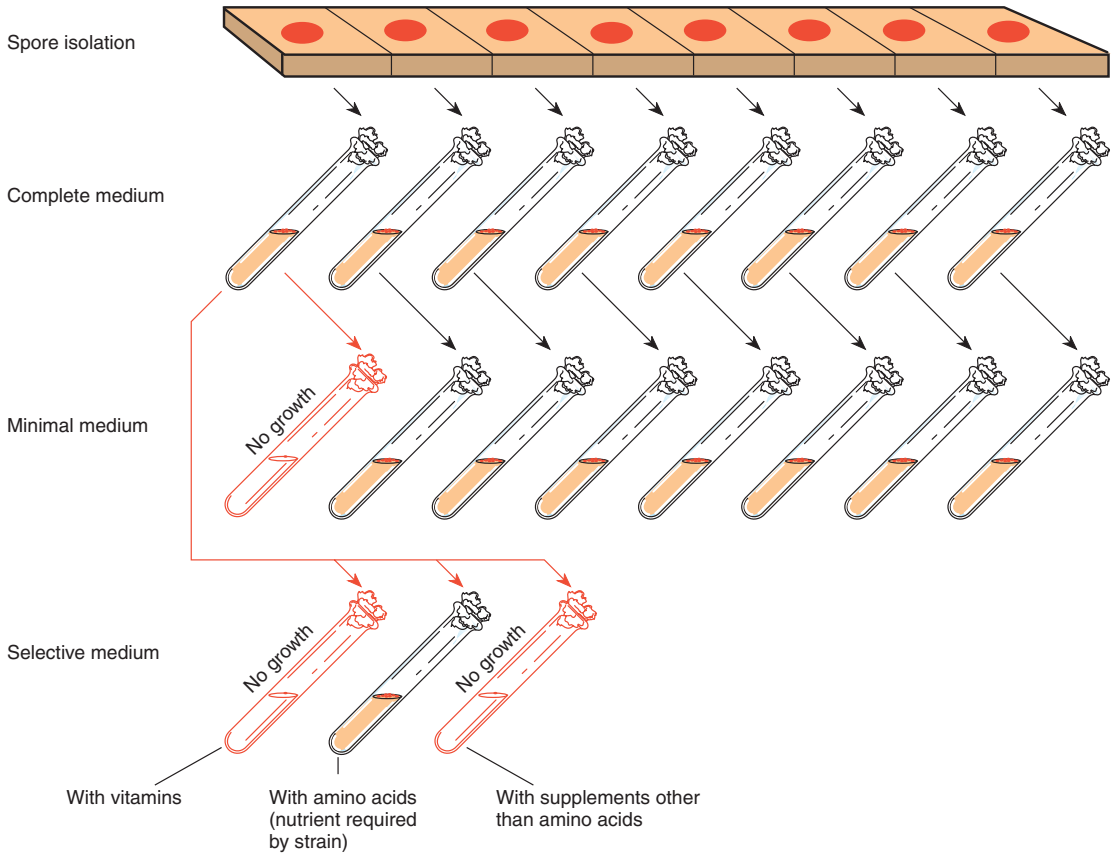


Figure 6.16 Isolation of nutritional-requirement mutants in *Neurospora.*

Class 1 has two types of spores, which are identical to the parental haploid spores. This ascus type is, therefore, referred to as a **parental ditype (PD).** The second class also has only two spore types, but they are recombinants. This ascus type is referred to as a **nonparental ditype (NPD).** The third class has all four possible spore types and is referred to as a **tetratype (TT).**

All three ascus types can be generated whether or not the two loci are linked. As figure 6.19 shows, if the loci are linked, parental ditypes come from the lack of a crossover, whereas nonparental ditypes come about from four-strand double crossovers (double crossovers involving all four chromatids). We should thus expect parental ditypes to be more numerous than nonparental ditypes for linked loci. However, if the loci are not linked, both parental and nonparental ditypes come about through independent assortment—they should occur in equal frequencies. We can therefore determine whether the loci are linked by comparing parental ditypes and nonparental ditypes. In table 6.4, the parental ditypes greatly outnumber the nonparental ditypes; the two loci are, therefore, linked. What is the map distance between the loci?

A return to figure 6.19 shows that in a nonparental ditype, all four chromatids are recombinant, whereas in a tetratype, only half the chromatids are recombinant. Remembering that 1% recombinant offspring equals 1 map unit, we can use the following formula:

$$\text{map units} = \frac{(1/2)\text{ the number of TT asci} + \text{the number of NPD asci}}{\text{total number of asci}} \times 100$$

Thus, for the data of table 6.4,

$$\text{map units} = \frac{(1/2)20 + 5}{100} \times 100 = \frac{10 + 5}{100} \times 100 = 15$$

Ordered Spores (Neurospora)

Unlike yeast, *Neurospora* has ordered spores; *Neurospora's* life cycle is shown in figure 6.20. Fertilization takes place within an immature fruiting body after a

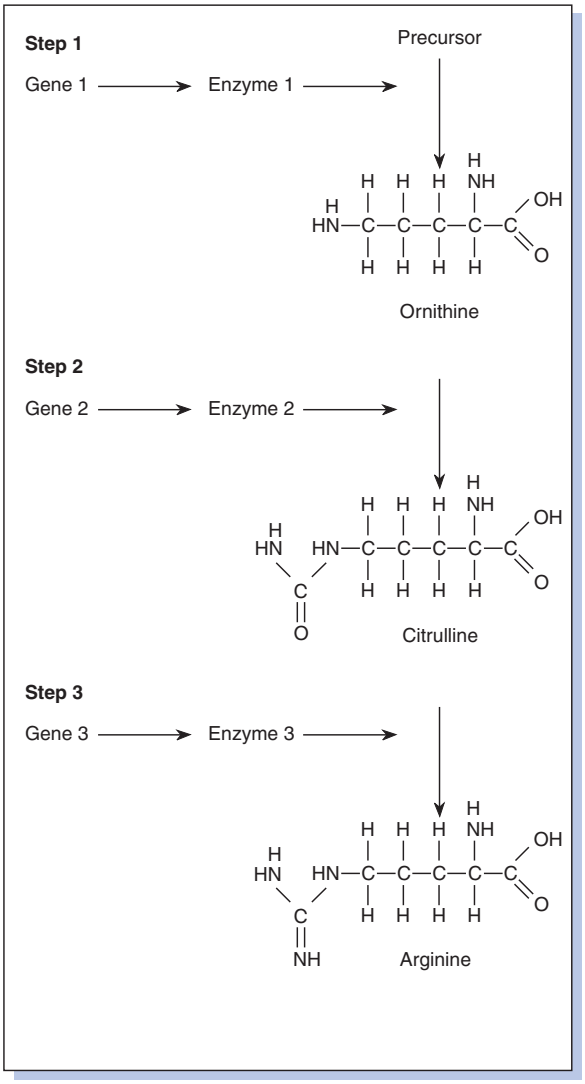


Figure 6.17 Arginine biosynthetic pathway of *Neurospora.*

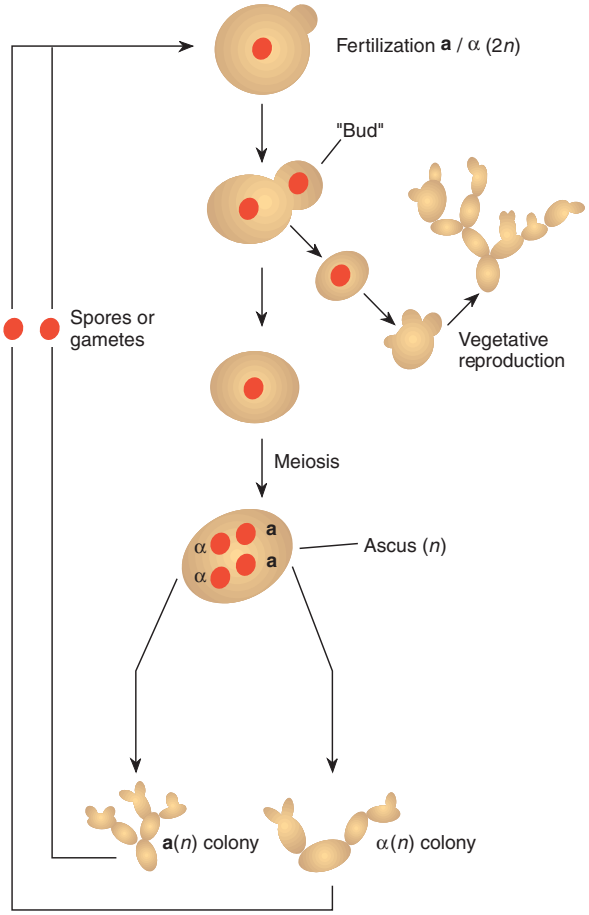


Figure 6.18 Life cycle of yeast. Mature cells are mating types **a** or α; *n* is the haploid stage; 2*n* is diploid.

Table 6.4 **The Three Ascus Types in Yeast Resulting from Meiosis in a Dihybrid, aa^+bb^+**

1 (PD)	2 (NPD)	3 (TT)
ab	ab^+	*ab*
ab	ab^+	ab^+
a^+b^+	a^+b	a^+b
a^+b^+	a^+b	a^+b^+
75	5	20

spore or filament of one mating type contacts a special filament extending from the fruiting body of the opposite mating type (mating types are referred to as *A* and *a*). The zygote's nucleus undergoes meiosis without any intervening mitosis. Unlike yeast, *Neurospora* does not have a diploid phase in its life cycle. Rather, it undergoes meiosis immediately after the diploid nuclei form.

Since the *Neurospora* ascus is narrow, the meiotic spindle is forced to lie along the cell's long axis. The two nuclei then undergo the second meiotic division, which is also oriented along the long axis of the ascus. The result is that the spores are ordered according to their centromeres (fig. 6.21). That is, if we label one centromere *A* and the other *a,* for the two mating types, a tetrad at meiosis I will consist of one *A* and one *a* centromere. At the end of meiosis in *Neurospora,* the four ascospores are in the order *A A a a* or *a a A A* in regard to centromeres. (We talk more simply of centromeres rather than chromosomes or chromatids because of the complications that

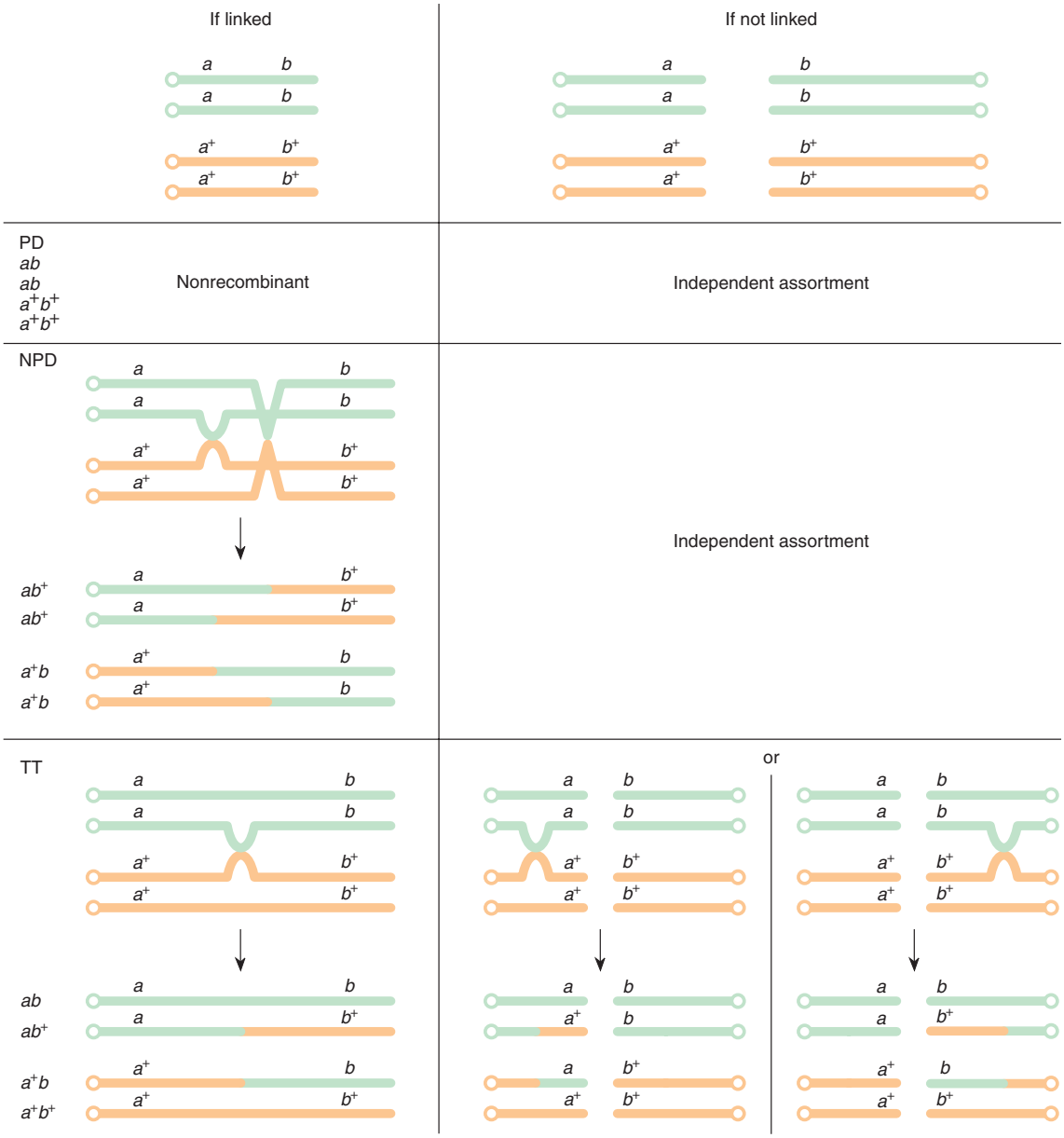


Figure 6.19 Formation of parental ditype (PD), nonparental ditype (NPD), and tetratype (TT) asci in a dihybrid yeast by linkage or independent assortment at meiosis. Open circles are centromeres.

crossing over adds. A type *A* centromere is always a type *A* centromere, whereas, due to crossing over, a chromosome attached to that centromere may be partly from the type *A* parent and partly from the type *a* parent.)

Before the ascospores mature in *Neurospora,* a mitosis takes place in each nucleus so that four pairs rather than just four spores are formed. In the absence of phenomena such as mutation or gene conversion, to be discussed later in the book, pairs are always identical (fig. 6.21). As we will see in a moment, because of the ordered spores, we can map loci in *Neurospora* in relation to their centromeres.

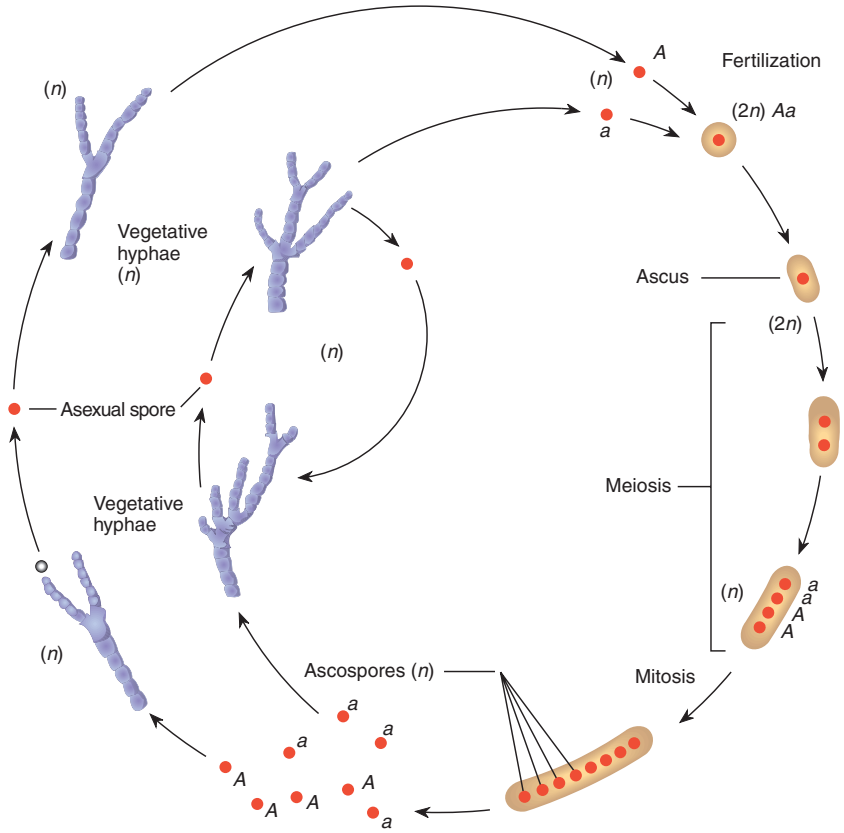


Figure 6.20 Life cycle of *Neurospora*. *A* and *a* are mating types; *n* is a haploid stage; 2*n* is diploid.

First and Second Division Segregation

Recall that there is a 4:4 segregation of the centromeres in the ascus of *Neurospora*. Two kinds of patterns appear among the loci on these chromosomes. These patterns depend on whether there was a crossover between the locus and its centromere (fig. 6.22). If there was no crossover between the locus and its centromere, the allelic pattern is the same as the centromeric pattern, which is referred to as **first-division segregation (FDS),** because the alleles separate from each other at meiosis I. If, however, a crossover has occurred between the locus and its centromere, patterns of a different type emerge (2:4:2 or 2:2:2:2), each of which is referred to as **second-division segregation (SDS).** Because the spores are ordered, the centromeres always follow a first-division segregation pattern. Hence, we should be able to map the distance of a locus to its centromere. Under the simplest circumstances (fig. 6.22), every second-division segregation configuration has four recombinant and four nonrecombinant chromatids (spores). Thus, half of the chromatids (spores) in a second-division segregation ascus are recombinant. Therefore, since 1% recombinant chromatids equal 1 map unit,

$$\text{map distance} = \frac{(1/2)\text{ the number of SDS asci}}{\text{total number of asci}} \times 100$$

An example using this calculation appears in table 6.5.

Three-point crosses in *Neurospora* can also be examined. Let us map two loci and their centromere. For simplicity, we will use the *a* and *b* loci. Dihybrids are formed from fused mycelia ($ab \times a^+b^+$), which then undergo meiosis. One thousand asci are analyzed, keeping the spore order intact. Before presenting the data, we should consider how to group them. Since each locus can show six different patterns (fig. 6.22), two loci scored together should give thirty-six possible spore arrangements (6 × 6). Some thought, however, tells us that many of these patterns are really random variants of each other. The tetrad in meiosis is a three-dimensional entity rather than a flat, four-rod object, as it is usually drawn. At the first meiotic division, either centromere can go to the left *or* the right, and when centromeres split at the second mei-

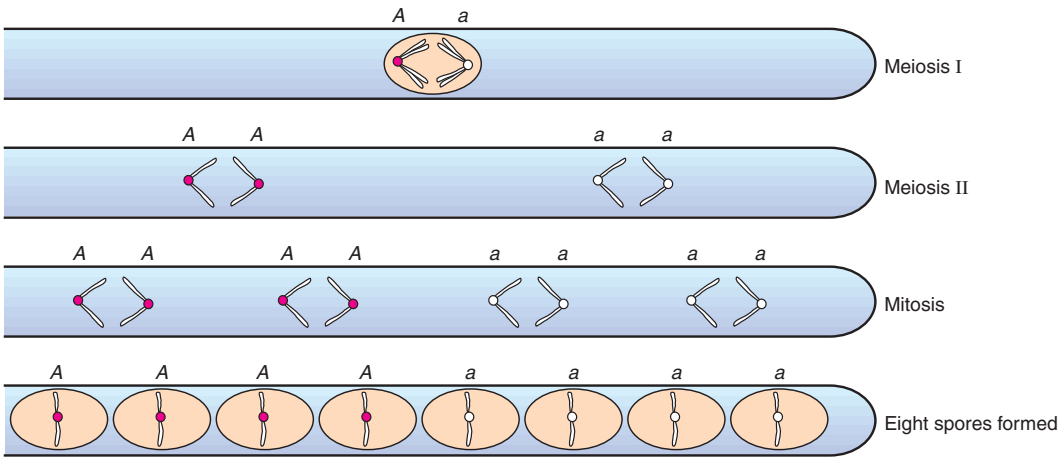


Figure 6.21 Meiosis in *Neurospora.* Although *Neurospora* has seven pairs of chromosomes at meiosis, only one pair is shown. *A* and *a,* the two mating types, represent the two centromeres of the tetrad.

otic division, movement within the future half-ascus (the four spores to the left or the four spores to the right) is also random. Thus, one genetic event can produce up to eight "different" patterns. For example, consider the arrangements figure 6.23 shows, in which a crossover occurs between the *a* and *b* loci. All eight arrangements, producing the ascus patterns of table 6.6, are equally likely. The thirty-six possible patterns then reduce to only the seven unique patterns shown in table 6.7. Note also that these asci can be grouped into the three types of asci found in yeast with unordered spores: parental ditypes, nonparental ditypes, and tetratypes. Had we not had the order of the spores from the asci, that would, in fact, be the only way we could score the asci (see the bottom of table 6.7).

Gene Order

We can now determine the distance from each locus to its centromere and the linkage arrangement of the loci if they are both linked to the same centromere. We can establish by inspection that the two loci are linked to each

Table 6.5 **Genetic Patterns Following Meiosis in an a^+a Heterozygous *Neurospora* (Ten Asci Examined)**

Spore Number	Ascus Number									
	1	2	3	4	5	6	7	8	9	10
1	a	a	a^+	a	a	a^+	a	a^+	a^+	a^+
2	a	a	a^+	a	a	a^+	a	a^+	a^+	a^+
3	a	a	a^+	a^+	a^+	a^+	a	a	a	a^+
4	a	a	a^+	a^+	a^+	a^+	a	a	a	a^+
5	a^+	a^+	a	a^+	a	a	a^+	a	a^+	a
6	a^+	a^+	a	a^+	a	a	a^+	a	a^+	a
7	a^+	a^+	a	a	a^+	a	a^+	a^+	a	a
8	a^+	a^+	a	a	a^+	a	a^+	a^+	a	a
	FDS	FDS	FDS	SDS	SDS	FDS	FDS	SDS	SDS	FDS

Note: Map distance (*a* locus to centromere) = (1/2)% SDS
= (1/2) 40%
= 20 map units

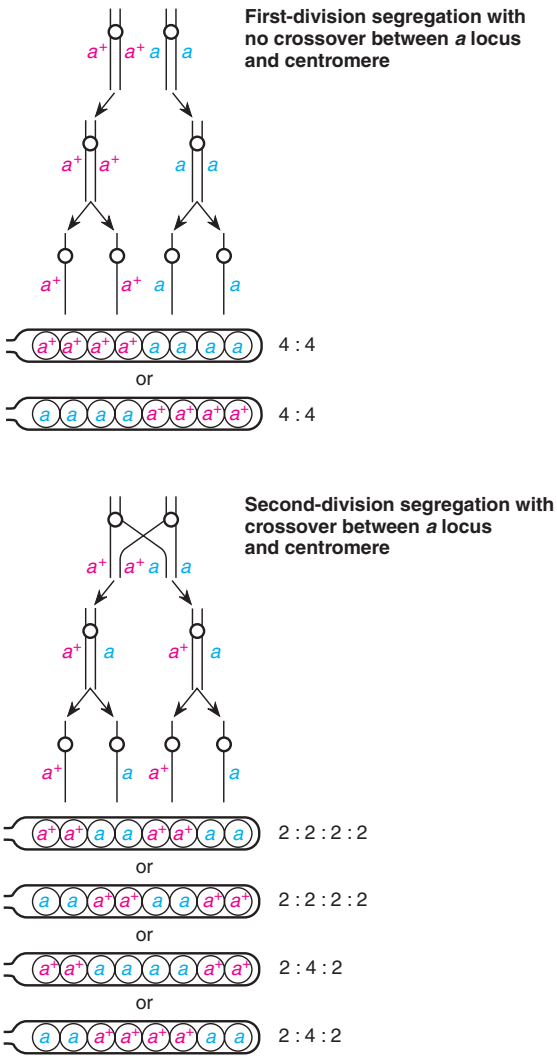


Figure 6.22 The six possible *Neurospora* ascospore patterns in respect to one locus.

Table 6.6 **Eight of the Thirty-Six Possible Spore Patterns in *Neurospora* Scored for Two Loci, *a* and *b* (All Random Variants of the Same Genetic Event)**

Spore Number	Ascus Number 1	2	3	4	5	6	7	8
1	ab	ab^+	ab	ab^+	a^+b^+	a^+b^+	a^+b	a^+b
2	ab	ab^+	ab	ab^+	a^+b^+	a^+b^+	a^+b	a^+b
3	ab^+	ab	ab^+	ab	a^+b	a^+b	a^+b^+	a^+b^+
4	ab^+	ab	ab^+	ab	a^+b	a^+b	a^+b^+	a^+b^+
5	a^+b	a^+b	a^+b^+	a^+b^+	ab^+	ab	ab^+	ab
6	a^+b	a^+b	a^+b^+	a^+b^+	ab^+	ab	ab^+	ab
7	a^+b^+	a^+b^+	a^+b	a^+b	ab	ab^+	ab	ab^+
8	a^+b^+	a^+b^+	a^+b	a^+b	ab	ab^+	ab	ab^+

Table 6.7 **The Seven Unique Classes of Asci Resulting from Meiosis in a Dihybrid *Neurospora*, ab/a^+b^+**

Spore Number	Ascus Number 1	2	3	4	5	6	7
1	ab	ab^+	ab	ab	ab	ab^+	ab
2	ab	ab^+	ab	ab	ab	ab^+	ab
3	ab	ab^+	ab^+	a^+b	a^+b^+	a^+b	a^+b^+
4	ab	ab^+	ab^+	a^+b	a^+b^+	a^+b	a^+b^+
5	a^+b^+	a^+b	a^+b^+	a^+b^+	a^+b^+	a^+b	a^+b
6	a^+b^+	a^+b	a^+b^+	a^+b^+	a^+b^+	a^+b	a^+b
7	a^+b^+	a^+b	a^+b	ab^+	ab	ab^+	ab^+
8	a^+b^+	a^+b	a^+b	ab^+	ab	ab^+	ab^+
	729	2	101	9	150	1	8
SDS for *a* locus				9	150	1	8
SDS for *b* locus			101		150	1	8
Unordered:	PD	NPD	TT	TT	PD	NPD	TT

other—and therefore to the same centromere—by examining classes 1 (parental ditype) and 2 (nonparental ditype) in table 6.7. If the two loci are unlinked, these two categories would represent two equally likely alternative events when no crossover takes place. Since category 1 represents almost 75% of all the asci, we can be sure the two loci are linked.

To determine the distance of each locus to the centromere, we calculate one-half the percentage of second-division segregation patterns for each locus. For the *a* locus, classes 4, 5, 6, and 7 are second-division segregation patterns. For the *b* locus, classes 3, 5, 6, and 7 are second-division segregation patterns. Therefore, the distances to the centromere, in map units, for each locus are

$$\text{for locus } a\text{: } (1/2)\,\frac{9 + 150 + 1 + 8}{1{,}000} \times 100$$

$$= 8.4 \text{ centimorgans}$$

$$\text{for locus } b\text{: } (1/2)\,\frac{101 + 150 + 1 + 8}{1{,}000} \times 100$$

$$= 13.0 \text{ centimorgans}$$

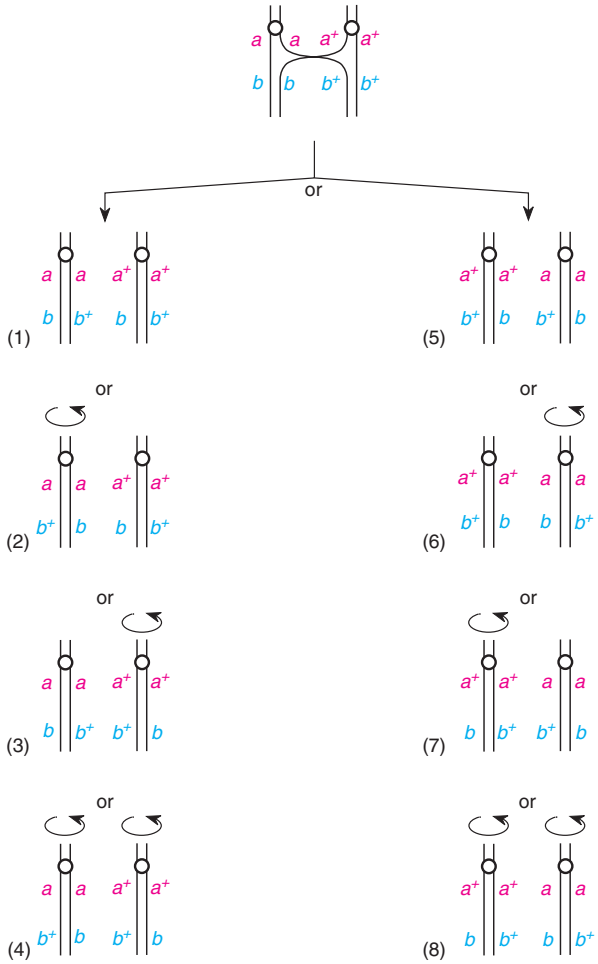


Figure 6.23 The eight random arrangements possible when a single crossover occurs between the *a* and *b* loci in *Neurospora* (see table 6.6). *Circular arrows* represent the rotation of a centromere from its position in the original configuration.

It should now be possible to describe exactly what type of crossover event produced each of the seven classes in table 6.7.

Unfortunately, these two distances do not provide a unique determination of gene order. In figure 6.24, we see that two alternatives are possible: one has a map distance between the loci of 21.4 map units; the other has 4.6 map units between loci. How do we determine which of these is correct? The simplest way is to calculate the *a*–*b* distance using the unordered spore information. That is, the map distance is

$$\text{map units} = \frac{(1/2)\text{ the number of TT asci} + \text{the number of NPD asci}}{\text{total number of asci}} \times 100$$

$$= \frac{(1/2)118 + 3}{1{,}000} \times 100 = 6.2$$

Since 6.2 map units is much closer to the *a*–*b* distance expected if both loci are on the same side of the centromere, we accept alternative 2 in figure 6.24.

A second way to choose between the alternatives in figure 6.24 is to find out what happens to the *b* locus when a crossover occurs between the *a* locus and its centromere. If the order in alternative 1 is correct, crossovers between the *a* locus and its centromere should have no effect on the *b* locus; if 2 is correct, most of the crossovers that move the *a* locus in relation to its centromere should also move the *b* locus.

Asci classes 4, 5, 6, and 7 include all the SDS patterns for the *a* locus. Of 168 asci, 150 (class 5) have similar SDS patterns for the *b* locus. Thus, 89% of the time, a crossover between the *a* locus and its centromere is also a crossover between the *b* locus and its centromere—compelling evidence in favor of alternative 2. (What form would the data take if alternative 1 were correct?)

In summary, mapping by tetrad analysis proceeds as follows. For both ordered and unordered spores, linkage is indicated by an excess of parental ditypes over nonparental ditypes. For unordered spores (yeast), the distance between two loci is one-half the number of

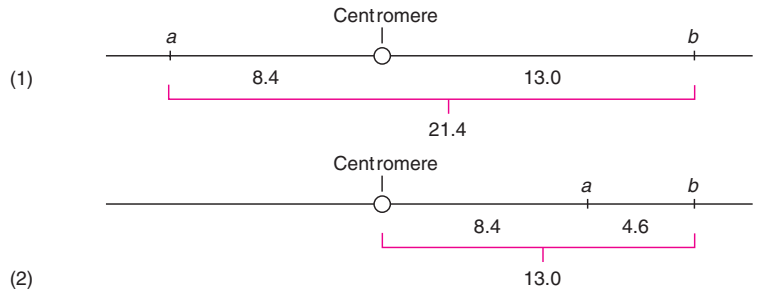


Figure 6.24 Two possible arrangements of the *a* and *b* loci and their centromere. Distances are in map units.

tetratypes plus the number of nonparental ditypes, all divided by the total number of asci, expressed as a percentage. For ordered spores (*Neurospora*), the distance from a locus to its centromere is one-half the percentage of second-division segregants. Mapping the distance between two loci is similar to the process in unordered spores.

SOMATIC (MITOTIC) CROSSING OVER

Crossing over is known to occur in somatic cells as well as during meiosis. It apparently occurs when two homologous chromatids come to lie next to each other and breakage and reunion follow, most likely as a consequence of DNA repair (see chapter 12). Unlike in meiosis, no synaptonemal complex forms. The occurrence of mitotic crossing over is relatively rare. In the fungus *Aspergillus nidulans,* mitotic crossing over occurs about once in every one hundred cell divisions.

Mitotic recombination was discovered in 1936 by Curt Stern, who noticed the occurrence of *twin spots* in fruit flies that were dihybrid for the yellow allele for body color (*y*) and the singed allele (*sn*) for bristle morphology (fig. 6.25). A twin spot could be explained by mitotic crossing over between the *sn* locus and its centromere (fig. 6.26). A crossover in the *sn*–*y* region would produce only a yellow spot, whereas a double crossover, one between *y* and *sn* and the other between *sn* and the centromere, would produce only a singed spot. (Verify this for yourself.) These three phenotypes were found in the relative frequencies expected. That is, given that the gene locations are drawn to scale in figure 6.26, we would expect double spots to be most common, followed by yellow spots, with singed spots rarest of all because they require a double crossover. This in fact occurred, and no other obvious explanation was consistent with these facts. Mitotic crossing over has been used in fungal genetics as a supplemental, or even a primary, method for determining linkage relations. Although gene orders are consistent between mitotic and meiotic mapping, relative distances are usually not, which is not totally unexpected. We know that neither meiotic nor mitotic crossing over is uniform along a chromosome. Apparently, the factors that cause deviation from uniformity differ in the two processes.

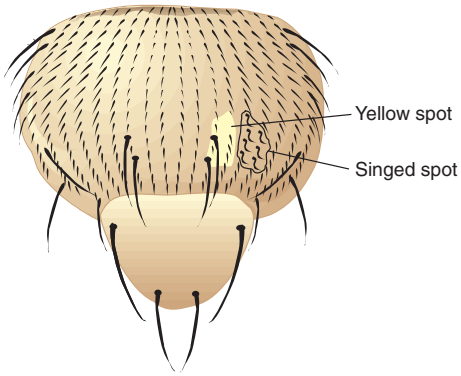
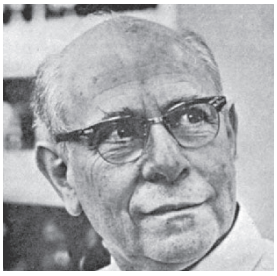


Figure 6.25 Yellow and singed twin spots on the thorax of a female *Drosophila.*



Curt Stern (1902–1981)
(Courtesy of the Science Council of Japan.)

HUMAN CHROMOSOMAL MAPS

In theory, we can map human chromosomes as we would those of any other organism. Realistically, the problems mentioned earlier (the inability to make specific crosses coupled with the relatively small number of offspring) make these techniques of human chromosome mapping very difficult. However, some progress has been made based on pedigrees, especially in assigning genes to the X chromosome. As the pedigree analysis in the previous chapter has shown, X chromosomal traits have unique patterns of inheritance, and loci on the X chromosome are easy to identify. Currently over four hundred loci are known to be on the X chromosome. It has been estimated, by several different methods, that between fifty and one hundred thousand loci exist on human chromosomes. In later chapters, we will discuss several additional methods of human chromosomal mapping that use molecular genetic techniques.

X Linkage

After determining that a human gene is X linked, the next problem is to determine the position of the locus on the X chromosome and the map units between loci. Sometimes we can do this with the proper pedigrees, if crossing over can be ascertained. An example of this "grand-

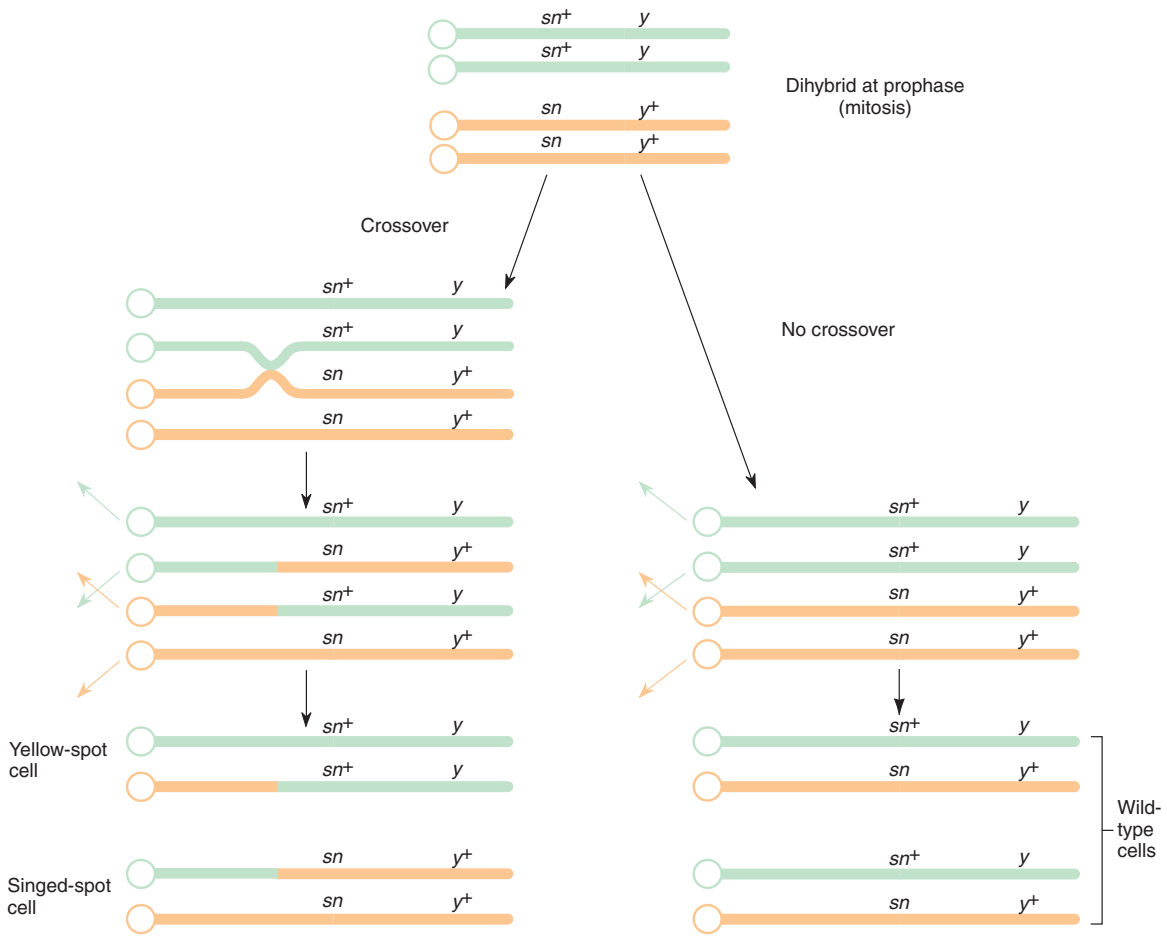


Figure 6.26 Formation of twin spots by somatic crossing over.

father method" appears in figure 6.27. In this example, a grandfather has one of the traits in question (here, color blindness). We then find that he has a grandson who is glucose-6-phosphate dehydrogenase (G-6-PD) deficient. From this we can infer that the mother (of the grandson) was dihybrid for the two alleles in the *trans* configuration. That is, she received her color-blindness allele on one of her X chromosomes from her father, and she must have received the G-6-PD-deficiency allele on the other X chromosome from her mother (why?). Thus, the two sons on the left in figure 6.27 are nonrecombinant, and the two on the right are recombinant. Theoretically, we can determine map distance by simply totaling the recombinant grandsons and dividing by the total number of grandsons. Of course, the methodology would be the same if the grandfather were both color-blind and G-6-PD deficient. The mother would then be dihybrid in the *cis* configuration, and the sons would be tabulated in the reverse manner. The point is that the grandfather's phenotype gives us information that allows us to infer that the mother was dihybrid, as well as telling us the *cis-trans* arrangement of her alleles. We can then score her sons as either recombinant or nonrecombinant.

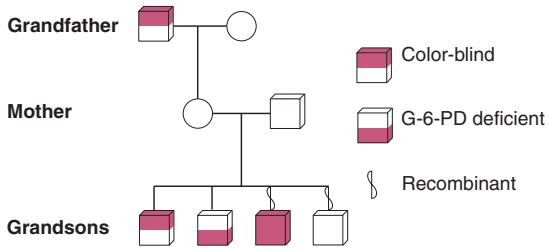


Figure 6.27 "Grandfather method" of determining crossing over between loci on the human X chromosome. G-6-PD is glucose-6-phosphate dehydrogenase.

Autosomal Linkage

From this we can see that it is relatively easy to map the X chromosome. The autosomes are another story. Since there are twenty-two autosomal linkage groups (twenty-two pairs of nonsex chromosomes), it is virtually impossible to determine from simple pedigrees which chromosome two loci are located on. Pedigrees can tell us if two loci are linked to each other, but not on which chromosome. In figure 6.28, the nail-patella syndrome includes, among other things, abnormal nail growth coupled with the absence or underdevelopment of kneecaps. It is a dominant trait. The male in generation II is dihybrid, with the *A* allele of the ABO blood type system associated with the nail-patella allele (*NPS1*) and the *B* allele with the normal nail-patella allele (*nps1*). Thus only one child in eight (III-5) is recombinant. Actually, the map distance is about 10%. In general, map distances appear greater in females than in males because more crossing over occurs in females (box 6.3).

We now turn our attention to the localization of loci to particular human chromosomes. The first locus that was definitely established to be on a particular autosome was the Duffy blood group on chromosome 1. This was ascertained in 1968 from a family that had a morphologically odd, or "uncoiled," chromosome 1. Inheritance in the Duffy blood group system followed the pattern of inheritance of the "uncoiled" chromosome. Real strides have been made since then. Two techniques, chromosomal banding and somatic-cell hybridization, have been crucial to autosomal mapping.

Chromosomal Banding

Techniques were developed around 1970 that make use of certain histochemical stains that produce repeatable banding patterns on the chromosomes. For example, Giemsa staining is one such technique; the resulting bands are called **G-bands.** More detail on these techniques is presented in chapter 15. Before these techniques, human and other mammalian chromosomes were grouped into general size categories because of the difficulty of differentiating many of them. With banding techniques came the ability to identify each human chromosome in a karyotype (see fig. 5.1).

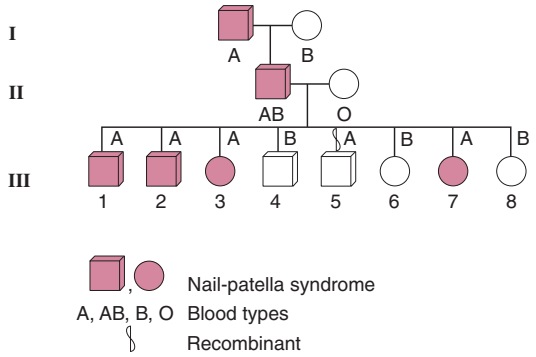


Figure 6.28 Linkage of the nail-patella syndrome and ABO loci.

Somatic-Cell Hybridization

The ability to distinguish each human chromosome is required to perform somatic-cell hybridization, in which human and mouse (or hamster) cells are fused in culture to form a hybrid. The fusion is usually mediated chemically with polyethylene glycol, which affects cell membranes; or with an inactivated virus, for example the Sendai virus, that is able to fuse to more than one cell at the same time. (The virus is able to do this because it has a lipid membrane derived from its host cells that easily fuses with new host cells. Because of this property, the virus can fuse to two cells close together, forming a cytoplasmic bridge between them that facilitates their fusion.) When two cells fuse, their nuclei are at first separate, forming a **heterokaryon,** a cell with nuclei from different sources. When the nuclei fuse, a hybrid cell is formed, and this hybrid tends to lose human chromosomes preferentially through succeeding generations. Upon stabilization, the result is a cell with one or more human chromosomes in addition to the original mouse or hamster chromosomal complement. Banding techniques allow the observer to recognize the human chromosomes. A geneticist looks for specific human phenotypes, such as enzyme products, and can then assign the phenotype to one of the human chromosomes in the cell line.

When cells are mixed together for hybridization, some cells do not hybridize. It is thus necessary to be able to select for study just those cells that are hybrids. One technique, originally devised by J. W. Littlefield in 1964, makes use of genetic differences in the way the cell lines synthesize DNA. Normally, in mammalian cells, aminopterin acts as an inhibitor of enzymes involved in DNA metabolism. Two enzymes, hypoxanthine phosphoribosyl transferase (HPRT) and thymidine kinase (TK), can bypass aminopterin inhibition by making use of secondary, or salvage, pathways in the cell. If hypoxanthine is provided, HPRT converts it to a purine, and if thymidine is provided, TK converts it to the nucleotide thymidylate. (Purines are converted to nucleotides and nucleotides are the subunits of DNA—see chapter 9.) Thus, normal cells in the absence of aminopterin synthesize DNA even if they lack HPRT activity ($HPRT^-$) or TK activity (TK^-). In the presence of aminopterin, $HPRT^-$ or TK^- cells die. However, in the presence of aminopterin, $HPRT^+$ TK^+ cells can synthesize DNA and survive. Using this information, the following selection system was developed.

Mouse cells that have the phenotype of $HPRT^+$ TK^- are mixed with human cells that have the phenotype of

BOX 6.3

Experimental Methods

Lod *Scores*

Human population geneticists can increase the accuracy of their linkage analysis by using a probability technique, developed by Newton Morton, called the ***lod* score method** (*L*og *Od*ds). The geneticist asks what the probability is of getting a particular pedigree assuming a particular recombination frequency (Θ), as compared with getting the same pedigree assuming independent assortment (Θ = 0.50). In other words, he or she calculates the ratio of the probability of genotypes in a family given a certain crossover frequency compared with the probability of those genotypes if the loci are unlinked. Logarithms are used for ease of calculation, and the parameter is called z, the *lod* score. Using this method, a researcher can try different crossover frequencies until the one giving the highest *lod* score is found.

For example, take the pedigree in figure 6.28. The father in generation II can have one of two allelic arrangements: *A NPS1/B nps1* or *A/B NPS1/nps1*. The former assumes linkage, whereas the latter does not. Our initial estimate of recombination, assuming linkage, was (1/8) × 100, or 12.5 map units. We now need to calculate the ratio of two probabilities:

$$z = \log \frac{\text{probability of birth sequence assuming 12.5 map units}}{\text{probability of birth sequence assuming independent assortment}}$$

Newton E. Morton (1929–).
(Courtesy of Dr. Newton E. Morton.)

Assuming 12.5 map units (or a probability of 0.125 of a crossover; Θ = 0.125), the probability of child III-1 is 0.4375. This child would be a nonrecombinant, so his probability of having the nail-patella syndrome and type A blood is half the probability of no crossover during meiosis, or (1 − 0.125)/2. We divide by two because there are two nonrecombinant types. This is the same probability for all children except III-5, whose probability of occurrence is 0.125/2 = 0.0625, since he is a recombinant. Thus, the numerator of the previous equation is $(0.4375)^7(0.0625)$.

If the two loci are not linked, then any genotype has a probability of 1/4, or 0.25. Thus, the sequence of the eight children has the probability of $(0.25)^8$. This is the denominator of the equation. Thus,

$$z = \log \frac{(0.4375)^7(0.0625)}{(0.25)^8}$$

$$z = \log [12.566] = 1.099$$

Any *lod* score greater than zero favors linkage. A *lod* score less than zero suggests that Θ has been underestimated. A *lod* of 3.0 or greater (10^3 or one thousand times more likely than independent assortment) is considered a strong likelihood of linkage. Thus, in our example, we have an indication of linkage with a recombination frequency of 0.125. Now we can calculate *lod* scores assuming other values of recombination, as table 1 does. You can see that the recombination frequency as calculated, 0.125 (12.5 map units), gives the highest *lod* score.

Table 1 ***Lod* Scores for the Cross in Figure 6.28**

Recombination Frequency (Θ)	*Lod* Score
0.05	0.951
0.10	1.088
0.125	1.099
0.15	1.090
0.20	1.031
0.25	0.932
0.30	0.801
0.35	0.643
0.40	0.457
0.45	0.244
0.50	0.000

$HPRT^-$ TK^+ in the presence of Sendai virus or polyethylene glycol. Fusion takes place in some of the cells, and the mixture is grown in a medium containing hypoxanthine, aminopterin, and thymidine (called **HAT medium**). In the presence of aminopterin, unfused mouse cells (TK^-) and unfused human cells ($HPRT^-$) die. Hybrid cells, however, survive because they are $HPRT^+$ TK^+. Eventually, the hybrid cells end up with random numbers of human chromosomes. There is one restriction: All cell lines selected are TK^+. This HAT method (using the HAT medium) not only selects for hybrid clones, but also localizes the *TK* gene to human chromosome 17, the one human chromosome found in every successful cell line.

Table 6.8 Assignment of the Gene for Blood Coagulating Factor III to Human Chromosome 1 Using Human-Mouse Hybrid Cell Lines

Hybrid Cell Line Designation	Tissue/ Factor Score	Human Chromosome Present																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
WILI	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	−	+	−	+
WIL6	−	−	+	−	+	+	+	+	+	−	+	+	−	−	+	−	−	+	−	+	+	+	−	+
WIL7	−	−	+	+	−	+	+	−	+	−	+	+	−	+	+	−	−	+	+	−	−	+	−	+
WIL14	+	+	−	+	−	−	−	+	+	−	+	−	+	−	+	+	−	+	−	−	−	−	−	+
SIR3	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+
SIR8	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+
SIR11	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+
REW7	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+
REW15	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	−	+	+	+	+	+	+	+
DUA1A	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	*
DUA1CsAzF	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
DUA1CsAzH	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
TSL1	−	−	−	+	+	−	−	−	−	−	+	+	−	+	+	−	+	+	+	−	+	−	−	−
TSL2	−	−	+	*	−	+	+	−	−	−	+	−	+	−	−	−	−	*	+	−	+	+	−	+
TSL2CsBF	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
XTR1	+	+	−	*	−	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+
XTR2	−	−	−	*	−	+	−	−	+	−	+	−	+	+	−	−	−	−	+	−	+	+	−	*
XTR3BsAgE	+	+	−	*	−	+	+	+	+	+	+	−	−	+	+	−	−	+	+	+	−	+	−	*
XTR22	−	−	+	*	+	+	+	−	+	−	+	+	−	−	−	+	−	−	+	+	+	+	+	*
XER9	−	−	+	−	+	−	−	−	+	−	+	*	+	−	+	−	−	+	+	−	−	+	−	*
XER11	+	+	−	+	+	−	+	+	+	−	+	*	+	+	−	+	+	+	+	+	+	+	+	*
REX12	−	−	−	+	−	−	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	*
JSR29	+	+	+	+	+	+	+	*	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+	+
JVR22	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
JWR22H	+	*	*	−	+	−	+	−	−	−	+	+	+	−	+	+	−	+	+	−	+	+	−	−
ALR2	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
ICL15	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	+	−	−
ICL15CsBF	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−
MH21	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
% Discord†		0	32	17	24	31	21	21	31	21	24	30	21	21	28	14	24	21	28	17	34	41	21	27

Source: Reprinted with permission from S.D. Carson, et al., "Tissue Factor Gene Localized to Human Chromosome 1 (after 1p21)," *Science*, 229:229–291. Copyright © 1985 American Association for the Advancement of Science.

* A translocation in which only part of the chromosome is present.

† Discord refers to cases in which the tissue factor score is plus, and the human chromosome is absent, or in which the score is minus and the chromosome is present.

After successful cell hybrids are formed, two particular tests are used to map human genes. A **synteny test** (same linkage group) determines whether two loci are in the same linkage group if the phenotypes of the two loci are either always together or always absent in various hybrid cell lines. An **assignment test** determines which chromosome a particular locus is on by the concordant appearance of the phenotype whenever that particular chromosome is in a cell line, or by the lack of the particular phenotype when a particular chromosome is absent from a cell line. The first autosomal synteny test, performed in 1970, demonstrated that the *B* locus of lactate dehydrogenase (LDH_B) was linked to the *B* locus of peptidase (PEP_B). (Both enzymes are formed from subunits

controlled by two loci each. In addition to the *B* locus, each protein has subunits controlled by an *A* locus.) Later, these loci were shown to reside on chromosome 12.

In another example, a blood-coagulating glycoprotein (a protein-polysaccharide complex) called tissue factor III was localized by assignment tests to chromosome 1. Table 6.8 shows twenty-nine human-mouse hybrid cell lines, or **clones,** the human chromosomes they contain, and their tissue factor score, the results of an assay for the presence of the coagulating factor. (Clones are cells arising from a single ancestor.) It is obvious from table 6.8 that the gene for tissue factor III is on human chromosome 1. Every time human chromosome 1 is present in a cell line, so is tissue factor III. Every time human chromosome 1 is absent, so is the tissue factor (zero discordance or 100% concordance). No other chromosome showed that pattern.

The human map as we know it now (compiled by Victor McKusick at Johns Hopkins University), containing over six thousand assigned loci of over twelve thousand known to exist, is shown in table 6.9 and figure 6.29. At

Victor A. McKusick (1921–). (Courtesy of Victor A. McKusick.)

Table 6.9 **Definition of Selected Loci of the Human Chromosome Map (figure 6.29)**

Locus	Protein Product	Chromosome	Locus	Protein Product	Chromosome
ABO	ABO blood group	9	*IGH*	Immunoglobulin heavy-chain gene family	14
AG	Alpha globin gene family	16	*IGK*	Immunoglobulin kappa-chain gene family	2
ALB	Albumin	4	*INS*	Insulin	11
AMY1	Amylase, salivary	1	*LDHA*	Lactate dehydrogenase A	11
AMY2	Amylase, pancreatic	1	*MDI*	Manic depressive illness	6
BCS	Breast cancer susceptibility	16	*MHC*	Major histocompatibility complex	6
C2	Complement component-2	6	*MN*	MN blood group	4
CAT	Catalase	11	*MYB*	Avian myeloblastosis virus oncogene	6
CBD	Color blindness, deutan	X	*NHCP1*	Nonhistone chromosomal protein-1	7
CBP	Color blindness, protan	X	*NPS1*	Nail-patella syndrome	9
CML	Chronic myeloid leukemia	22	*PEPA*	Peptidase A	18
DMD	Duchenne muscular dystrophy	X	*PVS*	Polio virus sensitivity	19
FES	Feline sarcoma virus oncogene	15	*Rh*	Rhesus blood group	1
FY	Duffy blood group	1	*RN5S*	5S RNA gene(s)	1
GLB1	Beta-galactosidase-1	3	*RNTMI*	Initiator methionine tRNA	6
H1	Histone-1	7	*RWS*	Ragweed sensitivity	6
HBB	Hemoglobin beta chain	11	*S1*	Surface antigen 1	11
HEMA	Classic hemophilia	X	*SIS*	Simian sarcoma virus oncogene	22
HEXA	Hexosaminidase A	15	*STA*	Stature	Y
HLA	Human leukocyte antigens	6	*TF*	Transferrin	3
HP	Haptoglobin	16	*XG*	Xg blood group	X
HYA	Y histocompatibility antigen, locus A	Y	*XRS*	X-ray sensitivity	13
IDDM	Insulin-dependent diabetes mellitus	6			
IFF	Interferon, fibroblast	9			

Note: A more complete list appears in V. A. McKusick, *Mendelian Inheritance in Man: A Catalog of Human Genes* (Baltimore: Johns Hopkins University Press, 1994).

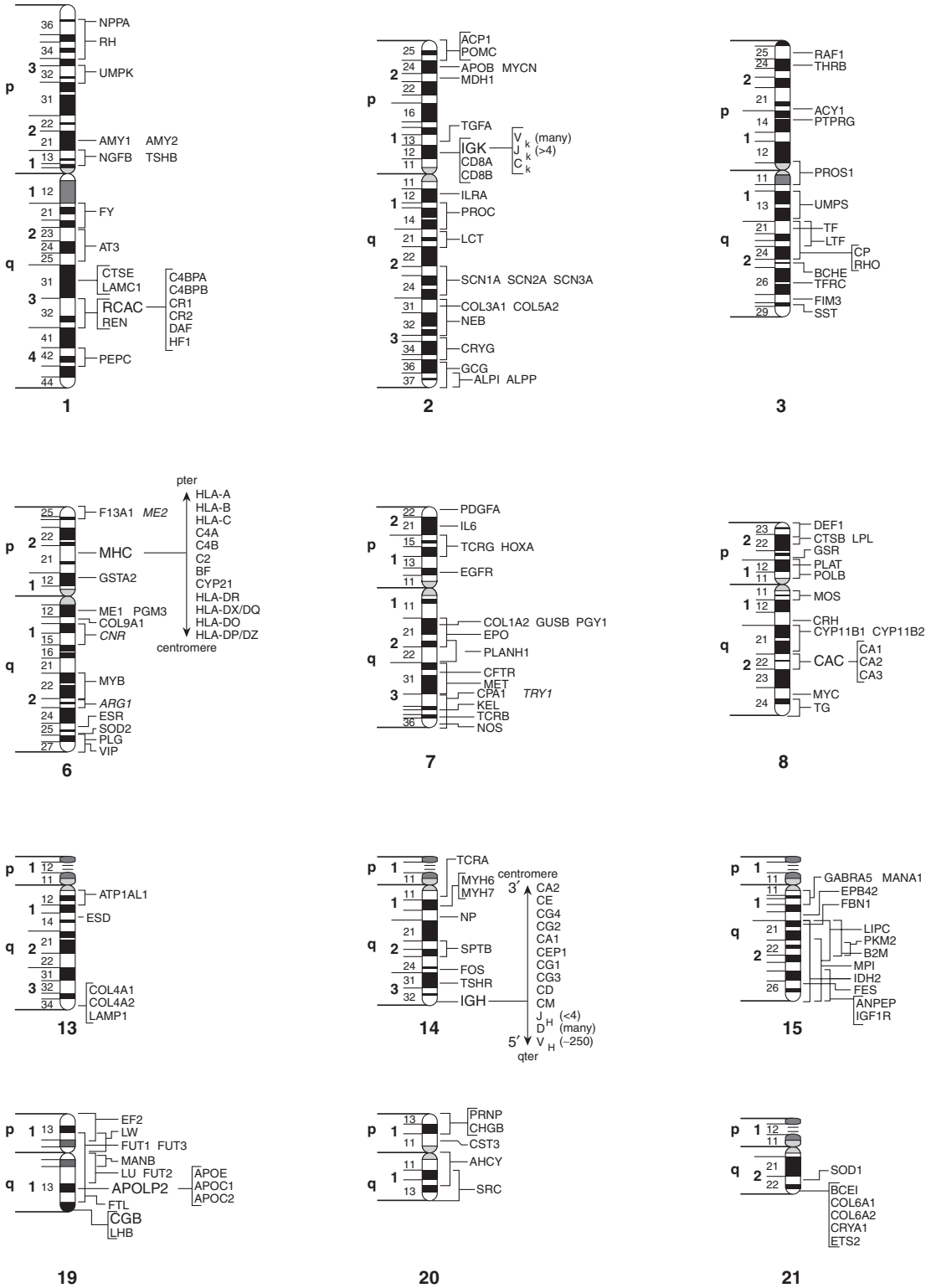
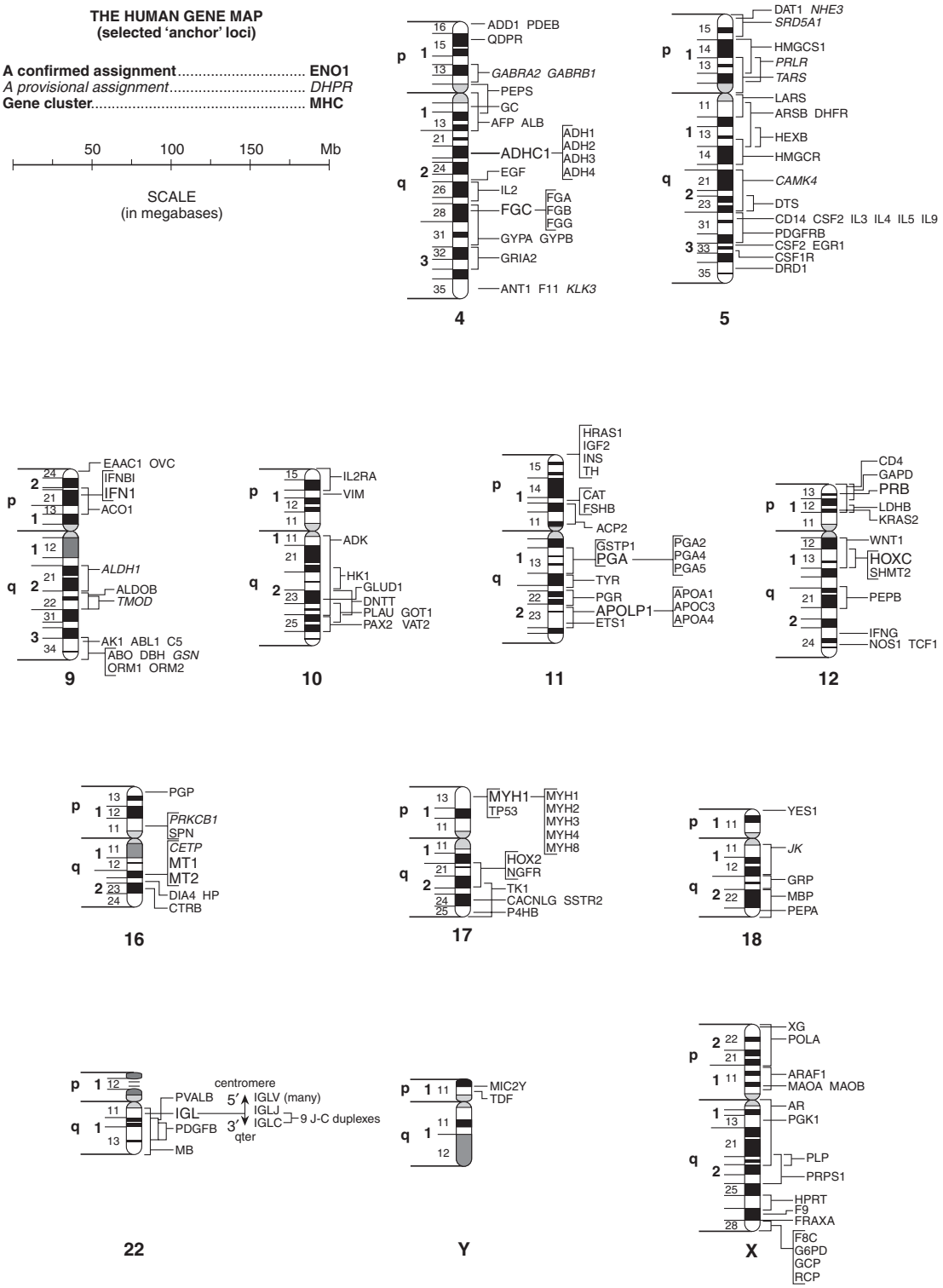


Figure 6.29 Human G-banded chromosomes with their accompanying assigned loci. The *p* and *q* refer to the short and long arms of the chromosomes, respectively. A key to the loci is given in McKusick (1994). (From Victor A. McKusick, *Mendelian inheritance in man,* 11th edition, 1994. Reprinted by permission of Johns Hopkins University Press, Baltimore, MD.)


THE HUMAN GENE MAP
(selected 'anchor' loci)
A confirmed assignment ENO1
A provisional assignment ..... DHPR
Gene cluster ..... MHC
50 100 150 Mb
SCALE
(in megabases)
ADD1 PDEB
QDPR
GABRA2 GABRB1
PEPS
GC
AFP ALB
ADH1
ADH2
ADH3
ADH4
ADHC1
EGF
IL2
FGA
FGB
FGG
FGC
GYPA GYPB
GRIA2
ANT1 F11 KLK3
4
DAT1 NHE3
SRD5A1
HMGCS1
PRLR
TARS
LARS
ARSB DHFR
HEXB
HMGCR
CAMK4
DTS
CD14 CSF2 IL3 IL4 IL5 IL9
PDGFRB
CSF2 EGR1
CSF1R
DRD1
5
EAAC1 OVC
IFNBI
IFN1
ACO1
ALDH1
ALDOB
TMOD
AK1 ABL1 C5
ABO DBH GSN
ORM1 ORM2
9
IL2RA
VIM
ADK
HK1
GLUD1
DNTT
PLAU GOT1
PAX2 VAT2
10
HRAS1
IGF2
INS
TH
CAT
FSHB
ACP2
GSTP1
PGA
PGA2
PGA4
PGA5
TYR
PGR
APOLP1
APOA1
APOC3
APOA4
ETS1
11
CD4
GAPD
PRB
LDHB
KRAS2
WNT1
HOXC
SHMT2
PEPB
IFNG
NOS1 TCF1
12
PGP
PRKCB1
SPN
CETP
MT1
MT2
DIA4 HP
CTRB
16
MYH1
TP53
MYH1
MYH2
MYH3
MYH4
MYH8
HOX2
NGFR
TK1
CACNLG SSTR2
P4HB
17
YES1
JK
GRP
MBP
PEPA
18
centromere
PVALB
IGL
PDGFB
MB
5′
3′
qter
IGLV (many)
IGLJ
IGLC
9 J-C duplexes
22
MIC2Y
TDF
Y
XG
POLA
ARAF1
MAOA MAOB
AR
PGK1
PLP
PRPS1
HPRT
F9
FRAXA
F8C
G6PD
GCP
RCP
X

present, geneticists studying human chromosomes are hampered not by a lack of techniques but by a lack of marker loci. When a new locus is discovered, it is now relatively easy to assign it to its proper chromosome.

The problem still exists of determining exactly where a particular locus belongs on a chromosome. This is facilitated by developing particular cell lines with broken chromosomes, so that parts are either missing or have moved to other chromosomes. These processes reveal new linkage arrangements and make it possible to determine the region in which a locus is situated on a particular chromosome. In chapter 13, we describe additional techniques used to locate genes on human chromosomes, including a description of the Human Genome Project, the program that sequenced the entire human genome as well as the genomes of other model organisms.

SUMMARY

STUDY OBJECTIVE 1: To learn about analytical techniques for locating the relative positions of genes on chromosomes in diploid eukaryotic organisms 110–122

The principle of independent assortment is violated when loci lie near each other on the same chromosome. Recombination between these loci results from the crossing over of chromosomes during meiosis. The amount of recombination provides a measure of the distance between these loci. One map unit (centimorgan) equals 1% recombinant gametes. Map units can be determined by testcrossing a dihybrid and recording the percentage of recombinant offspring. If three loci are used (a three-point cross), double crossovers will be revealed. A coefficient of coincidence, the ratio of observed to expected double crossovers, can be calculated to determine if one crossover changes the probability that a second one will occur nearby.

A chiasma seen during prophase I of meiosis represents both a physical and a genetic crossing over. This can be demonstrated by using homologous chromosomes with morphological distinctions.

Because of multiple crossovers, the measured percentage recombination underestimates the true map distance, especially for loci relatively far apart; the best map estimates come from summing the distances between closely linked loci. A mapping function can be used to translate observed map distances into more accurate ones.

STUDY OBJECTIVE 2: To learn about analytical techniques for locating the relative positions of genes on chromosomes in ascomycete fungi 122–132

Organisms that retain all the products of meiosis lend themselves to chromosome mapping by haploid mapping (tetrad analysis). With unordered spores, such as in yeast, we use

$$\text{map units} = \frac{(1/2)\text{ the number of TT asci} + \text{the number of NPD asci}}{\text{total number or asci}} \times 100$$

Map units between a locus and its centromere in organisms with ordered spores, such as *Neurospora,* can be calculated as

$$\text{map units} = \frac{(1/2)\text{ the number of SDS asci}}{\text{total number of asci}} \times 100$$

Crossing over also occurs during mitosis, but at a much reduced rate. Somatic (mitotic) crossing over can be used to map loci.

STUDY OBJECTIVE 3: To learn about analytical techniques for locating the relative positions of genes on human chromosomes 132–140

Human chromosomes can be mapped. Recombination distances can be established by pedigrees, and loci can be attributed to specific chromosomes by synteny and assignment tests in hybrid cell lines.

SOLVED PROBLEMS

PROBLEM 1: A homozygous claret (*ca,* claret eye color), curled (*cu,* upcurved wings), fluted (*fl,* creased wings) fruit fly is crossed with a pure-breeding wild-type fly. The F_1 females are testcrossed with the following results:

fluted	4
claret	173
curled	26
fluted, claret	24
fluted, curled	167
claret, curled	6
fluted, claret, curled	298
wild-type	302

a. Are the loci linked?
b. If so, give the gene order, map distances, and coefficient of coincidence.

Answer: The pattern of numbers among the eight offspring classes is the pattern we are used to seeing for linkage of three loci. We can tell from the two groups in largest numbers (the nonrecombinants—fluted, claret, curled and wild-type) that the alleles are in the coupling (*cis*) arrangement. If we compare either of the nonrecombinant classes with either of the double crossover classes (fluted and claret, curled), we see that the fluted locus is in the center. For example, compare fluted, a double crossover offspring, with the wild-type, a nonrecombinant; clearly, fluted has the odd pattern. Thus the trihybrid female parent had the following arrangement of alleles:

$$\frac{ca\ fl\ cu}{ca^+\ fl^+\ cu^+}$$

A crossover in the *ca–fl* region produces claret and fluted, curled offspring, and a crossover in the *fl–cu* region produces fluted, claret and curled offspring. Counting the crossovers in each region, including the double crossovers in each, and then converting to percentages, yields a claret-to-fluted distance of 35.0 map units (173 + 167 + 6 + 4) and a fluted-to-curled distance of 6.0 map units (26 + 24 + 6 + 4). We expect 0.35 × 0.06 × 1,000 = 21 double crossovers, but we observed only 6 + 4 = 10. Thus, the coefficient of coincidence is 10/21 = 0.48.

PROBLEM 2: The *ad5* locus in *Neurospora* is a gene for an enzyme in the synthesis pathway for the DNA base adenine. A wild-type strain ($ad5^+$) is crossed with an adenine-requiring strain, $ad5^-$. The diploid undergoes meiosis, and one hundred asci are scored for their segregation patterns with the following results:

$ad5^+$	$ad5^+$	$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	$ad5^-$	$ad5^-$	40
$ad5^-$	$ad5^-$	$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	$ad5^+$	$ad5^+$	46
$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	5
$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	3
$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	4
$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	$ad5^+$	$ad5^+$	$ad5^-$	$ad5^-$	2

What can you say about the linkage arrangements at this locus?

Answer: You can see that 14 (5 + 3 + 4 + 2) asci are of the second-division segregation type (SDS) and 86 (40 + 46) are of the first-division segregation type (FDS). To map the distance of the locus to its centromere, we divide the percentage of SDS types by 2: 14/100 = 14%; divided by 2 is 7%. Thus, the *ad5* locus is 7 map units from its centromere.

PROBLEM 3: In yeast, the *his5* locus is a gene for an enzyme in the synthesis pathway for the amino acid histidine, and the *lys11* locus is a gene for an enzyme in the synthesis pathway for the amino acid lysine. A haploid wild-type strain ($his5^+\ lys11^+$) is crossed with the double mutant ($his5^-\ lys11^-$). The diploid is allowed to undergo meiosis, and 100 asci are scored with the following results:

$his5^+\ lys11^+$	$his5^+\ lys11^+$	$his5^+\ lys11^-$
$his5^+\ lys11^+$	$his5^-\ lys11^-$	$his5^+\ lys11^-$
$his5^-\ lys11^-$	$his5^-\ lys11^+$	$his5^-\ lys11^+$
$his5^-\ lys11^-$	$his5^+\ lys11^-$	$his5^-\ lys11^+$
62	30	8

What is the linkage arrangement of these loci?

Answer: Of the 100 asci analyzed, 62 were parental ditypes (PD), 30 were tetratypes (TT), and 8 were nonparental ditypes (NPD). To map the distance between the two loci, we take the percentage of NPD (8%) plus half the percentage of TT (1/2 of 30 = 15%) = 23% or 23 centimorgans between loci.

PROBLEM 4: A particular human enzyme is present only in clone B. The human chromosomes present in clones A, B, and C appear as pluses in the following table. Determine the probable chromosomal location of the gene for the enzyme.

	Human Chromosome							
Clone	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
A	+	+	+	+	−	−	−	−
B	+	+	−	−	+	+	−	−
C	+	−	+	−	+	−	+	−

Answer: If a gene is located on a chromosome, the gene must be present in the clones with the chromosome (+). Chromosomes 1, 2, 5, 6 are present in B. If the gene in question were located on chromosome 1, the enzyme should have been present in all three clones. A similar argument holds for chromosome 2, in which the enzyme should have been present in clones A and B, and so on for the rest of the chromosomes. The only chromosome that is unique to clone B is 6. Therefore, the gene is located on chromosome 6.

EXERCISES AND PROBLEMS*

DIPLOID MAPPING

1. A homozygous groucho fly (*gro,* bristles clumped above the eyes) is crossed with a homozygous rough fly (*ro,* eye abnormality). The F_1 females are testcrossed, producing these offspring:

groucho	518
rough	471
groucho, rough	6
wild-type	5
	1,000

a. What is the linkage arrangement of these loci?
b. What offspring would result if the F_1 dihybrids were crossed among themselves instead of being testcrossed?

2. A female fruit fly with abnormal eyes (*abe*) of a brown color (*bis,* bistre) is crossed with a wild-type male. Her sons have abnormal, brown eyes; her daughters are of the wild-type. When these F_1 flies are crossed among themselves, the following offspring are produced:

	Sons	Daughters
abnormal, brown	219	197
abnormal	43	45
brown	37	35
wild-type	201	223

What is the linkage arrangement of these loci?

3. In *Drosophila,* the loci inflated (*if*, small, inflated wings) and warty (*wa*, abnormal eyes) are about 10 map units apart on the X chromosome. Construct a data set that would allow you to determine this linkage arrangement. What differences would be involved if the loci were located on an autosome?

4. A geneticist crossed female fruit flies that were heterozygous at three electrophoretic loci, each with fast and slow alleles, with males homozygous for the slow alleles. The three loci were *got1* (glutamate oxaloacetate transaminase-1), *amy* (alpha-amylase), and *sdh* (succinate dehydrogenase). The first 1,000 offspring isolated had the following genotypes:

Class 1	$got^s\ got^s\ amy^s\ amy^s\ sdh^s\ sdh^s$	441
Class 2	$got^f\ got^s\ amy^f\ amy^s\ sdh^f\ sdh^s$	421
Class 3	$got^f\ got^s\ amy^s\ amy^s\ sdh^s\ sdh^s$	11
Class 4	$got^s\ got^s\ amy^f\ amy^s\ sdh^f\ sdh^s$	14
Class 5	$got^f\ got^s\ amy^f\ amy^s\ sdh^s\ sdh^s$	58
Class 6	$got^s\ got^s\ amy^s\ amy^s\ sdh^f\ sdh^s$	53
Class 7	$got^f\ got^s\ amy^s\ amy^s\ sdh^f\ sdh^s$	1
Class 8	$got^s\ got^s\ amy^f\ amy^s\ sdh^s\ sdh^s$	1

What are the linkage arrangements of these three loci, including map units? If the three loci are linked, what is the coefficient of coincidence?

5. The following three recessive markers are known in lab mice: *h*, hotfoot; *o*, obese; and *wa*, waved. A trihybrid of unknown origin is testcrossed, producing the following offspring:

hotfoot, obese, waved	357
hotfoot, obese	74
waved	66
obese	79
wild-type	343
hotfoot, waved	61
obese, waved	11
hotfoot	9
	1,000

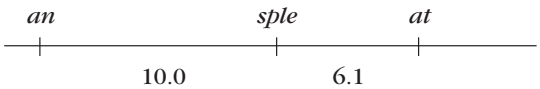
a. If the genes are linked, determine the relative order and the map distances between them.
b. What was the *cis-trans* allele arrangement in the trihybrid parent?
c. Is there any crossover interference? If yes, how much?

6. The following three recessive genes are found in corn: *bt1*, brittle endosperm; *gl17*, glossy leaf; *rgdl,* ragged seedling. A trihybrid of unknown origin is testcrossed, producing the following offspring:

brittle, glossy, ragged	236
brittle, glossy	241
ragged	219
glossy	23
wild-type	224
brittle, ragged	17
glossy, ragged	21
brittle	19
	1,000

a. If the genes are linked, determine the relative order and map distances.
b. Reconstruct the chromosomes of the trihybrid.
c. Is there any crossover interference? If yes, how much?

7. In *Drosophila,* ancon (*an*, legs and wings short), spiny legs (*sple*, irregular leg hairs), and arctus oculus (*at*, small narrow eyes) have the following linkage arrangement on chromosome 3:



*Answers to selected exercises and problems are on page A-6.

a. Devise a data set with no crossover interference that would yield these map units.
b. What data would yield the same map units but with a coefficient of coincidence of 0.60?

8. Ancon (*an*) and spiny legs (*sple*), from problem 7, are 10 map units apart on chromosome 3. Notchy (*ny,* wing tips nicked) is on the X chromosome (chromosome 1). Create a data set that would result if you were making crosses to determine the linkage arrangement of these three loci. How would you know that the notchy locus is on the X chromosome?

9. In the house mouse, the autosomal alleles Trembling and Rex (short hair) are dominant to not trembling (normal) and long hair, respectively. Heterozygous Trembling, Rex females were crossed with normal, long-haired males and yielded the following offspring:

Trembling, Rex	42
Trembling, long-haired	105
normal, Rex	109
normal, long-haired	44

a. Are the two genes linked? How do you know?
b. In the heterozygous females, were Trembling and Rex in *cis* or *trans* position? Explain.
c. Calculate the percent recombination between the two genes.

10. In corn, a trihybrid Tunicate (*T*), Glossy (*G*), Liguled (*L*) plant was crossed with a nontunicate, nonglossy, liguleless plant, producing the following offspring:

Tunicate, liguleless, Glossy	58
Tunicate, liguleless, nonglossy	15
Tunicate, Liguled, Glossy	55
Tunicate, Liguled, nonglossy	13
nontunicate, Liguled, Glossy	16
nontunicate, Liguled, nonglossy	53
nontunicate, liguleless, Glossy	14
nontunicate, liguleless, nonglossy	59

a. Determine which genes are linked.
b. Determine the genotype of the heterozygote; be sure to indicate which alleles are on which chromosome.
c. Calculate the map distances between the linked genes.

11. In *Drosophila*, kidney-shaped eye (*k*), cardinal eye (*cd*), and ebony body (*e*) are three recessive genes. If homozygous kidney, cardinal females are crossed with homozygous ebony males, the F_1 offspring are all wild-type. If heterozygous F_1 females are mated with kidney, cardinal, ebony males, the following 2,000 progeny appear:

880	kidney, cardinal
887	ebony
64	kidney, ebony
67	cardinal
49	kidney
46	ebony, cardinal
3	kidney, ebony, cardinal
4	wild-type

a. Determine the chromosomal composition of the F_1 females.
b. Derive a map of the three genes.

12. Following is a partial map of the third chromosome in *Drosophila.*

19.2 javelin bristles (*jv*)
43.2 thread arista (*th*)
66.2 Delta veins (*Dl*)
70.7 ebony body (*e*)

a. If flies heterozygous in *cis* position for javelin and ebony are mated among themselves, what phenotypic ratio do you expect in the progeny?
b. A true-breeding thread, ebony fly is crossed with a true-breeding Delta fly. An F_1 female is test-crossed to a thread, ebony male. Predict the expected progeny and their frequencies for this cross. Assume no interference.
c. Repeat *b,* but assume a coefficient of coincidence of 0.4.

13. Suppose that you have determined the order of three genes to be *a*, *c*, *b*, and that by doing two-point crosses you have determined map distances as $a–c = 10$ and $c–b = 5$. If interference is -1.5, and the three-point cross is

$$\frac{A\ C\ B}{a\ c\ b} \times \frac{a\ c\ b}{a\ c\ b}$$

what frequency of double crossovers do you expect?

HAPLOID MAPPING (TETRAD ANALYSIS)

14. Given the following cross in *Neurospora: ab* × a^+b^+, construct results showing that crossing over occurs in two of the four chromatids of a tetrad at meiosis. What would the results be if crossing over occurred during interphase before each chromosome became two chromatids? if each crossover event involved three or four chromatids?

15. A strain of yeast requiring both tyrosine (tyr^-) and arginine (arg^-) is crossed to the wild-type. After meiosis, the following ten asci are dissected. Classify each ascus as to segregational type (PD, NPD, TT). What is the linkage relationship between these two loci?

1	$arg^-\ tyr^-$	$arg^+\ tyr^+$	$arg^+\ tyr^+$	$arg^-\ tyr^-$
2	$arg^+\ tyr^+$	$arg^+\ tyr^+$	$arg^-\ tyr^-$	$arg^-\ tyr^-$
3	$arg^-\ tyr^+$	$arg^-\ tyr^+$	$arg^+\ tyr^-$	$arg^+\ tyr^-$
4	$arg^-\ tyr^-$	$arg^-\ tyr^-$	$arg^+\ tyr^+$	$arg^+\ tyr^+$
5	$arg^-\ tyr^-$	$arg^-\ tyr^+$	$arg^+\ tyr^-$	$arg^+\ tyr^+$
6	$arg^+\ tyr^+$	$arg^+\ tyr^+$	$arg^-\ tyr^-$	$arg^-\ tyr^-$
7	$arg^-\ tyr^-$	$arg^+\ tyr^+$	$arg^-\ tyr^+$	$arg^+\ tyr^-$
8	$arg^+\ tyr^+$	$arg^+\ tyr^+$	$arg^-\ tyr^-$	$arg^-\ tyr^-$
9	$arg^+\ tyr^+$	$arg^-\ tyr^-$	$arg^-\ tyr^-$	$arg^+\ tyr^+$
10	$arg^-\ tyr^-$	$arg^+\ tyr^+$	$arg^+\ tyr^+$	$arg^-\ tyr^-$

16. A certain haploid strain of yeast was deficient for the synthesis of the amino acids tryptophan (try^-) and methionine (met^-). It was crossed to the wild-type, and meiosis occurred. One dozen asci were analyzed for their tryptophan and methionine requirements. The following results, with the inevitable lost spores, were obtained:

1	$try^-\ met^-$	?	?	$try^-\ met^-$
2	?	$try^-\ met^-$	$try^+\ met^+$	$try^+\ met^+$
3	$try^-\ met^+$	$try^-\ met^-$	$try^+\ met^-$	$try^+\ met^+$
4	$try^-\ met^-$	$try^+\ met^+$	?	$try^+\ met^-$
5	$try^-\ met^+$	?	?	$try^+\ met^-$
6	$try^+\ met^+$	$try^+\ met^+$	$try^-\ met^-$	$try^-\ met^-$
7	$try^+\ met^+$	$try^+\ met^-$	?	$try^-\ met^-$
8	$try^+\ met^+$	$try^-\ met^-$	?	$try^+\ met^+$
9	$try^-\ met^+$	$try^+\ met^-$	$try^-\ met^+$	$try^+\ met^-$
10	$try^-\ met^-$	$try^+\ met^+$	$try^-\ met^-$	$try^+\ met^+$
11	$try^+\ met^+$	$try^+\ met^+$	?	?
12	?	$try^+\ met^-$	?	$try^-\ met^+$

a. Classify each ascus as to segregational type (note that some asci may not be classifiable).
b. Are the genes linked?
c. If so, how far apart are they?

17. In *Neurospora*, a haploid strain requiring arginine (arg^-) is crossed with the wild-type (arg^+). Meiosis occurs, and ten asci are dissected with the following results. Map the *arg* locus.

1	arg^+	arg^+	arg^-	arg^-	arg^+	arg^+	arg^-	arg^-
2	arg^-	arg^-	arg^+	arg^+	arg^-	arg^-	arg^+	arg^+
3	arg^+	arg^+	arg^+	arg^+	arg^-	arg^-	arg^-	arg^-
4	arg^+	arg^+	arg^+	arg^+	arg^-	arg^-	arg^-	arg^-
5	arg^-	arg^-	arg^-	arg^-	arg^+	arg^+	arg^+	arg^+
6	arg^+	arg^+	arg^-	arg^-	arg^-	arg^-	arg^+	arg^+
7	arg^-	arg^-	arg^+	arg^+	arg^+	arg^+	arg^-	arg^-
8	arg^+	arg^+	arg^+	arg^+	arg^-	arg^-	arg^-	arg^-
9	arg^-	arg^-	arg^+	arg^+	arg^+	arg^+	arg^-	arg^-
10	arg^-	arg^-	arg^-	arg^-	arg^+	arg^+	arg^+	arg^+

18. A haploid strain of *Neurospora* with fuzzy colony morphology (f) was crossed with the wild-type (f^+). Twelve asci were scored. The following results, with the inevitable lost spores were obtained:

1	?	f	f	?	?	f^+	f^+	f^+
2	f	f	f^+	f^+	f^+	f^+	f	f
3	f	?	?	?	f^+	?	?	?
4	f^+	?	?	?	f	f	f	f
5	f	f	?	?	?	f^+	?	f^+
6	?	f	f	?	?	?	?	?
7	f^+	f^+	f	f	f	f	f^+	f^+
8	f	f	f	?	?	f^+	f^+	f^+
9	f^+	?	?	?	?	f	f	?
10	f	f	f^+	f^+	f	f	f^+	f^+
11	f	f	f	f	f^+	f^+	f^+	f^+
12	f	f	?	?	?	?	f^+	f^+

a. Classify each ascus as to segregational type, and note which asci cannot be classified.
b. Map the chromosome containing the f locus with all the relevant measurements.

19. Draw ten of the remaining twenty-eight ascus patterns not included in table 6.6. To which of the seven major categories of table 6.7 does each belong?

20. In yeast, the a and b loci are 12 map units apart. Construct a data set to demonstrate this.

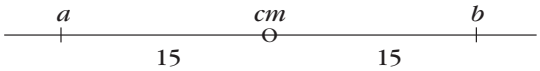
21. In *Neurospora*, the a locus is 12 map units from its centromere. Construct a data set to show this.

22. An *ab Neurospora* was crossed with an a^+b^+ form. Meiosis occurred, and 1,000 asci were dissected. Using the classes of table 6.7, the following data resulted:

Class 1	700	Class 5	5
Class 2	0	Class 6	5
Class 3	190	Class 7	10
Class 4	90		

What is the linkage arrangement of these loci?

23. Given the following linkage arrangement in *Neurospora*, construct a data set similar to that in table 6.7 that is consistent with it (*cm* is centromere).



24. Determine crossover events that led to each of the seven classes in table 6.7.

25. In *Neurospora,* a cross is made between ab^+ and a^+b individuals. The following one hundred ordered tetrads are obtained:

Spores	I	II	III	IV	V	VI	VII	VIII
1, 2	a^+b	a^+b	a^+b	a^+b^+	a^+b^+	a^+b	a^+b	ab^+
3, 4	a^+b	a^+b^+	a^+b^+	a^+b	a^+b	ab^+	ab^+	a^+b
5, 6	ab^+	ab	ab^+	ab	ab^+	a^+b	ab^+	a^+b
7, 8	ab^+	ab^+	ab	ab^+	ab	ab^+	a^+b	ab^+
	85	2	3	2	3	3	1	1

a. Are genes a and b linked? How do you know?
b. Calculate the gene-to-centromere distances for a and b.

26. *Neurospora* has four genes—*a*, *b*, *c*, and *d*—that control four different phenotypes. Your job is to map these genes by performing pairwise crosses. You obtain the following ordered tetrads:

	$ab^+ \times a^+b$				$bc^+ \times b^+c$		
Spores	**I**	**II**	**III**	**Spores**	**I**	**II**	**III**
1, 2	ab^+	ab	ab^+	1, 2	bc^+	b^+c^+	b^+c
3, 4	ab^+	ab	a^+b^+	3, 4	bc^+	b^+c^+	b^+c^+
5, 6	a^+b	a^+b^+	a^+b	5, 6	b^+c	bc	bc
7, 8	a^+b	a^+b^+	ab	7, 8	b^+c	bc	bc^+
	45	43	12		70	4	26

	$cd^+ \times c^+d$						
Spores	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
1, 2	cd^+	cd	cd	cd	cd^+	cd	cd^+
3, 4	cd^+	cd	cd^+	c^+d	c^+d	c^+d^+	c^+d
5, 6	c^+d	c^+d^+	c^+d^+	c^+d^+	c^+d	c^+d^+	c^+d^+
7, 8	c^+d	c^+d^+	c^+d	cd^+	cd^+	cd	cd
	42	2	30	15	5	1	5

a. Calculate the gene-to-centromere distances.
b. Which genes are linked? Explain.
c. Derive a complete map for all four genes.

27. You have isolated a new fungus and have obtained a strain that requires both arginine (arg^-) and adenine (ad^-). You cross these two strains and collect four hundred random spores that you plate on minimal medium. If twenty-five spores grow, what is the distance between these two genes?

28. Three distinct genes, *pab*, *pk*, and *ad*, were scored in a cross of *Neurospora.* From the cross $pab\ pk^+\ ad^+ \times pab^+\ pk\ ad$, the following ordered tetrads were recovered:

Spores	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
1, 2	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad^+$	$pab\ pk^+\ ad$	$pab\ pk^+\ ad$
3, 4	$pab\ pk^+\ ad^+$	$pab^+pk\ ad$	$pab\ pk\ ad$	$pab\ pk^+ad$	$pab^+\ pk\ ad$	$pab^+\ pk\ ad$	$pab^+\ pk\ ad$	$pab^+pk\ ad^+$
5, 6	$pab^+pk\ ad$	$pab\ pk^+ad^+$	$pab^+pk^+ad^+$	$pab^+pk\ ad^+$	$pab\ pk\ ad$	$pab\ pk^+ad$	$pab^+pk\ ad^+$	$pab\ pk^+ad^+$
7, 8	$pab^+pk\ ad$	$pab^+pk\ ad$	$pab^+pk\ ad$	$pab^+pk\ ad$	$pab^+pk^+\ ad^+$	$pab^+pk\ ad^+$	$pab\ pk^+ad^+$	$pab^+pk\ ad$
	34	35	9	7	2	2	1	3

Based on the data, construct a map of the three genes. Be sure to indicate centromeres.

HUMAN CHROMOSOMAL MAPS

29. The Duffy blood group with alleles FY^a and FY^b was localized to chromosome 1 in human beings when an "uncoiled" chromosome was associated with it. Construct a pedigree that would verify this.

30. What pattern of scores would you expect to get, using the hybrid clones in table 6.8, for a locus on human chromosome 6? 14? X?

31. A man with X-linked color blindness and X-linked Fabry disease (alpha-galactosidase-A deficiency) mates with a normal woman and has a normal daughter. This daughter then mates with a normal man and produces ten sons (as well as eight normal daughters). Of the sons, five are normal, three are like their grandfather, one is only color-blind, and one has Fabry disease. From these data, what can you say about the relationship of these two X-linked loci?

32. In people, the ABO system (I^A, I^B, i alleles) is linked to the aldolase-B locus (*ALDOB*), a gene that functions in the liver. Deficiency, which is recessive, results in fructose intolerance. A man with blood type AB has a fructose-intolerant, type B father and a normal, type AB mother. He and a woman with blood type O and fructose intolerance have ten children. Five are type A and normal, three are fructose intolerant and type B, and two are type A and intolerant to fructose. Draw a pedigree of this family and determine the map distances involved. (Calculate a *lod* score to determine the most likely recombination frequency between the loci.)

33. Hemophilia and color-blindness are X-linked recessive traits. A normal woman whose mother was color-blind and whose father was a hemophiliac mates with a normal man whose father was color-blind. They have the following children:

4 normal daughters
1 normal son
2 color-blind sons
2 hemophiliac sons
1 color-blind, hemophiliac son

Estimate the distance between the two genes.

34. The results of an analysis of five human-mouse hybrids for five enzymes are given in table along with the human chromosomal content of each clone (+ = enzyme or chromosome present; − = absent). Deduce which chromosome carries which gene.

	Clone				
Human Enzyme	**A**	**B**	**C**	**D**	**E**
glutathione reductase	+	+	−	−	−
malate dehydrogenase	−	+	−	−	−
adenosine deaminase	−	+	−	+	+
galactokinase	−	+	+	−	−
hexosaminidase	+	−	−	+	−

	Human Chromosome																					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Clone A	−	−	−	−	+	+	+	+	−	+	−	−	−	−	+	+	−	−	−	−	+	+
Clone B	+	+	−	+	−	−	−	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−
Clone C	−	−	−	+	−	−	+	−	−	+	−	+	+	+	+	−	+	−	+	−	−	+
Clone D	+	−	+	−	+	−	−	−	−	+	−	−	−	+	+	−	−	+	+	+	+	−
Clone E	−	−	−	+	−	−	−	−	+	+	+	+	−	+	−	+	−	+	−	+	+	+

35. You have selected three mouse-human hybrid clones and analyzed them for the presence of human chromosomes. You then analyze each clone for the presence or absence of particular human enzymes (+ = presence of human chromosome or enzyme activity). Based on the following results indicate the probable chromosomal location for each enzyme.

	Human Chromosomes						
Clone	**3**	**7**	**9**	**11**	**15**	**18**	**20**
X	−	+	−	+	+	−	+
Y	+	+	−	+	−	+	−
Z	−	+	+	−	−	+	+

	Enzyme				
Clone	**A**	**B**	**C**	**D**	**E**
X	+	+	−	−	+
Y	+	−	+	+	+
Z	−	−	+	−	+

36. Three mouse-human cell lines were scored for the presence (+) or absence (−) of human chromosomes, with the results as follows:

	Human Chromosomes							
Clone	**1**	**2**	**3**	**4**	**5**	**14**	**15**	**18**
A	+	+	+	+	−	−	−	−
B	+	+	−	−	+	+	−	−
C	+	−	+	−	+	−	+	−

If a particular gene is located on chromosome 3, which clones should be positive for the enzyme from that gene?

CRITICAL THINKING QUESTIONS

1. Do three-point crosses in fruit flies capture all the multiple crossovers in a region?
2. If 4% of all tetrads have a single crossover between two loci: (a) What is the map distance between these loci if these are fruit flies? (b) What is the proportion of second-division segregants if these are *Neurospora?* (c) What is the proportion of nonparental ditypes if these are yeast?

Suggested Readings for chapter 6 are on page B-3.

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7

LINKAGE AND MAPPING IN PROKARYOTES AND BACTERIAL VIRUSES

STUDY OBJECTIVES

1. To define bacteria and bacterial viruses and learn about methods of studying them 149
2. To study life cycles and sexual processes in bacteria and bacteriophages 154, 163
3. To make use of the sexual processes of bacteria and their viruses to map their chromosomes 155, 166

STUDY OUTLINE

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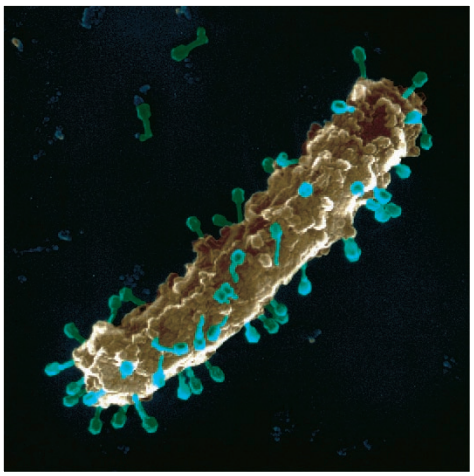


Scanning electron micrograph (color enhanced) of an *Escherichia coli* bacterium with adsorbed T-family bacteriophages (36,000x). (© Oliver Meckes/MPI-Tubingen/Photo Researchers.)

All organisms and viruses have genes located sequentially in their genetic material; and almost all can undergo recombination between homologous (equivalent) pieces of genetic material. Because recombination can occur, it is possible to map, by analytical methods, the locations and sequence of genes along the chromosomes of all organisms and almost all viruses. In this chapter, the viruses we look at are those that attack bacteria. Through work with bacteria and viruses, we have entered the modern era of molecular genetics, the subject of the next section of this book.

Bacteria (including the cyanobacteria, the blue-green algae) are prokaryotes. The prokaryotes also include the **archaea,** or archaebacteria, a kingdom recognized in 1980. These highly specialized organisms (previously classified as bacteria), along with the bacteria and eukaryotes, make up the three domains of life on Earth.

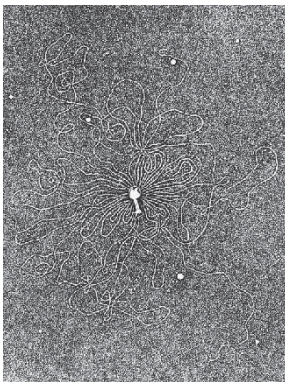
The true bacteria can be classified according to shape: a spherical bacterium is called a **coccus;** a rod-shaped bacterium is called a **bacillus;** and a spiral bacterium is called a **spirillum.** Prokaryotes do not undergo mitosis or meiosis but simply divide in two after their chromosome (usually only one), most often a circle of DNA, has replicated (see chapter 9). Bacterial viruses do not even divide; they are mass-produced within a host cell.

BACTERIA AND BACTERIAL VIRUSES IN GENETIC RESEARCH

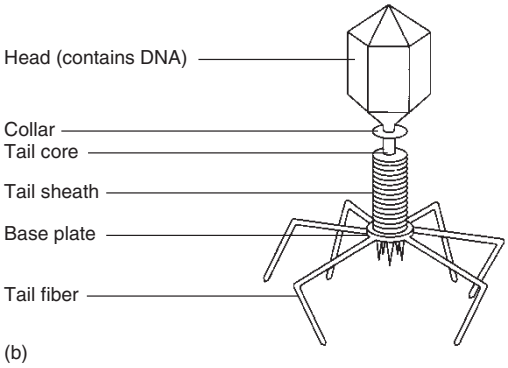
Several properties of bacteria and viruses make them especially suitable for genetic research. First, bacteria and their viruses generally have a short generation time. Some viruses increase three-hundredfold in about a half hour; an *Escherichia coli* cell divides every twenty minutes. In contrast, generation time is fourteen days in fruit flies, a year in corn, and twenty years or so in human beings. (*E. coli,* the common intestinal bacterium, was discovered by Theodor Escherich in 1885.)

Another reason bacteria and bacterial viruses are so well-suited for genetic research is because they have much less genetic material than eukaryotes do, and the organization of this material is much simpler. The term *prokaryote* arises from the lack of a true nucleus (*pro* means before and *karyon* means kernel or nucleus); they have no nuclear membranes (see fig. 3.2) and usually have only a single, relatively "naked" chromosome, so they are haploid. Bacteria may, however, contain small, auxiliary circles of DNA, called **plasmids.** Bacterial viruses are even simpler. Although animal and plant viruses, discussed in more detail later in the book (chapters 13 and 16), can be more complicated, the viruses we are interested in studying in this chapter—the bacterial viruses, **bacteriophages,** or just **phages** (Greek: eating)—are exclusively genetic material surrounded by a protein coat.

Bacteriophages are usually classified first by the type of genetic material (nucleic acid) they have (DNA or RNA, single- or double-stranded), then by structural features of their protein surfaces **(capsids)** such as type or symmetry and number of discrete protein subunits **(capsomeres)** in the capsid, and general size. Most bacteriophages are complex, like T2 (fig. 7.1), or made up of a headlike capsule like T2 without the tail appendages, or filamentous. Most contain double-stranded DNA. Bacteriophages are obligate parasites; outside of a host, they are inert molecules. Once their genetic material penetrates a host cell, they can take over the metabolism of that cell and construct multiple copies of themselves. We will discuss details of this and alternative infection



(a)



(b)

Figure 7.1 Phage T2 and its chromosome. (*a*) The chromosome, which is about 50 μm long, has burst from the head. (*b*) The intact phage. The phage attaches to a bacterium using its tail fibers and base plate and then injects its genetic material into the host cell. ([*a*] A. K. Kleinschmidt, et al., "Darstellung und Langen messungen des gesamten Deoxyribose-nucleinsaure Inhaltes von T2-Bacteriophagen" *Biochemica et Biophysica Acta,* 61:857–64, 1962. Reproduced by permission of Elsevier Science Publishers.)

pathways later in the chapter. The smallest bacteriophages (e.g., R17) have RNA as their genetic material and contain just three genes, one each for a coat protein, an attachment protein, and an enzyme to replicate their RNA. The larger bacteriophages (T2, T4) have DNA as their genetic material and contain up to 130 genes.

A third reason for the use of bacteria and viruses in genetic study is their ease of handling. A researcher can handle millions of bacteria in a single culture with a minimal amount of work compared with the effort required to grow the same number of eukaryotic organisms such as fruit flies or corn. (Some eukaryotes, such as yeast or *Neurospora,* can, of course, be handled using prokaryotic techniques, as we saw in chapter 6.) Let us look at an expansion of the techniques, introduced in chapter 6, that geneticists use in bacterial and viral studies.

TECHNIQUES OF CULTIVATION

All organisms need an energy source, a carbon source, nitrogen, sulfur, phosphorus, several metallic ions, and water. Those that require an organic form of carbon are termed **heterotrophs.** Those that can utilize carbon as carbon dioxide are termed **autotrophs.** All bacteria obtain their energy either by photosynthesis or chemical oxidation. Bacteria are usually grown in or on a chemically defined **synthetic medium,** either in liquid in flasks or test tubes, or on petri plates using an agar base to supply rigidity. When one cell is placed on the medium in the plate, it will begin to divide. After incubation, often overnight, a colony, or clone, will exist where previously was only one cell. Overlapping colonies form a confluent growth (fig. 7.2). A culture medium that has only the minimal necessities required by the bacterial species is referred to as *minimal medium* (table 7.1).

Alternatively, bacteria can grow on a medium that supplies, in addition to their minimal requirements, the more complex substances that the bacteria normally synthesize, including amino acids, vitamins, and so on. A medium of this kind allows the growth of strains of bacteria, called **auxotrophs,** that have particular nutritional requirements. (The parent, or wild-type, strain is referred to as a **prototroph.**) For example, a strain that has an enzyme defect in the pathway that produces the amino acid histidine will not grow on a minimal medium because it has no way of obtaining histidine; it is a histidine-requiring auxotroph. If, however, histidine were provided in the medium, the organisms could grow. This type of mutant is called a **conditional-lethal mutant.** The organism would normally die, but under appropriate conditions, such as the addition of histidine, the organism can survive.

This histidine-requiring auxotrophic mutant can grow only on an **enriched** or **complete medium,** whereas the parent prototroph could grow on a minimal medium. Media are often enriched by adding complex mixtures of organic substances such as blood, beef extract, yeast extract, or peptone, a digestion product. Many media, however, are made up of a minimal medium with the addition of only one other substance, such as an amino acid or a vitamin. These are called **selective media;** we will discuss their uses later in the chapter. In addition to minimal, complete, and selective media, other media exist for specific purposes such as aiding in counting colonies, helping maintain cells in a nongrowth phase, and so on.

Table 7.1 **Minimal Synthetic Medium for Growing *E. coli,* a Heterotroph**

Component	Quantity
$NH_4H_2PO_4$	1 g
Glucose	5 g
NaCl	5 g
$MgSO_4 \cdot 7H_2O$	0.2 g
K_2HPO_4	1 g
H_2O	1,000 ml

Source: Data from M. Rogosa, et al., *Journal of Bacteriology,* 54:13, 1947.

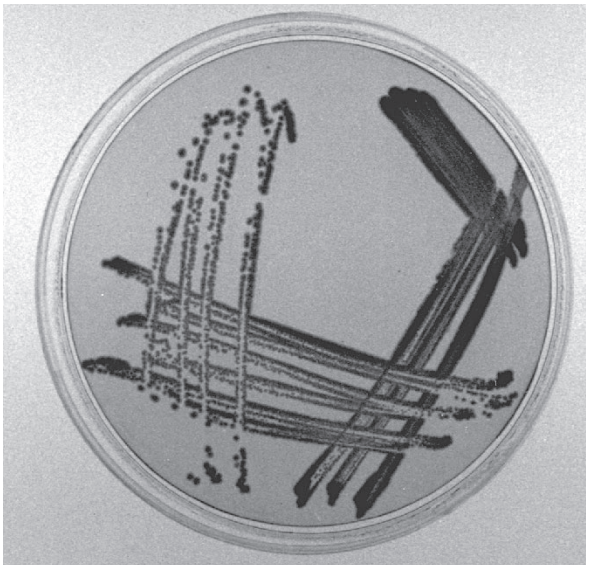


Figure 7.2 Confluent growth of bacterial colonies on a petri plate. Bacteria were streaked on the petri plate with an inoculation loop—a metal wire with a looped end—covered with bacteria. Streaks began at the upper right and continued around clockwise. With a heavy inoculation on the loop, bacterial growth is confluent. Eventually, only a few bacteria are left; they form single colonies at the upper left. (Photo by Robert Tamarin.)

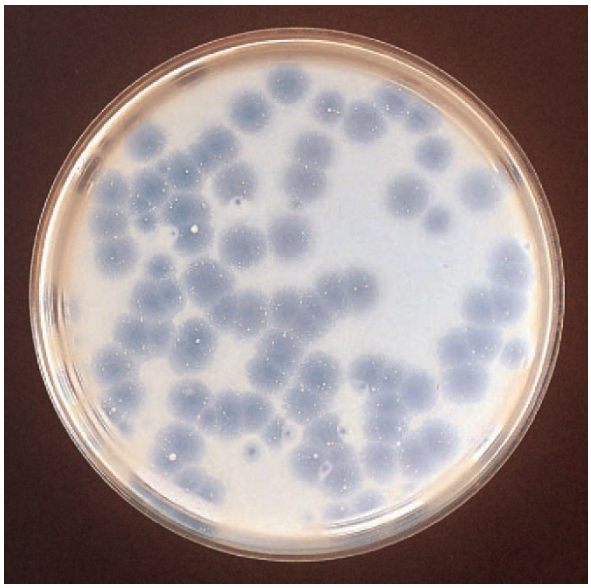


Figure 7.3 Viral plaques (phage T1) on a bacterial lawn of *E. coli.* (© Bruce Iverson, BSc.)

The experimental cultivation of viruses is somewhat different. Since viruses are obligate parasites, they can grow only in living cells. Thus, for the cultivation of phages, petri plates of appropriate media are inoculated with enough bacteria to form a continuous cover, or **bacterial lawn.** This bacterial culture serves as a medium for the growth of viruses added to the plate. Since the virus attack usually results in rupture, or **lysis,** of the bacterial cell, addition of the virus usually produces clear spots, known as **plaques,** on the petri plates (fig. 7.3). Large quantities of viruses can be grown in flasks of bacteria.

BACTERIAL PHENOTYPES

Bacterial phenotypes fall into three general classes: colony morphology, nutritional requirements, and drug or infection resistance.

Colony Morphology

The first of these classes, colony morphology, relates simply to the form, color, and size of the colony that grows from a single cell. A bacterial cell growing on a petri plate in an incubator at 37° C divides as frequently as once every twenty minutes. Each cell gives rise to a colony, or clone, at its original position. In a relatively short amount of time (e.g., overnight), the colonies will consist of enough cells to be seen with the unaided eye. The different morphologies observed among the colonies are usually under genetic control (fig. 7.4).

Nutritional Requirements

The second basis for classifying bacteria—by their nutritional requirements—reflects the failure of one or more enzymes in the bacteria's biosynthetic pathways. If an auxotroph has a requirement for the amino acid cysteine that the parent strain (prototroph) does not have, then that auxotroph most likely has a nonfunctional enzyme in the pathway for the synthesis of cysteine. Figure 7.5 shows five steps in cysteine synthesis; a different enzyme controls each step. All enzymes are proteins, and the information in one or more genes determines the sequences in the strings of amino acids that make up those proteins (chapter 11). A normal or wild-type allele

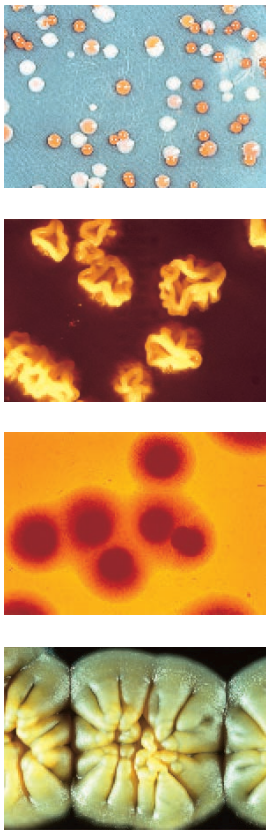


Figure 7.4 Various bacterial colony forms on agar petri plates. (*a*) Red and white colonies of *Serratia marcescens.* (*b*) Irregular raised folds of *Streptomyces griseus*. (*c*) Round colonies with concentrated centers and diffuse edges of *Mycoplasma.* (*d*) Irregularly folded raised colonies of *Streptomyces antibioticus.* ([*a*] © Dr. E. Buttone/Peter Arnold, Inc., [*b*] © C. Case/Visuals Unlimited, [*c*] © Michael G. Gabridge/Visuals Unlimited, [*d*] © Cabisco/Visuals Unlimited.)

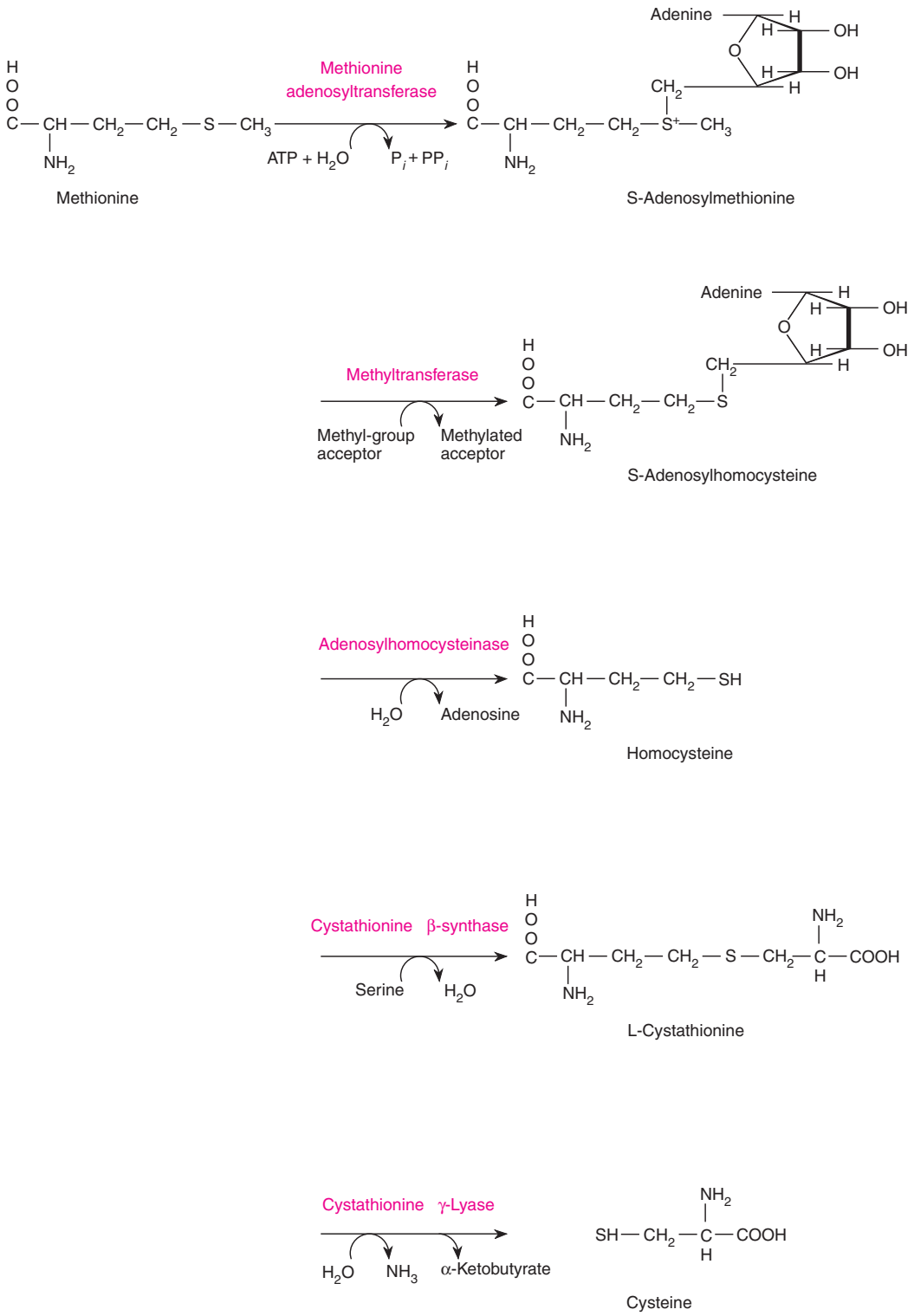
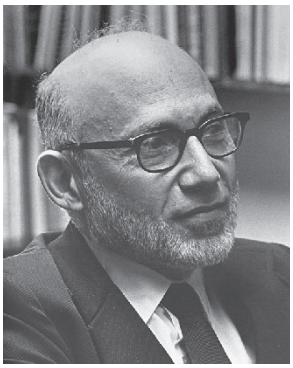


Figure 7.5 Five-step conversion of methionine to cysteine. Each step is controlled by a different enzyme (*red*).



Joshua Lederberg (1925–).
(Courtesy of Dr. Joshua Lederberg.)

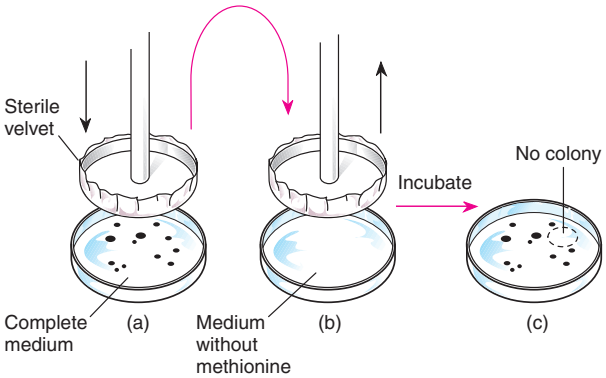


Figure 7.6 Replica-plating technique. (*a*) A pattern of colonies from a plate of complete medium is transferred (*b*) to a second plate of medium that lacks methionine. (*c*) In the locations where colonies fail to grow on the second plate, we can infer that the original colony was a methionine-requiring auxotroph.

produces a normal, functional enzyme. The alternative allele may produce a nonfunctional enzyme. Recall the one-gene-one-enzyme hypothesis from chapter 2.

A technique known as **replica-plating,** devised by Joshua Lederberg, is a rapid **screening technique** that makes it possible to determine quickly whether a given strain of bacteria is auxotrophic for a particular metabolite. In this technique, a petri plate of complete medium is inoculated with bacteria. The resulting growth will have a certain configuration of colonies. This plate of colonies is pressed onto a piece of sterilized velvet. Then any number of petri plates, each containing a medium that lacks some specific metabolite, can be pressed onto this velvet to pick up inocula in the same pattern as the growth on the original plate (fig. 7.6). If a colony grows on the complete medium but does not grow on a plate with a medium missing a metabolite, the inference is that the colony is made of auxotrophic cells that require the absent metabolite. Samples of this bacterial strain can be obtained from the colony growing on complete medium for further study. The nutritional requirement of this strain is its phenotype. The methionine-requiring auxotroph of figure 7.6 would be designated as Met^- (methionine-minus or Met-minus).

In terms of energy sources, the plus or minus notation has a different meaning. For example, a strain of bacteria that can utilize the sugar galactose as an energy source would be Gal^+. If it could not utilize galactose, it would be called Gal^-. The latter strain will not grow if galactose is its sole carbon source. It will grow if a sugar other than galactose is present. Note that a Met^- strain needs methionine to grow, whereas a Gal^- strain needs a carbon source *other* than galactose; it cannot use galactose.

Resistance and Sensitivity

The third common classification of phenotypes in bacteria involves resistance and sensitivity to drugs, phages, and other environmental insults. For example, penicillin, an antibiotic that prevents the final stage of cell-wall construction in bacteria, will kill growing bacterial cells. Nevertheless, we frequently find a number of cells that do grow in the presence of penicillin. These colonies are resistant to the drug, and this resistance is under simple genetic control. The phenotype is penicillin resistant (Pen^r) as compared with penicillin sensitive (Pen^s), the normal condition, or wild-type. Numerous antibiotics are used in bacterial studies (table 7.2).

Table 7.2 **Some Antibiotics and Their Antibacterial Mechanisms**

Antibiotic	Microbial Origin	Mode of Action
Penicillin G	*Penicillium chrysogenum*	Blocks cell-wall synthesis
Tetracycline	*Streptomyces aureofaciens*	Blocks protein synthesis
Streptomycin	*Streptomyces griseus*	Interferes with protein synthesis
Terramycin	*Streptomyces rimosus*	Blocks protein synthesis
Erythromycin	*Streptomyces erythraeus*	Blocks protein synthesis
Bacitracin	*Bacillus subtilis*	Blocks cell-wall synthesis

Drug sensitivity provides another screening technique for isolating nutritional mutations. For example, if we were looking for mutants that lacked the ability to synthesize a particular amino acid (e.g., methionine), we could grow large quantities of bacteria (prototrophs) and then place them on a medium that lacked methionine but contained penicillin. Here, any growing cells would be killed. But methionine auxotrophs would not grow, and, therefore, they would not be killed. The penicillin could then be washed out and the cells reinoculated onto a complete medium. The only colonies that form should be composed of methionine auxotrophs (Met^-).

Screening for resistance to phages is similar to screening for drug resistance. When bacteria are placed in a medium containing phages, only those bacteria that are resistant to the phages will grow and produce colonies. They can thus be easily isolated and studied.

VIRAL PHENOTYPES

Bacteriophage phenotypes fall generally into two categories: plaque morphology and growth characteristics on different bacterial strains. For example, T2, an *E. coli* phage (see fig. 7.1), produces small plaques with fuzzy edges (genotype r^+). Rapid-lysis mutants (genotype r) produce large, smooth-edged plaques (fig. 7.7). Similarly, T4, another *E. coli* phage, has rapid-lysis mutants that produce large, smooth-edged plaques on *E. coli* B but will not grow at all on *E. coli* K12, a different strain. Here, rapid-lysis mutants illustrate both the colony morphology phenotypes and the growth-restriction phenotypes of phages.

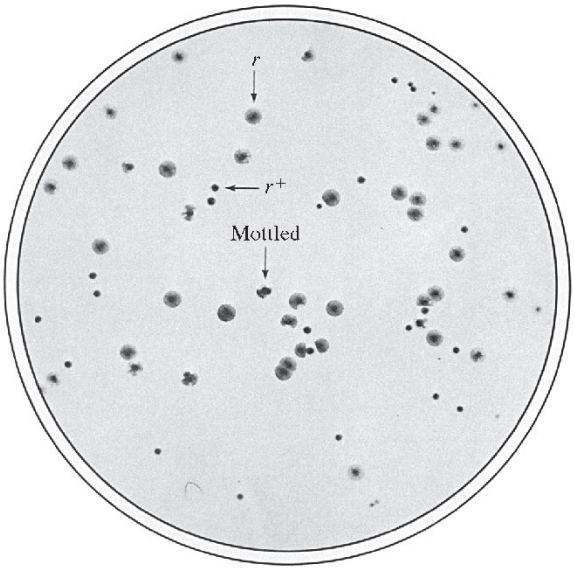


Figure 7.7 Normal (r^+) and rapid-lysis (r) mutants of phage T2. Mottled plaques occur when r and r^+ phages grow together. (From *Molecular Biology of Bacterial Viruses* by Gunther S. Stent. © 1963, 1978 by W. H. Freeman and Company. Used with permission.)

SEXUAL PROCESSES IN BACTERIA AND BACTERIOPHAGES

Although bacteria and viruses are ideal subjects for biochemical analysis, they would not be useful for genetic study if they did not have sexual processes. If we define a sexual process as combining genetic material from two individuals, then the life cycles of bacteria and viruses include sexual processes. Although they do not undergo sexual reproduction by the fusion of haploid gametes, bacteria and viruses do undergo processes that incorporate genetic material from one cell or virus into another cell or virus, forming recombinants. Actually, bacteria have three different methods to gain access to foreign genetic material: **transformation, conjugation,** and **transduction** (fig. 7.8).

Phages can exchange genetic material when a bacterium is infected by more than one virus particle **(virion).** During the process of viral infection, the genetic material of different phages can exchange parts (or recombine; see fig. 7.8). We will examine the exchange processes in bacteria and then in bacteriophages, and then proceed to the use of these methods for mapping bacterial and viral chromosomes. (*Chromosome* refers to the structural entity in the cell or virus made up of the genetic material. In eukaryotes, it is double-stranded DNA complexed with proteins [chapter 15]. Staining of this eukaryotic organelle led to the term *chromosome,* which means "colored body." In prokaryotes, the chromosome is a circle [usually] of double-stranded DNA. In viruses, it is virtually any combination of linear or circular, single- or double-stranded RNA or DNA. Sometimes the term **genophore** is used for the prokaryotic and viral genetic material, limiting the word *chromosome* to the eukaryotic version. We will use the term *chromosome* for the intact genetic material of any organism or virus.)

Transformation

Transformation was first observed in 1928 by F. Griffith and was examined at the molecular level in 1944 by O. Avery and his colleagues, who used the process to demonstrate that DNA was the genetic material of bacteria. Chapter 9 presents the details of these experiments. In transformation, a cell takes up extraneous DNA found in the environment and incorporates it into its genome

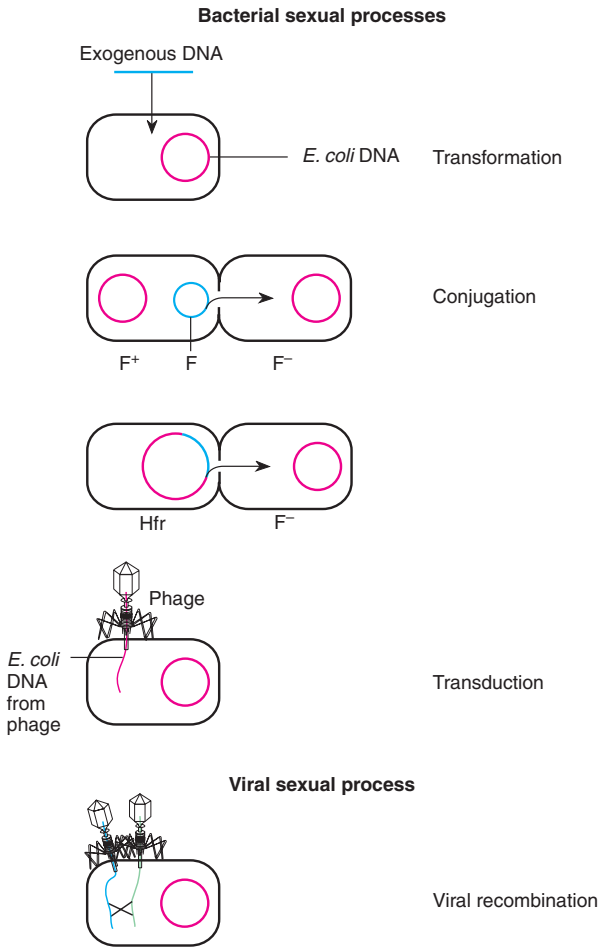


Figure 7.8 Summary of bacterial and viral sexual processes.

(genetic material) through recombination. Not all bacteria are competent to be transformed, and not all extracellular DNA is competent to transform. To be competent to transform, the extracellular DNA must be double-stranded. To be competent to be transformed, a cell must have the surface protein, **competence factor,** which binds to the extracellular DNA in an energy-requiring reaction. However, bacteria that are not naturally competent can be treated to make them competent, usually by treatment with calcium chloride, which makes them more permeable.

Mechanisms of Transformation

Under natural conditions, only one of the strands of extracellular DNA is brought into the cell. The single strand brought into the cell can then be incorporated into the host genome by two crossovers (fig. 7.9). (The molecular mechanisms of crossing over are presented in chapter 12.) Note that unlike eukaryotic crossing over, this is not a reciprocal process. The bacterial chromosome incorporates part of the foreign DNA. The remaining single-stranded DNA, originally part of the bacterial chromosome, is degraded by host enzymes called exonucleases; linear DNA is degraded rapidly in prokaryotes.

Transformation is a very efficient method of mapping in some bacteria, especially those that are inefficient in other mechanisms of DNA intake (such as transduction, discussed later in this chapter). For example, a good deal of the mapping of the soil bacterium, *Bacillus subtilis,* has been done through the process of transformation; *E. coli,* however, is inefficient in transformation, so other methods are used to map its chromosome.

Transformation Mapping

The general idea of transformation mapping is to add DNA from a bacterial strain with known genotype to another strain, also with known genotype, but with different alleles at two or more loci. We then look for incorporation of the donor alleles into the recipient strain of bacteria. The more often alleles from two loci are incorporated together into the host, the closer together these loci must be to each other. Thus, we can use an index of co-occurrence that is in inverse relationship to map distance: the larger the co-occurrence of alleles of two loci, the closer together the loci must be. This is another way of looking at the mapping concepts we discussed in

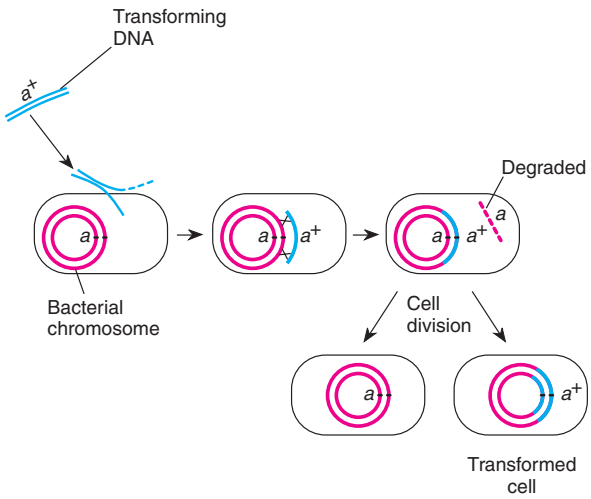


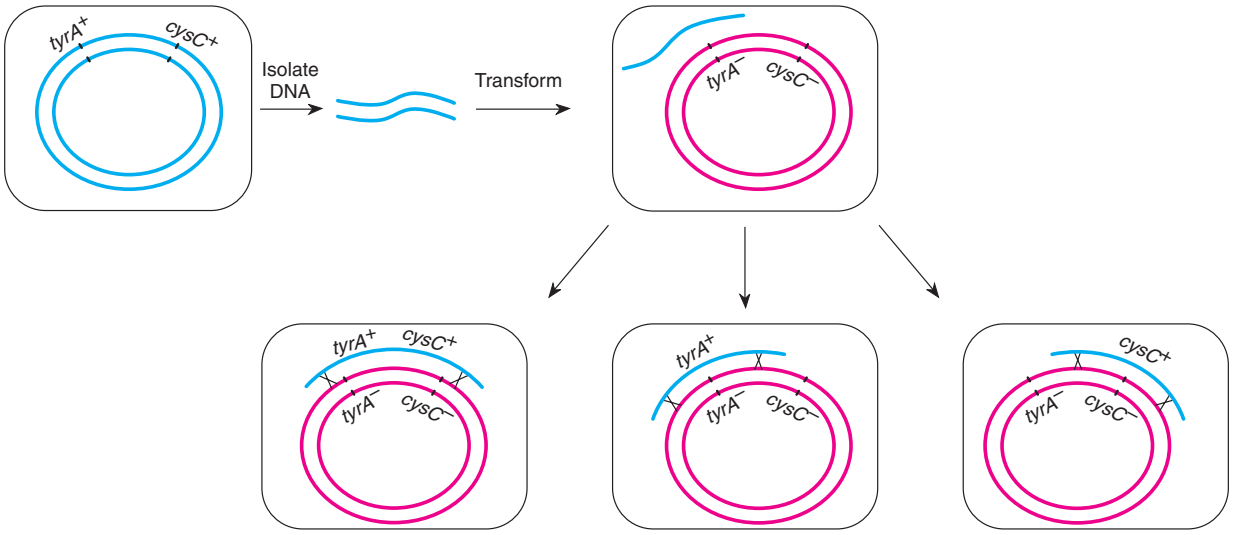
Figure 7.9 A single strand of transforming DNA (*blue* with a^+ allele) enters a bacterial cell (*red* chromosome with *a* allele). Two crossovers bring the foreign DNA into the bacterial chromosome. After DNA replication and cell division, one cell has the *a* allele and the other the a^+ allele. The chromosome is drawn as a double circle, symbolizing the double-stranded structure of DNA.

chapter 6, where we discovered that the closer two loci are, the fewer the recombinations between them and thus the higher the co-occurrence.

Now, we also must look at another concept, that is, selecting for recombinant cells. In fruit flies, every offspring of a mated pair represents a sampling of the meiotic tetrad, and thus a part of the total, whether or not recombination took place. Here, however, many cells are present that do not take part in the transformation process. In a bacterial culture, for example, only one cell in a thousand might be transformed. We must thus always be sure when working with bacterial gene transfer that we count only those cells that have taken part in the process. Let us look at an example.

A recipient strain of *B. subtilis* is auxotrophic for the amino acids tyrosine ($tyrA^-$) and cysteine ($cysC^-$). We are interested in how close these loci are on the bacterial chromosome. We thus isolate DNA from a prototrophic strain of bacteria ($tyrA^+$ $cysC^+$). We add this donor DNA to the auxotrophic strain and allow time for transformation to take place (fig. 7.10). If the experiment is successful, and the loci are close enough together, then some of the recipient bacteria may incorporate donor DNA that has either both donor alleles or one or the other donor allele. Thus, some of the recipient cells will now have the $tyrA^+$ and $cysC^+$ alleles, some will have just the donor $tyrA^+$ allele, some will have just the donor $cysC^+$ allele, and the overwhelming majority will be of the untransformed auxotrophic genotype, $tyrA^-$ $cysC^-$. We thus need to count the transformed cells.

We do this by removing any extraneous transforming DNA and then pouring the cells out onto a complete medium so that all cells can grow. These cells are then replica-plated onto three plates—a minimal medium plate, a minimal medium plus tyrosine plate, and a minimal medium plus cysteine plate—and allowed to grow overnight in an incubator at 37° C. We then count colonies (fig. 7.11). Those growing on minimal medium are of genotype $tyrA^+$ $cysC^+$; those growing on minimal medium with tyrosine but not growing on minimal medium are $tyrA^-$ $cysC^+$; and those growing on minimal medium with cysteine but not growing on minimal medium are $tyrA^+$ $cysC^-$. The overwhelming majority will grow on complete medium, but not on minimal medium or minimal media with just tyrosine or cysteine added. This majority is made up of the nontransformants, that is, auxotrophs that were not involved in a transformation event—they took up no foreign DNA.



Growth	$tyrA^+$ $cysC^+$	$tyrA^+$ $cysC^-$	$tyrA^-$ $cysC^+$
Complete medium	+	+	+
Minimal medium	+	−	−
Minimal + tyrosine	+	−	+
Minimal + cysteine	+	+	−

Figure 7.10 Transformation experiment with *B. subtilis*. A $tyrA^-$ $cysC^-$ strain is transformed with DNA from a $tyrA^+$ $cysC^+$ strain. Nontransformants as well as three types of transformants (two single and one double) result. Genotypes are determined by growth characteristics on four different types of petri plates (see fig. 7.11).

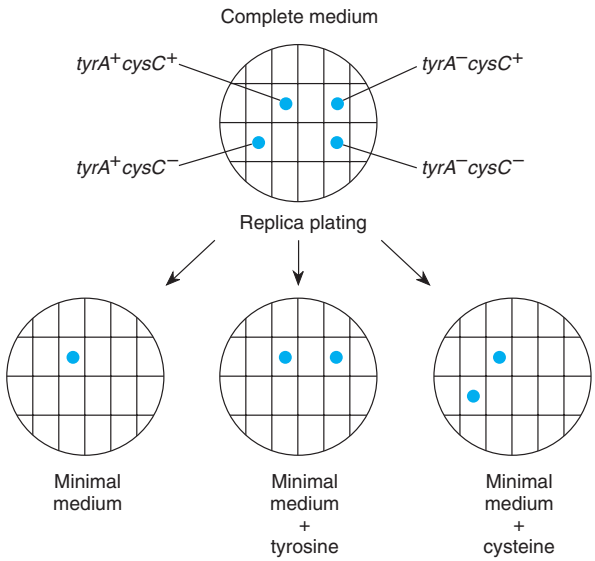


Figure 7.11 Four patterns of growth on different media reveal the genotypes of transformed and untransformed cells. Only four colonies are shown, and a grid is added for ease of identification. After transformation (see fig. 7.10), cells are plated on complete medium and then replica-plated onto minimal medium with either tyrosine or cysteine added.

As a control against reversion, the normal mutation of $tyrA^-$ to $tyrA^+$ or $cysC^-$ to $cysC^+$, we grow several plates of auxotrophs in minimal medium and minimal medium with tyrosine or cysteine added. These are auxotrophs that were not exposed to prototrophic donor DNA. We then count the number of natural revertants and correct our experimental numbers by the natural reversion rate. Thus, we are sure that what we measure is the actual transformation rate rather than just a mutation rate that we mistake for transformation. This control should *always* be carried out.

From the experiment (see figs. 7.10 and 7.11), we count twelve double transformants ($tyrA^+$ $cysC^+$), thirty-one $tyrA^+$ $cysC^-$, and twenty-seven $tyrA^-$ $cysC^+$. From these data, we calculate the co-occurrence, or cotransfer index, (r) as

$$r = \frac{\text{number of double transformants}}{\text{number of double transformants} + \text{number of single transformants}}$$

From our data

$$r = 12/(12 + 31 + 27) = 0.17.$$

This is a relative number indicating the co-occurrence of the two loci and thus their relative distance apart on the bacterial chromosome. Remember that as this number increases for different pairs of loci, the loci are closer and closer together.

By systematically examining many loci, we can establish their relative order. For example, if locus *A* is closely linked to locus *B* and *B* to *C,* we can establish the order *A B C.* It is not possible by this method to determine exact order for very closely linked genes. For this information we need to rely upon transduction, which we will consider shortly. However, transformation has allowed us to determine that the map of *B. subtilis* is circular, a phenomenon found in all prokaryotes and many phages. (The *E. coli* map is shown later.)

Conjugation

In 1946, Joshua Lederberg and Edward L. Tatum (later to be Nobel laureates) discovered that *E. coli* cells can exchange genetic material through the process of conjugation. They mixed two auxotrophic strains of *E. coli.* One strain required methionine and biotin (Met^- Bio^-), and the other required threonine and leucine (Thr^- Leu^-). This cross is shown in figure 7.12. Remember that if a strain is Met^- Bio^-, it is, without saying, wild-type for all other loci. Thus, a cell with the Met^- Bio^- phenotype actually has the genotype of met^- bio^- thr^+ leu^+. Similarly, the Thr^- Leu^- strain is actually met^+ bio^+ thr^- leu^-. (Note that symbols such as "Thr^-" represent phenotypes; symbols such as "thr^-" represent genotypes.)

Lederberg and Tatum used multiple auxotrophs in order to rule out spontaneous reversion (mutation). About one in 10^6 Met^- cells will spontaneously become prototrophic (Met^+) every generation. However, with multiple auxotrophs, the probability that several loci will simultaneously and spontaneously revert (e.g., $met^- \rightarrow met^+$) becomes vanishingly small. (In fact, the control plates in the experiment, illustrated in fig. 7.12, showed no growth for parental double mutants.) After mixing the strains, Lederberg and Tatum found that about one cell in 10^7 was prototrophic (met^+ bio^+ thr^+ leu^+).

To rule out transformation, one strain was put in each arm of a U-tube with a sintered glass filter at the bottom. (fig. 7.13). The liquid and large molecules, including DNA, were mixed by alternate application of pressure and suction to one arm of the tube; whole cells did not pass through the filter. The result was that the fluids surrounding the cells, as well as any large molecules (e.g., DNA), could be freely mixed while the cells were kept separate. After cell growth stopped in the two arms (in complete medium), the contents were plated out on minimal medium. There were no prototrophs in either arm. Therefore, cell-to-cell contact was required for the genetic material of the two cells to recombine.

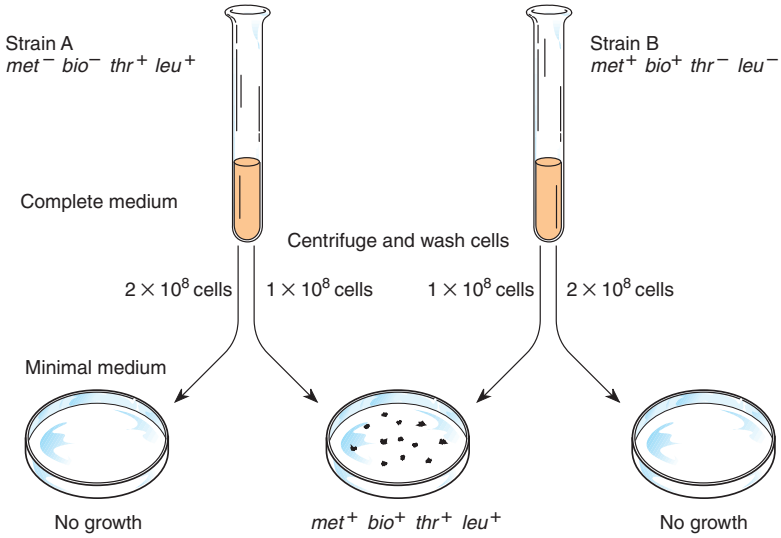


Figure 7.12 Lederberg and Tatum's cross showing that *E. coli* undergoes genetic recombination.

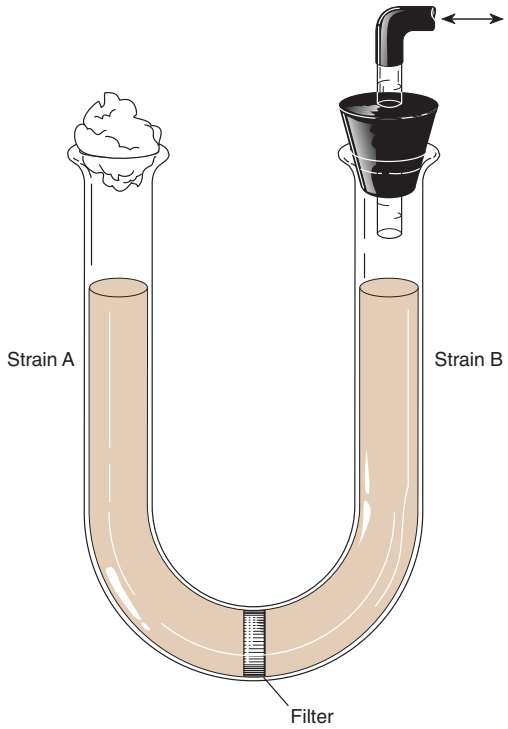


Figure 7.13 The U-tube experiment. Alternating suction and pressure force liquid and macromolecules back and forth across the filter.

F Factor

In bacteria, conjugation is a one-way transfer, with one strain acting as donor and the other as recipient. Sometimes donor cells, if stored for a long time, lose the ability to be donors, but they can regain the ability if they are mated with other donor strains. This discovery led to the hypothesis that a **fertility factor, F,** made any strain that carried it a male (donor) strain, termed F^+. The strain that did not have the F factor, referred to as a female or F^- strain, served as a recipient for genetic material during conjugation. Research supports this hypothesis.

The F factor is a *plasmid,* a term originally coined by Lederberg to refer to independent, self-replicating genetic particles. Plasmids are usually circles of double-stranded DNA. (Plasmids are at the heart of recombinant DNA technology, which is discussed in detail in chapter 13.) They are auxiliary circles of DNA that many bacteria carry. They are usually much smaller than the bacterial chromosome.

Researchers found that the transfer of the F factor occurred far more frequently than the transfer of other genes from the donor. That is, during conjugation, about one recombinant occurred in 10^7 cells, whereas transfer of the F factor occurred at a rate of about one conversion of F^- to F^+ in every five conjugations. An *E. coli* strain was then discovered that transferred its genetic material at a rate about one thousand times that of the normal F^+ strain. This strain was called **Hfr,** for *h*igh *f*requency of *r*ecombination. Several other phenomena occurred with this high rate of transfer. First, the ability to transfer the F factor itself dropped to almost zero in this strain. Second,

not all loci were transferred at the same rate. Some loci were transferred much more frequently than others.

Escherichia coli cells are normally coated with hair-like **pili (fimbriae).** F^+ and Hfr cells have one to three additional pili (singular: pilus) called **F-pili,** or sex pili. During conjugation, these sex pili form a connecting bridge between the F^+ (or Hfr) and F^- cells (fig. 7.14). Once a connection is made, the sex pilus then contracts to bring the two cells into contact. DNA transfer takes place through a nick in either the plasmid (in F^+ cells) or the bacterial chromosome (in Hfr cells). A single strand of the DNA double-stranded donor DNA then passes from the F^+ or Hfr cell to the F^- cell across the cell membranes. DNA replication in both the donor and recipient cells reestablishes double-stranded DNA in both. The F factor itself has the genes for sex-pilus formation and DNA transfer to a conjugating F^- cell. At least twenty-two genes are involved in the transfer process, including genes for the pilus protein, nicking the DNA, and regulation of the process.

In the transfer process of conjugation, the donor cell does not lose its F factor or its chromosome because only a single strand of the DNA double helix is transferred; the remaining single strand is quickly replicated. (The process of DNA replication is described in chapter 9.) For a short while, the F^- cell that has conjugated with an Hfr cell has two copies of whatever chromosomal loci were transferred: one copy of its own and one transferred in. With these two copies, the cell is a partial diploid, or a **merozygote.** The new foreign DNA **(exogenote)** can be incorporated into the host chromosome **(endogenote)** by an even number of breakages and reunions between the two, just as in transformation. The unincorporated linear DNA is soon degraded by enzymes. The conjugation process is diagrammed in figure 7.15.

Interrupted Mating

To demonstrate that the transfer of genetic material from the donor to the recipient cell during conjugation is a linear event, F. Jacob and E. Wollman devised the technique of **interrupted mating.** In this technique, F^- and Hfr strains were mixed together in a food blender.

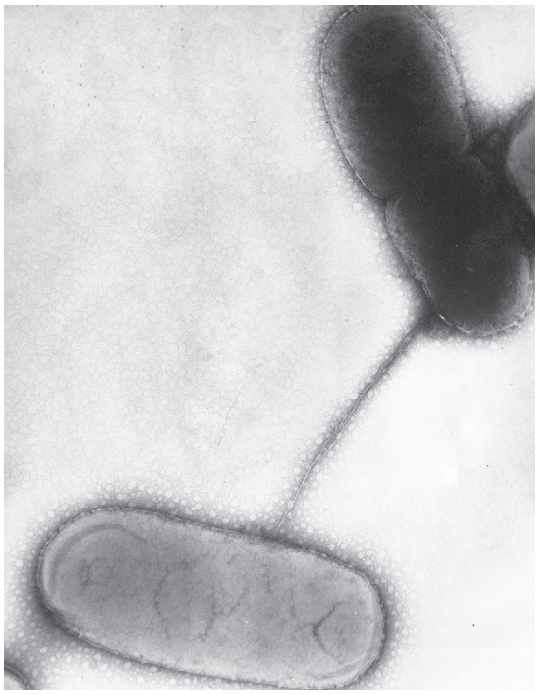


Figure 7.14 Electron micrograph of conjugation between an F^+ (*upper right*) and an F^- (*lower left*) cell with the F-pilus between them. Magnification 3,700×. (Courtesy of Wayne Rosenkrans and Dr. Sonia Guterman.)

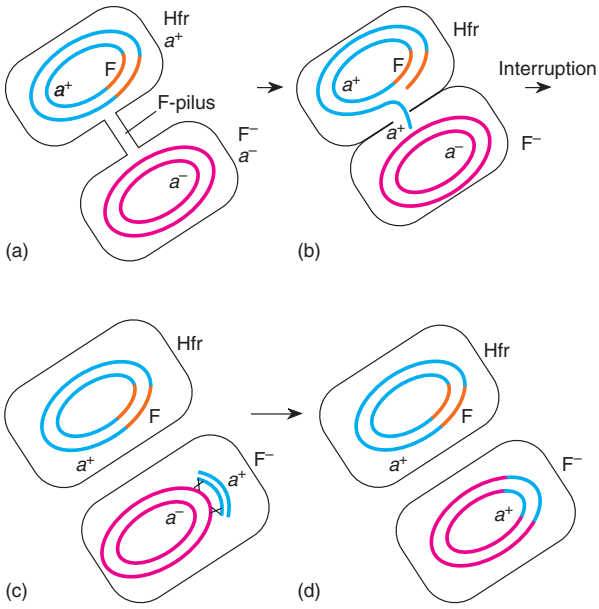


Figure 7.15 Bacterial conjugation. (*a*) The F-pilus draws an Hfr and an F^- cell close together. (*b*) The Hfr chromosome then begins to pass into the F^- cell, beginning at the F region of the Hfr chromosome but in the direction away from the F factor. Only a single strand passes into the F^- cell; this strand and the single strand remaining in the Hfr cell are replicated. After the process is interrupted (*c*), two crossovers bring the a^+ allele into the F^- a^- chromosome (*d*).

Elie Wollman (1917–). (Courtesy of Dr. Elie Wollman and the Pasteur Institute.)

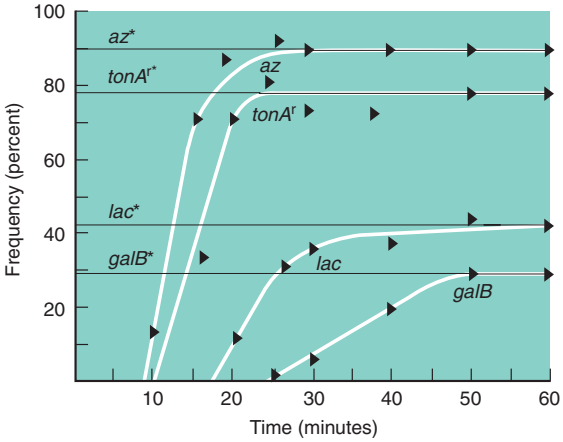
After waiting a specific amount of time, Jacob and Wollman turned the blender on. The spinning motion separated conjugating cells and thereby interrupted their mating. Then the researchers tested the F^- cells for various alleles originally in the Hfr cell. In an experiment like this, the Hfr strain is usually sensitive to an antibiotic such as streptomycin. After conjugation is interrupted, the cells are plated onto a medium containing the antibiotic, which kills all the Hfr cells. Then the genotypes of the F^- cells can be determined by replica-plating without fear of contamination by Hfr cells.

The mating outlined in table 7.3 was carried out. In the food blender, an Hfr strain sensitive to streptomycin (str^s) but resistant to azide (azi^r), resistant to phage T1 ($tonA^r$), and prototrophic for the amino acid leucine (leu^+) and the sugars galactose ($galB^+$) and lactose (lac^+) was added to an F^- strain that was resistant to streptomycin (str^r), sensitive to azide (azi^s), sensitive to T1 ($tonA^s$), and auxotrophic for leucine, galactose, and lactose (leu^-, $galB^-$, and lac^-). After a specific number of minutes (ranging from zero to sixty), the food blender was turned on. To kill all the Hfr cells, the cell suspension was plated on a medium containing streptomycin. The remaining cells were then plated on medium without leucine. The only colonies that resulted were F^- recombinants. They must have received the leu^+ allele from the Hfr in order to grow on a medium lacking leucine. Hence, all colonies had been selected to be F^- recombinants. By replica-plating onto specific media, investigators were able to determine the *azi*, *tonA*, *lac*, and *galB* alleles and the percentage of recombinant colonies that had the original Hfr allele (leu^+). (Note that by trial and error, it was determined that leucine should be the locus to use to select for recombinants. As we will see, the leucine locus entered first.)

Figure 7.16 shows that as time of mating increases, two things happen. First, new alleles enter the F^- cells from the Hfr cells. The $tonA^r$ allele first appears among recombinants after about ten minutes of mating, whereas $galB^+$ first enters the F^- cells after about twenty-five minutes. This suggests a sequential entry of loci into the F^- cells from the Hfr (fig. 7.17). Second, as time proceeds, the percentage of recombinants with a given allele from the Hfr increases. At ten minutes, $tonA^r$ is first found among recombinants. After fifteen minutes, about 40% of recombinants have the $tonA^r$ allele from the Hfr; and after about twenty-five minutes, about 80% of the recombinants have the $tonA^r$ allele. This limiting percent-

Table 7.3 **Genotypes of Hfr and F^- Cells Used in an Interrupted Mating Experiment**

Hfr	F^-
str^s	str^r
azi^r	azi^s
$tonA^r$	$tonA^s$
leu^+	leu^-
$galB^+$	$galB^-$
lac^+	lac^-



*Limiting percentage for *az*, $tonA^r$, *lac*, and *galB* loci.

Figure 7.16 Frequency of Hfr genetic characters among recombinants after interrupted mating. As time proceeds, new alleles appear and then increase in frequency. Interruption of the mating limits the frequency of successful passage. (From F. Jacobs and E. L. Wollman, *Sexuality and the Genetics of Bacteria*, Academic Press, 1961.)

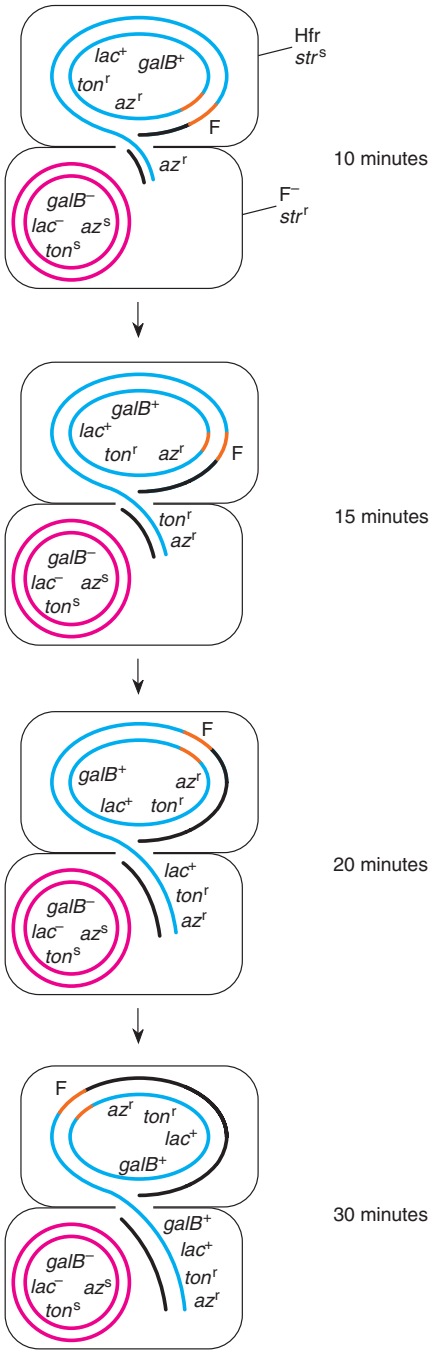


Figure 7.17 Conjugation in *E. coli.* Hfr chromosome is *blue;* F^- chromosome is *red;* and new DNA replication is *black.* As time proceeds, alleles from the Hfr enter the F^- cell in an orderly, sequential fashion. After the cells separate, two crossovers can bring Hfr alleles into the F^- chromosome. The F factor (*orange*) is the last part of the Hfr chromosome to enter the F^- cell.

age does not increase with additional time. The limiting percentage is lower for loci that enter later, a fact explained by the assumption that even without the food blender, mating is usually interrupted before completion by normal agitation alone.

Mapping and Conjugation

Jacob and Wollman, working with several different Hfr strains, collected data that indicated that the bacterial chromosome was circular. The strains were of independent origin, and the results were quite striking (table 7.4).

If we ponder this table for a short while, one fact becomes obvious: The relative order of the loci is always the same. What differs is the point of origin and the direction of the transfer. Jacob and Wollman proposed that normally the F factor is an independent circular DNA entity in the F^+ cell, and that during conjugation only the F factor is passed to the F^- cell. Since it is a small fragment of DNA, it can be passed entirely in a high proportion of conjugations before the cells separate. Every once in a while, however, the F factor becomes integrated into the chromosome of the host, which then becomes an Hfr cell. The point of integration can be different in different strains. However, once the F factor is integrated, it determines the initiation point of transfer for the *E. coli* chromosome, as well as the direction of transfer.

The F factor is the last part of the *E. coli* chromosome to be passed from the Hfr cell. This explains why an Hfr, in contrast to an F^+, rarely passes the F factor itself. In the original work of Lederberg and Tatum, the one recombinant in 10^7 cells most likely came from a conjugation between an F^- cell and an Hfr that had formed spontaneously from an F^+ cell. Integration of the F factor is diagrammed in figure 7.18. The F factor can also reverse this process and loop out of the *E. coli* chromosome. (Sometimes the F factor loops out incorrectly, as in figure 7.19, forming an F′ [F-prime] factor. The passage of this F′ factor to an F^- cell is called **F-duction** or **sexduction.** Not really useful in mapping, the process has proved exceptionally useful in studies of gene expression because of the formation of stable merozygotes, which we will examine in chapter 14.)

We could now diagram the *E. coli* chromosome and show the map location of all known loci. The map units would be in minutes, obtained by interrupted mating. However, at this point, the map would not be complete. Interrupted mating is most accurate in giving the relative position of loci that are not very close to each other. With this method alone, a great deal of ambiguity would arise as to the specific order of very close genes on the chromosome. The remaining sexual process in bacteria, transduction, provides the details that interrupted mating or transformation don't explain.

Table 7.4 **Gene Order of Various Hfr Strains Determined by Means of Interrupted Mating**

Types of Hfr	Order of Transfer of Genetic Characters*																		
HfrH	0	T	L	Az	T_1	Pro	Lac	Ad	Gal	Try	H	S-G	Sm	Mal	Xyl	Mtl	Isol	M	B_1
1	0	L	T	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal	Ad	Lac	Pro	T_1	Az
2	0	Pro	T_1	Az	L	T	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal	Ad	Lac
3	0	Ad	Lac	Pro	T_1	Az	L	T	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal
4	0	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal	Ad	Lac	Pro	T_1	Az	L	T
5	0	M	B_1	T	L	Az	T_1	Pro	Lac	Ad	Gal	Try	H	S-G	Sm	Mal	Xyl	Mtl	Isol
6	0	Isol	M	B_1	T	L	Az	T_1	Pro	Lac	Ad	Gal	Try	H	S-G	Sm	Mal	Xyl	Mtl
7	0	T_1	Az	L	T	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal	Ad	Lac	Pro
AB311	0	H	Try	Gal	Ad	Lac	Pro	T_1	Az	L	T	B_1	M	Isol	Mtl	Xyl	Mal	Sm	S-G
AB312	0	Sm	Mal	Xyl	Mtl	Isol	M	B_1	T	L	Az	T_1	Pro	Lac	Ad	Gal	Try	H	S-G
AB313	0	Mtl	Xyl	Mal	Sm	S-G	H	Try	Gal	Ad	Lac	Pro	T_1	Az	L	T	B_1	M	Isol

Source: From F. Jacobs and E. L. Wollman, *Sexuality and the Genetics of Bacteria,* Academic Press, 1961.

* The 0 refers to the origin of transfer.

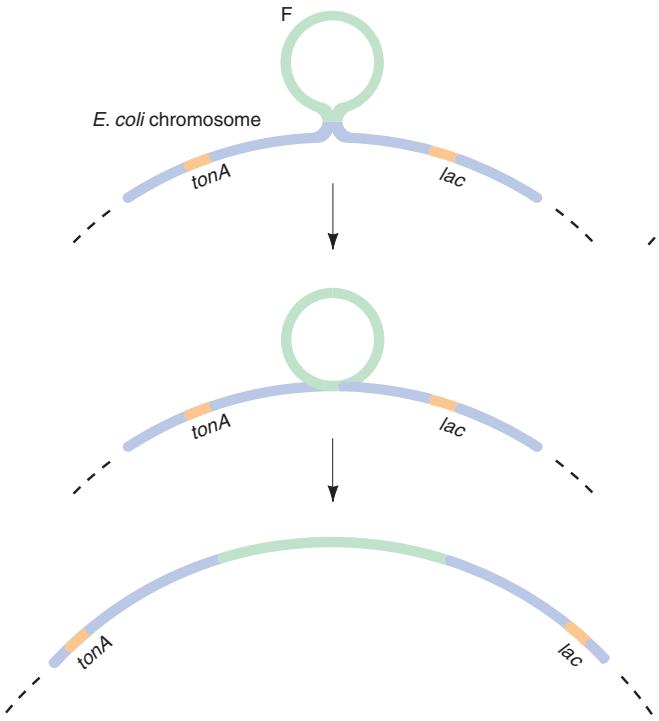


Figure 7.18 Integration of the F factor by a single crossover. After a simultaneous breakage in both the F factor and the *E. coli* chromosome, the two broken circles reunite to make one large circle, the Hfr chromosome. In this case, the integration occurs between the *tonA* and *lac* loci.

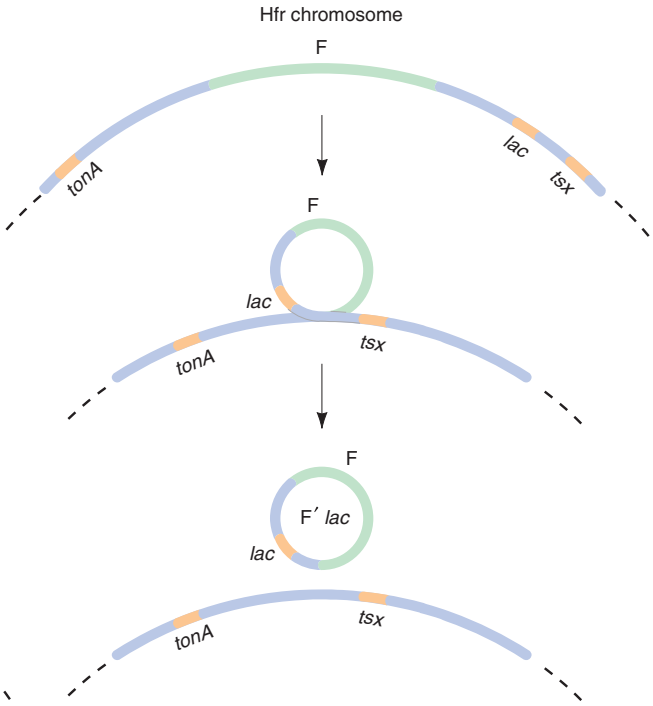


Figure 7.19 Occasionally, the F factor loops out imprecisely, taking part of the cell's genome in the loop. The circular F factor is freed by a single recombination (crossover) at the loop point.

LIFE CYCLES OF BACTERIOPHAGES

Phages are obligate intracellular parasites. Phage genetic material enters the bacterial cell after the phage has adsorbed to the cell surface. Once inside, the viral genetic material takes over the metabolism of the host cell. During the infection process, the cell's genetic material is destroyed, while the viral genetic material is replicated many times. The viral genetic material then controls the mass production of various protein components of the virus. New virus particles are assembled within the host cell, which bursts open (is lysed), releasing a **lysate** of hundreds of viral particles to infect other bacteria. This life cycle appears in figure 7.20.

Recombination

Much genetic work on phages has been done with a group of seven *E. coli* phages called the T series (T-odd: T1, T3, T5, T7; T-even: T2, T4, and T6) and several others, including phage λ (lambda; fig. 7.21). Figure 7.1 diagrammed the complex structure of T2. Phages can undergo recombination processes when a cell is infected with two genetically distinct virions. Hence, the phage genome can be mapped by recombination. As an example, consider the host-range and rapid-lysis loci. Rapid-lysis mutants (*r*) of the T-even phages produce large, sharp-edged plaques. The wild-type produces a smaller, more fuzzy-edged plaque (see fig. 7.7).

Alternative alleles are known also for host-range loci, phage loci that determine the strains of bacteria the phage can infect. For example, T2 can infect *E. coli* cells. These phages can be designated as $T2h^+$ for the normal host range. The *E. coli* is then called Tto^s, referring to their sensitivity to the T2 phage. In the course of evolution, an *E. coli* mutant arose that is resistant to the normal phage. This mutant strain is named Tto^r for T2 resistance. In the further course of evolution, the phages have produced mutant forms that can grow on the Tto^r strain of *E. coli.* These phage mutants are designated as T2h for host-range mutant. Remember, *host-range* signifies a mutation in the phage genome, whereas *phage resistance* indicates a mutation in the bacterial genome.

In 1945, Max Delbrück (a 1969 Nobel laureate) developed mixed indicators, which can be used to demonstrate four phage phenotypes on the same petri plate (fig. 7.22). A bacterial lawn of mixed Tto^r and Tto^s is grown. On this lawn, the rapid-lysis phage mutants (*r*) produce large plaques, whereas the wild-type (r^+) produce smaller plaques. Phages with host-range mutation (*h*) lyse both Tto^r and Tto^s bacteria. They produce the plaques that are clear (but appear dark) in figure 7.22. Since phages with the wild-type host-range allele (h^+) can only infect the Tto^s bacteria, they produce turbid plaques. The Tto^r bacteria growing within these plaques (which appear light-colored in fig. 7.22) produce the turbidity.

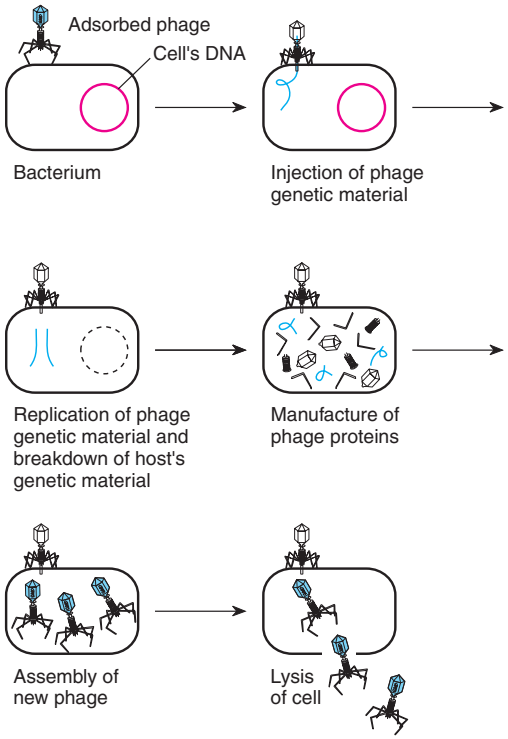


Figure 7.20 The viral life cycle, using T4 infection of *E. coli* as an example.

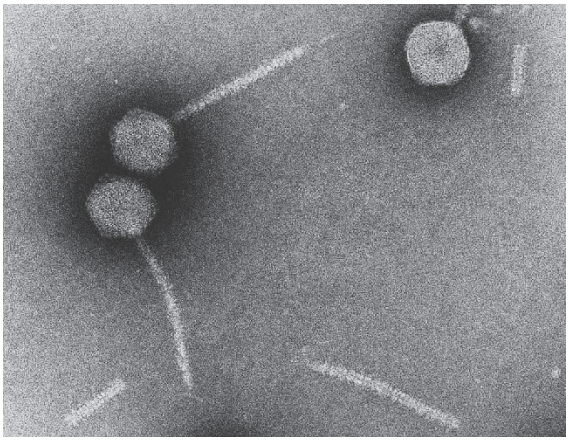


Figure 7.21 Phage λ. Magnification 167,300×. Note that phage λ lacks the tail fibers and base plate of phage T2 (see fig. 7.1). (Courtesy of Robley Williams.)

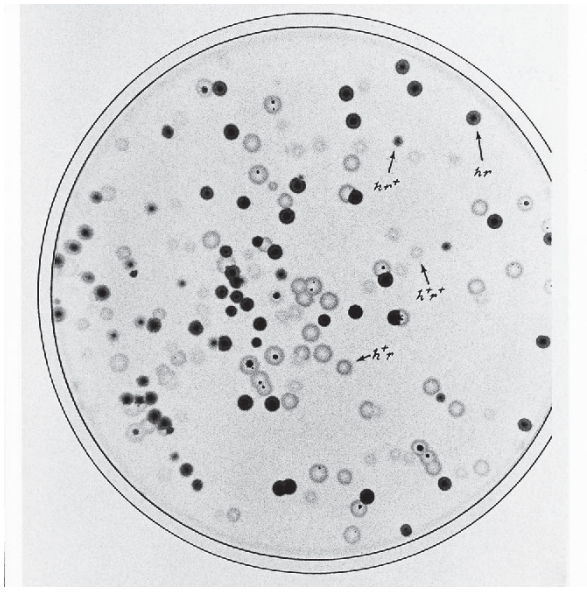


Figure 7.22 Four types of plaques produced by mixed phage T2 on a mixed lawn of *E. coli*. (From *Molecular Biology of Bacterial Viruses* by Gunther S. Stent, © 1963, 1978 by W. H. Freeman & Company. Used with permission.)

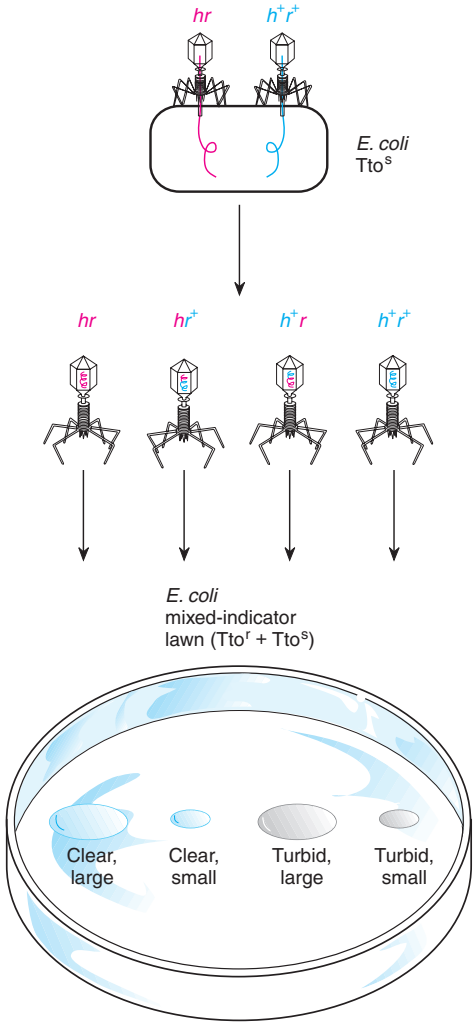


Figure 7.23 Crossing *hr* and h^+r^+ phage. Enough of both types are added to sensitive bacterial cells (Tto^s) to ensure multiple infections. The lysate, consisting of four genotypes, is grown on a mixed-indicator bacterial lawn (Tto^s and Tto^r). Plaques of four types appear (see fig. 7.22), indicating the genotypes of the parental and recombinant phages.

From the wild stock of phages, we can isolate host-range mutants by looking for plaques on a Tto^r bacterial lawn. Only *h* mutants will grow. These phages can then be tested for the *r* phenotype and the double mutants isolated. Once the two strains (double mutant and wild-type) are available, they can be added in large numbers to sensitive bacteria (fig. 7.23). Large numbers of phages are used to ensure that each bacterium is infected by at least one of each phage type, creating the possibility of recombination within the host bacterium. After a round of phage multiplication, the phages are isolated and plated out on Delbrück's mixed-indicator stock. From this growth, the phenotype (and, hence, genotype) of each phage can be recorded. The percentage of recombinants can be read directly from the plate. For example, on a given petri plate (e.g., fig. 7.22) there might be

hr	46	h^+r^+	52
h^+r	34	hr^+	26

The first two, *hr* and h^+r^+, are the original, or parental, phage genotypes. The second two categories result from recombination between the *h* and *r* loci on the phage chromosome. A single crossover in this region produces the recombinants. Note that with phage recombination, parental phages are counted, since every opportunity was provided for recombination within each bacterium. Thus, every progeny phage arises from a situation in which recombination could have taken place. The proportion of recombinants is

$$(34 + 26)/(46 + 52 + 34 + 26)$$
$$= 60/158 = 0.38 \text{ or } 38\% \text{ or } 38 \text{ map units}$$

This percentage recombination is the map distance, which (as in eukaryotes) is a relative index of distance between loci: The greater the physical distance, the greater the amount of recombination, and thus the larger the map distance. One map unit (1 centimorgan) is equal to 1% recombinant offspring.

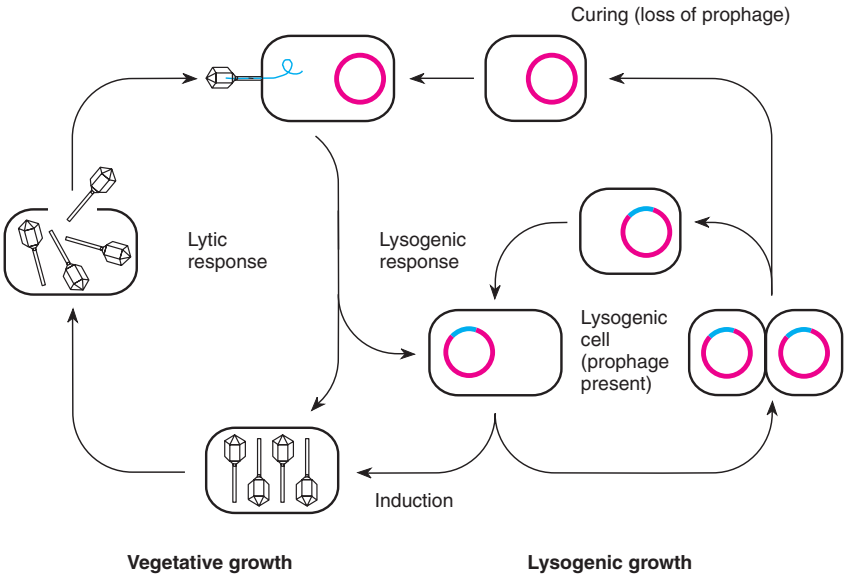


Figure 7.24 Alternative life-cycle stages of a temperate phage (lysogenic and vegetative growth).

Lysogeny

Certain phages are capable of two different life-cycle stages. Some of the time, they replicate in the host cytoplasm and destroy the host cell. At other times, these phages are capable of surviving in the host cell. The host is then referred to as **lysogenic** and the phage as **temperate.** (The term *lysogeny* means "giving birth to lysis." A lysogenic bacterium can be induced to initiate the virulent phase of the phage life cycle.)

The majority of research on lysogeny has been done on phage λ (see fig. 7.21). The λ prophage integrates into the host chromosome; other prophages, like P1, exist as independent plasmids. Phage λ, unlike the F factor, attaches at a specific point, termed *att*λ. This locus can be mapped on the *E. coli* chromosome; it lies between the galactose (*gal*) and biotin (*bio*) loci. When the phage is integrated, it protects the host from further infection (superinfection) by other λ phages. The integrated phage is now termed a **prophage.** Presumably it becomes integrated by a single crossover between itself and the host after apposition at the *att*λ site. (This process resembles the F-factor integration shown in fig. 7.18.)

A prophage can enter the lytic cycle of growth by a process of **induction,** which involves the excision of the prophage followed by the virulent or lytic stage of the viral life cycle. We consider the interesting and complex control mechanisms of life cycle in detail in chapter 14. Induction can take place through a variety of mechanisms, including UV irradiation and passage of the integrated prophage during conjugation **(zygotic induction).** The complete life cycle of a temperate phage is shown in figure 7.24.

TRANSDUCTION

Before lysis, when phage DNA is being packaged into phage heads, an occasional error occurs that causes bacterial DNA to be incorporated into the phage head instead. When this happens, bacterial genes can be transferred to another bacterium via the phage coat. This process, called transduction, has been of great use in mapping the bacterial chromosome. Transduction occurs in two patterns: specialized and generalized.

Specialized Transduction

The process of **specialized** or **restricted transduction** was first discovered in phage λ by Lederberg and his students. Specialized transduction is analogous to sexduction—it depends upon a mistake made during a looping-out process. In sexduction, the error is in the F factor. In specialized transduction, the error is in the λ prophage. Figure 7.25 shows the λ prophage looping out incorrectly to create a defective phage carrying the adjacent *gal* locus. Since only loci adjacent to the phage attachment site can be transduced in this process, specialized transduction has not proven very useful for mapping the host chromosome.

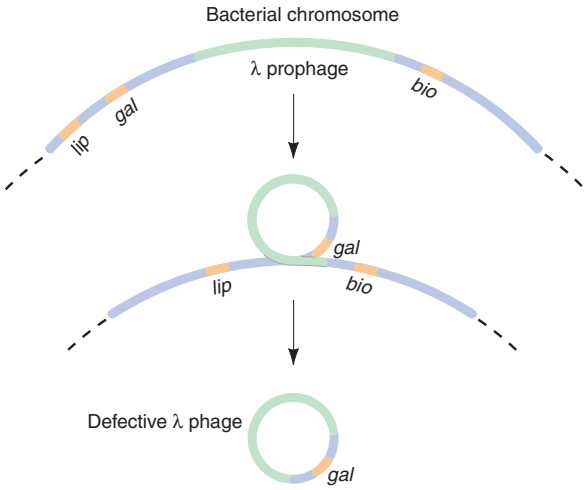


Figure 7.25 Imprecise excision, or looping out, of the λ prophage, resulting in a defective phage carrying the *gal* locus.

Generalized Transduction

Generalized transduction, which Zinder and Lederberg discovered, was the first mode of transduction discovered. The bacterium was *Salmonella typhimurium* and the phage was P22. Virtually any locus can be transduced by generalized transduction. The mechanism, therefore, does not depend on a faulty excision, but rather on the random inclusion of a piece of the host chromosome within the phage protein coat. A defective phage, one that carries bacterial DNA rather than phage DNA, is called a **transducing particle.** Transduction is complete when the genetic material from the transducing particle is injected into a new host and enters the new host's chromosome by recombination.

For P22, the rate of transduction is about once for every 10^5 infecting phages. Since a transducing phage can carry only 2 to 2.5% of the host chromosome, only genes very close to each other can be transduced together **(cotransduced).** Cotransduction can thus help to fill in the details of gene order over short distances after interrupted mating or transformation is used to ascertain the general pattern. Transduction is similar to transformation in that cotransduction, like co-occurrence in transformation, is a relative indicator of map distance.

Mapping with Transduction

Transduction can be used to establish gene order and map distance. Gene order can be established by two-factor transduction. For example, if gene *A* is cotransduced with gene *B* and *B* with gene *C*, but *A* is never cotransduced with *C*, we have established the order *A B C*

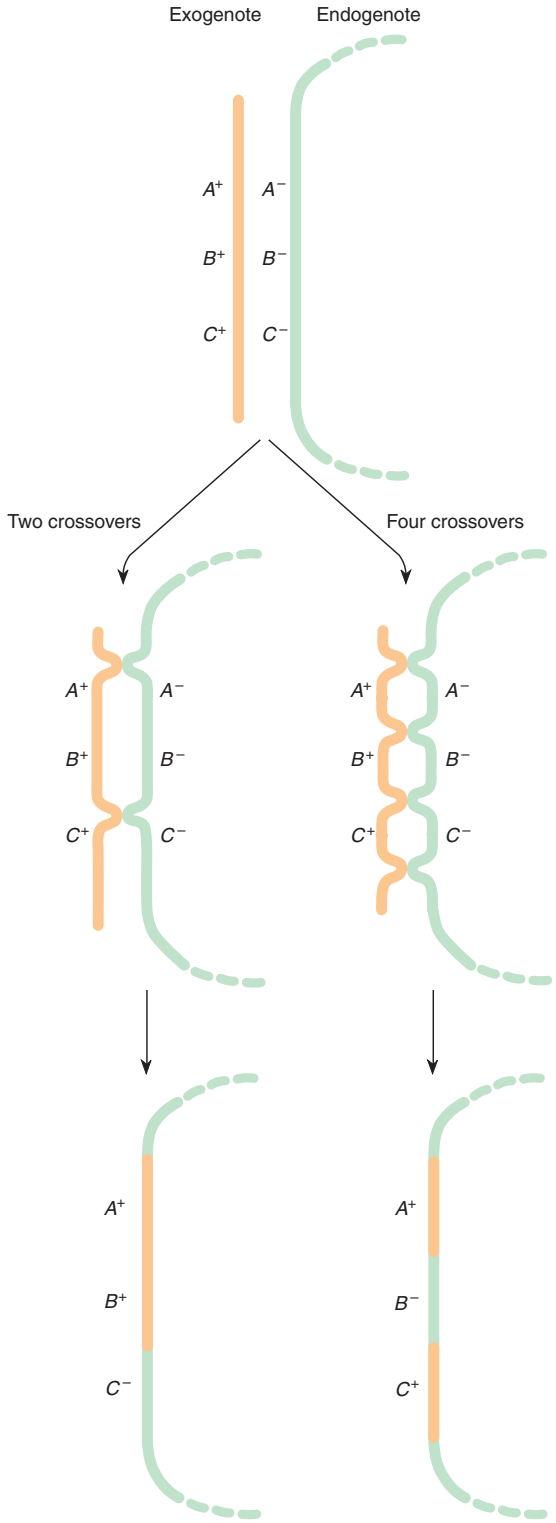


Figure 7.26 The rarest transductant requires four crossovers.

Table 7.5 **Gene Order Established by Two-Factor Cotransduction***

Transductants	Number
A^+B^+	30
A^+C^+	0
B^+C^+	25
$A^+ B^+ C^+$	0

* An $A^+ B^+ C^+$ strain of bacteria is infected with phage. The lysate is used to infect an $A^- B^- C^-$ strain. The transductants are scored for the wild-type alleles they contain. These data include only those bacteria transduced for two or more of the loci. Since *AB* cotransductants and *BC* cotransductants occur, but no *AC* types, we can infer the *A B C* order.

(table 7.5). This would also apply to quantitative differences in cotransduction. For example, if *E* is often cotransduced with *F* and *F* often with *G*, but *E* is very rarely cotransduced with *G*, then we have established the order *E F G*.

However, even more valuable is three-factor transduction, in which we can simultaneously establish gene order and relative distance. Three-factor transduction is especially valuable when the three loci are so close that it is very difficult to make ordering decisions on the basis of two-factor transduction or interrupted mating. For example, if genes *A*, *B*, and *C* are usually cotransduced, we can find the order and relative distances by taking advantage of the rarity of multiple crossovers. Let us use the prototroph ($A^+ B^+ C^+$) to make transducing phages that then infect the $A^- B^- C^-$ strain of bacteria.

To detect cells that have been transduced for one, two, or all three of the loci, we need to eliminate the nontransduced cells. In other words, after transduction, there will be $A^- B^- C^-$ cells in which no transduction event has taken place. There will also be seven classes of bacteria that have been transduced for one, two, or all three loci ($A^+ B^+ C^+$, $A^+ B^+ C^-$, $A^+ B^- C^+$, $A^- B^+ C^+$, $A^+ B^- C^-$, $A^- B^+ C^-$, and $A^- B^- C^+$). The simplest way to select for transduced bacteria is to select bacteria in which the wild-type has replaced at least one of the loci. For example, if, after transduction, we grow the bacteria in minimal media with the requirements of B^- and C^- added, all the bacteria that are A^+ will grow. (Without the requirement of A^- bacteria, no A^- bacteria will grow.) Hence, although we lose the $A^- B^+ C^+$, $A^- B^+ C^-$, and $A^- B^- C^+$ categories, we also lose the $A^- B^- C^-$, untransduced bacteria. In this example, the *A* locus is the selected locus; we must keep in mind that we have an incomplete, although informative, data set. Replica-plating allows us to determine genotypes at the *B* and *C* loci for the A^+ bacteria.

In this example, colonies that grow on complete medium without the requirement of the *A* mutant are replica-plated onto complete medium without the requirement of the *B* mutant and then onto complete medium without the requirement of the *C* mutant. In this way, each transductant can be scored for the other two loci (table 7.6). Now let us take all these selected transductants in which the A^+ allele was incorporated. These can be of four categories: $A^+ B^+ C^+$, $A^+ B^+ C^-$, $A^+ B^- C^+$, and $A^+ B^- C^-$. We can now compare the relative numbers of each of these four categories. The rarest category will be caused by the event that brings in the outer two markers, but not the center one, because this event requires four crossovers (fig. 7.26). Thus, by looking at the number of transductants in the various categories, we can determine that the gene order is *A B C* (table 7.7), since the $A^+ B^- C^+$ category is the rarest.

Table 7.6 **Method of Scoring Three-Factor Transductants**

	Minimal Medium			
Colony Number	Without *A* Requirement	Without *B* Requirement	Without *C* Requirement	Genotype
1	+	+	−	$A^+ B^+ C^-$
2	+	−	−	$A^+ B^- C^-$
3	+	−	−	$A^+ B^- C^-$
4	+	+	+	$A^+ B^+ C^+$
5	+	−	+	$A^+ B^- C^+$
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.

Note: The plus indicates growth, the minus lack of growth. An $A^- B^- C^-$ strain was transduced by phage from an $A^+ B^+ C^+$ strain.

Table 7.7 **Numbers of Transductants and Relative Cotransduction Frequencies in the Experiment Used to Determine the *A B C* Gene Order (Table 7.6)**

Class	Number
$A^+ B^+ C^+$	50
$A^+ B^+ C^-$	75
$A^+ B^- C^+$	1
$A^+ B^- C^-$	300
	426
Relative Cotransductance	
A-B: (50 + 75)/426 = 0.29	
A-C: (50 + 1)/426 = 0.12	

Table 7.7 also includes calculations of the relative cotransduction frequencies. Remember that in all organisms and viruses, the higher the frequency of co-occurrence between the alleles of two loci, the closer those loci are on the chromosome. We usually measure the separation of loci by crossing over between them; the closer together, the lower those crossing-over values are and, hence, the smaller the measure of map units apart. Here, as with transformation, we are measuring the co-occurrence directly; therefore, the measure—cotransductance—is the inverse of map distance. In other words, the greater the cotransduction rate, the closer the two loci are; the more frequently two loci are transduced together, the closer they are and the higher the cotransduction value will be.

The data in table 7.7 should not be used to calculate the *B-C* cotransduction rate because the data are selected values, all of which are A^+; they do not encompass the total data. Missing is the $A^- B^+ C^+$ group that would contribute to the *B-C* cotransduction rate. The $A^- B^+ C^-$ and $A^- B^- C^+$ groups, also missing, would contribute only to the totals in the denominator, not the numerator, of the cotransductance index.

From these sorts of transduction experiments, it is possible to round out the details of map relations in *E. coli* after obtaining the overall picture by interrupted mating. The partial map of *E. coli* appears in figure 7.27. Definitions of loci can be found in table 7.8. Unlike the measurements in eukaryotic mapping, prokaryotic map distances are not generally thought of in map units (centimorgans). Rather, the general distance between loci is determined in *minutes* with cotransduction values used for loci that are very close to each other. (In chapter 13, we discuss mapping methods that rely on directly sequencing the DNA.)

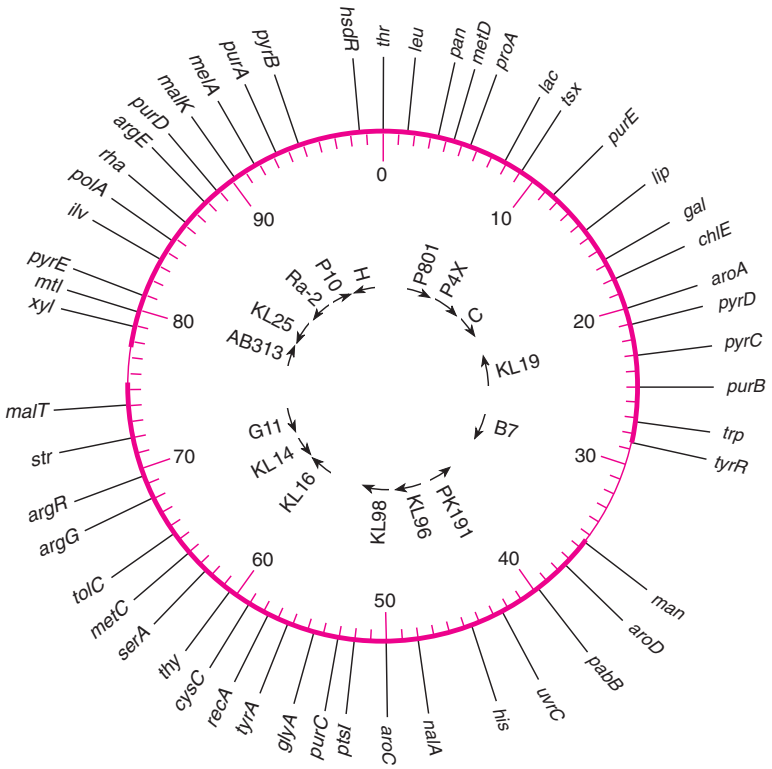


Figure 7.27 Selected loci on a circular map of *E. coli*. Definitions of loci not found in the text can be found in table 7.8. Units on the map are in minutes. *Arrows* within the circle refer to Hfr-strain transfer starting points, with directions indicated. The two thin regions on the outer circle are the only areas not covered by P1 transducing phages. (From B. J. Bachmann et al., "Recalibrated linkage map of *Escherichia coli* K-12," *Bacteriological Reviews*, 40:116–17. Copyright © 1976 American Society for Microbiology, Washington, D.C. Reprinted by permission.)

Table 7.8 Symbols Used in the Gene Map of the *E. coli* Chromosome

Genetic Symbols	Mutant Character	Enzyme or Reaction Affected
araD	Cannot use the sugar arabinose as a carbon source	L-Ribulose-5-phosphate-4-epimerase
araA		L-Arabinose isomerase
araB		L-Ribulokinase
araC		
argB	Requires the amino acid arginine for growth	N-Acetylglutamate synthetase
argC		N-Acetyl-γ-glutamokinase
argH		N-Acetylglutamic-γ-semialdehyde dehydrogenase
argG		Acetylornithine-*d*-transaminase
argA		Acetylornithinase
argD		Ornithine transcarbamylase
argE		Argininosuccinic acid synthetase
argF		Argininosuccinase
argR	Arginine operon regulator	
aroA, B, C	Requires several aromatic amino acids and vitamins for growth	Shikimic acid to 3-Enolpyruvyl-shikimate-5-phosphate
aroD		Biosynthesis of shikimic acid
azi	Resistant to sodium azide	
bio	Requires the vitamin biotin for growth	
carA	Requires uracil and arginine	Carbamate kinase
carB		
chlA-E	Cannot reduce chlorate	Nitrate-chlorate reductase and hydrogen lysase
cysA	Requires the amino acid cysteine for growth	3-Phosphoadenosine-5-phosphosulfate to sulfide
cysB		Sulfate to sulfide; four known enzymes
cysC		
dapA	Requires the cell-wall component diaminopimelic acid	Dihydrodipicolinic acid synthetase
dapB		N-Succinyl-diaminopimelic acid deacylase
dap + *hom*	Requires the amino acid precursor homoserine and the cell-wall component diaminopimelic acid for growth	Aspartic semialdehyde dehydrogenase
dnaA-Z	Mutation, DNA replication	DNA biosynthesis
Dsd	Cannot use the amino acid D-serine as a nitrogen source	D-Serine deaminase
fla	Flagella are absent	
galA	Cannot use the sugar galactose as a carbon source	Galactokinase
galB		Galactose-1-phosphate uridyl transferase
galD		Uridine-diphosphogalactose-4-epimerase
glyA	Requires glycine	Serine hydroxymethyl transferase
gua	Requires the purine guanine for growth	
H	The H antigen is present	
his	Requires the amino acid histidine for growth	Ten known enzymes*
hsdR	Host restriction	Endonuclease R
ile	Requires the amino acid isoleucine for growth	Threonine deaminase
ilvA	Requires the amino acids isoleucine and valine for growth	α-Hydroxy-β-keto acid rectoisomerase
ilvB		α, β-Dihydroxyisovaleric dehydrase*
ilvC		Transaminase B
ind (indole)	Cannot grow on tryptophan as a carbon source	Tryptophanase
λ (*att*λ)	Chromosomal location where prophage λ is normally inserted	
lacI	*Lac* operon regulator	
lacY	Unable to concentrate β-galactosides	Galactoside permease
lacZ	Cannot use the sugar lactose as a carbon source	β-Galactosidase

continued

Table 7.8 ***continued***

Genetic Symbols	Mutant Character	Enzyme or Reaction Affected
lacO	Constitutive synthesis of lactose operon proteins	Defective operator
leu	Requires the amino acid leucine for growth	Three known enzymes*
lip	Requires lipoate	
lon (long form)	Filament formation and radiation sensitivity are affected	
lys	Requires the amino acid lysine for growth	Diaminopimelic acid decarboxylase
lys + met	Requires the amino acids lysine and methionine for growth	
λrec, malT	Resistant to phage λ and cannot use the sugar maltose	Regulator for two operons
malK	Cannot use the sugar maltose as a carbon source	Maltose permease
man	Cannot use mannose sugar	Phosphomannose isomerase
melA	Cannot use melibiose sugar	Alpha-galactosidase
met A-M	Requires the amino acid methionine for growth	Ten or more genes
mtl	Cannot use the sugar mannitol as a carbon source	Two enzymes
muc	Forms mucoid colonies	Regulation of capsular polysaccharide synthesis
nalA	Resistant to nalidixic acid	
O	The O antigen is present	
pan	Requires the vitamin pantothenic acid for growth	
pabB	Requires *p*-aminobenzoate	
phe A, B	Requires the amino acid phenylalanine for growth	
pho	Cannot use phosphate esters	Alkaline phosphatase
pil	Has filaments (pili) attached to the cell wall	
plsB	Deficient phospholipid synthesis	Glycerol 3-phosphate acyltransferase
polA	Repairs deficiencies	DNA polymerase I
proA, *proB*, *proC*	Requires the amino acid proline for growth	
ptsI	Defective phosphotransferase system	Pts-system enzyme I
purA	Requires certain purines for growth	Adenylosuccinate synthetase
purB		Adenylosuccinase
purC, E		5-Aminoimidazole ribotide (AIR) to 5-aminoimidazole-4-(N-succino carboximide) ribotide
purD		Biosynthesis of AIR
pyrB	Requires the pyrimidine uracil for growth	Aspartate transcarbamylase
pyrC		Dihydroorotase
pyrD		Dihydroorotic acid dehydrogenase
pyrE		Orotidylic acid pyrophosphorylase
pyrF		Orotidylic acid decarboxylase
R gal	Constitutive production of galactose	Repressor for enzymes involved in galactose production
R1 pho, R2 pho	Constitutive synthesis of phosphatase	Alkaline phosphatase repressor
R try	Constitutive synthesis of tryptophan	Repressor for enzymes involved in tryptophan synthesis
RC (RNA control)	Uncontrolled synthesis of RNA	
recA	Cannot repair DNA radiation damage or recombine	
rhaA-D	Cannot use the sugar rhamnose as a carbon source	Isomerase, kinase, aldolase, and regulator
rpoA-D	Problems of transcription	Subunits of RNA polymerase
serA	Requires the amino acid serine for growth	3-Phosphoglycerate dehydrogenase
serB		Phosphoserine phosphatase
str	Resistant to or dependent on streptomycin	
suc	Requires succinic acid	

continued

Table 7.8 ***continued***

Genetic Symbols	Mutant Character	Enzyme or Reaction Affected
supB	Suppresses ochre mutations	t-RNA
tonA	Resistant to phages T1 and T5 (mutants called B/1, 5)	T1, T5 receptor sites absent
tonB	Resistant to phage T1 (mutants called B/1)	T1 receptor site absent
T6, colK rec	Resistant to phage T6 and colicine K	T6 and colicine receptor sites absent
T4 rec	Resistant to phage T4 (mutants called B/4)	T4 receptor site absent
tsx	T6 resistance	
thi	Requires the vitamin thiamine for growth	
tolC	Tolerance to colicine E1	
thr	Requires the amino acid threonine for growth	
thy	Requires the pyrimidine thymine for growth	Thymidylate synthetase
trpA	Requires the amino acid tryptophan for growth	Tryptophan synthetase, A protein
trpB		Tryptophan synthetase, B protein
trpC		Indole-3-glycerolphosphate synthetase
trpD		Phosphoribosyl anthranilate transferase
trpE		Anthranilate synthetase
tyrA	Requires the amino acid tyrosine for growth	Chorismate mutase T-prephenate dehydrogenase
tyrR		Regulates three genes
uvrA-E	Resistant to ultraviolet radiation	Ultraviolet-induced lesions in DNA are reactivated
valS	Cannot charge Valyl-tRNA	Valyl-tRNA synthetase
xyl	Cannot use the sugar xylose as a carbon source	

Source: B. J. Bachmann and K. B. Low, "Linkage map of *Escherichia coli* K-12," *Microbiological Reviews,* 44:1–56. Copyright © 1990 American Society for Microbiology, Washington, D.C. Reprinted by permission.

* Denotes enzymes controlled by the homologous gene loci of *Salmonella typhimurium.*

SUMMARY

STUDY OBJECTIVE 1: To define bacteria and bacterial viruses and learn about methods of studying them 149–154

Prokaryotes (bacteria) usually have a single circular chromosome of double-stranded DNA. A bacteriophage consists of a chromosome wrapped in a protein coat. Its chromosome can be DNA or RNA. Phenotypes of bacteria include colony morphology, nutritional requirements, and drug resistance. Phage phenotypes include plaque morphology and host range. Replica-plating is a rapid screening technique for assessing the phenotype of a bacterial clone.

STUDY OBJECTIVE 2: To study life cycles and sexual processes in bacteria and bacteriophages 154–166

In transformation, a competent bacterium can take up relatively large pieces of DNA from the medium. This DNA can be incorporated into the bacterial chromosome.

During the process of conjugation, the fertility factor, F, is passed from an F^+ to an F^- cell. If the F factor integrates into the host chromosome, an Hfr cell results that can pass its entire chromosome into an F^- cell. The F factor is the last region to cross into the F^- cell.

In transduction, a phage protein coat containing some of the host chromosome passes to a new host bacterium. Again, recombination with this new chromosomal segment can take place.

STUDY OBJECTIVE 3: To make use of the sexual processes of bacteria and their viruses to map their chromosomes 155–171

We can map the phage chromosome by measuring recombination after a bacterium has been simultaneously infected by two strains of the virus carrying different alleles. In *E. coli,* mapping is most efficiently accomplished via interrupted mating and transduction. The former provides information on general gene arrangement and the latter provides finer details.

SOLVED PROBLEMS

PROBLEM 1: A wild-type strain of *B. subtilis* is transformed by DNA from a strain that cannot grow on galactose (gal^-) and also needs biotin for growth (bio^-). Transformants are isolated by exposing the transformed cells to minimal medium with penicillin, killing the wild-type cells. After the penicillin is removed, replica-plating is used to establish the genotypes of 30 transformants:

Class 1 gal^- bio^-	17
Class 2 gal^- bio^+	4
Class 3 gal^+ bio^-	9

What is the relative co-occurrence of these two loci?

Answer: The three classes of colonies represent the three possible transformant groups. Classes 2 and 3 are single transformants and class 1 is the double transformant. We are interested in the relative co-occurrence of the two loci. Therefore we divide the number of double transformants by the total: $r = 17/(17 + 4 + 9) = 0.57$. This is a relative value inverse to a map distance; the larger it is, the closer the loci are to each other.

PROBLEM 2: A gal^- bio^- $att\lambda^-$ strain of *E. coli* is transduced by P22 phages from a wild-type strain. Transductants are selected for by growing the cells with galactose as the sole energy source. Replica-plating and testing for lysogenic ability gives the genotypes of 106 transformants:

Class 1 gal^+ bio^- $att\lambda^-$	71
Class 2 gal^+ bio^+ $att\lambda^-$	0
Class 3 gal^+ bio^- $att\lambda^+$	9
Class 4 gal^+ bio^+ $att\lambda^+$	26

What is the gene order, and what are the relative cotransduction frequencies?

Answer: We have selected all transductants that are gal^+. Class 2 is in the lowest frequency (0) and therefore represents the quadruple crossover between the transducing DNA and the host chromosome. From this, we see that *att*λ must be in the middle because this low-probability event is the one that would have switched only the middle locus. In other words, the two end loci would be recombinant, and the middle locus would have the host allele. We can only calculate two cotransduction frequencies because these are selected data. Note that in class 1, there is no cotransduction between *gal* and either of the other two loci; class 2 would show the cotransduction of *gal* and *bio;* class 3 represents the cotransduction of *gal* and *att*λ; and class 4 represents the cotransduction of *gal* and both other loci. Therefore, cotransduction values are

$$gal\text{–}att\lambda = (9 + 26)/106 = 35/106 = 0.33$$
$$gal\text{–}bio = (0 + 26)/106 = 26/106 = 0.25.$$

EXERCISES AND PROBLEMS*

BACTERIA AND BACTERIAL VIRUSES IN GENETIC RESEARCH

1. What is the nature and substance of prokaryotic chromosomes and viral chromosomes? Are viruses alive?

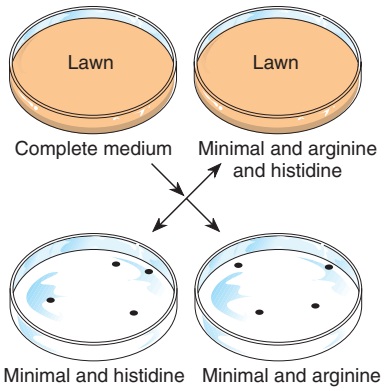
TECHNIQUES OF CULTIVATION

2. What are the differences between a heterotroph and an auxotroph? a minimal and a complete medium? an enriched and a selective medium?
3. What are the differences between a plaque and a colony?

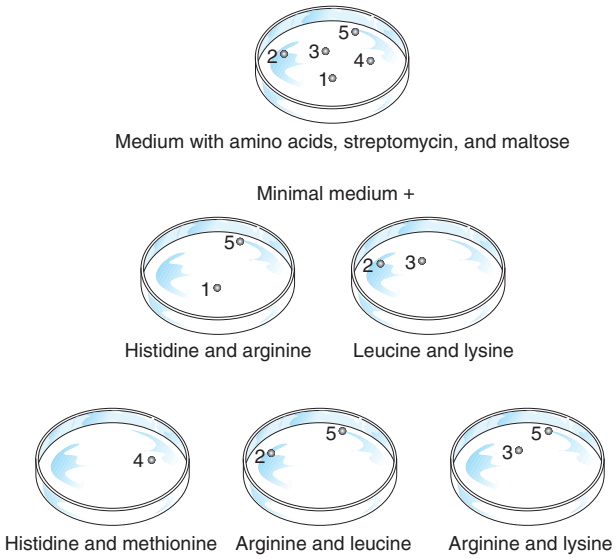
BACTERIAL PHENOTYPES

4. What genotypic notation indicates alleles that make a bacterium
   a. resistant to penicillin?
   b. sensitive to azide?
   c. require histidine for growth?
   d. unable to grow on galactose?
   e. able to grow on glucose?
   f. susceptible to phage T1 infection?
5. An *E. coli* cell is placed on a petri plate containing λ phages. It produces a colony overnight. By what mechanisms might it have survived?
6. An *E. coli* lawn is formed on a petri plate containing complete medium. Replica-plating is used to transfer material to plates containing minimal medium and combinations of the amino acids arginine and histidine (see the figure). Give the genotype of the original strain as well as the genotypes of the odd colonies found growing on the plates.

*Answers to selected exercises and problems are on page A-7.



7. Prototrophic Hfr *E. coli* strain G11, sensitive to streptomycin and $malT^+$ (can use maltose) is used in a conjugation experiment. The *str* locus is one of the last to be transferred, whereas the *malT* locus is one of the first. This strain is mated to an F^- strain resistant to streptomycin, $malT^-$ (cannot utilize maltose), and requiring five amino acids (histidine, arginine, leucine, lysine, and methionine). Recombinants are selected for by plating on a medium with streptomycin, with maltose as the sole carbon source, and all five amino acids present. Thus, all recombinant F^- cells will grow irrespective of their amino acid requirements. Five colonies are grown on the original plate with streptomycin, maltose, and all five amino acids in question (see the figure). These colonies are replica-plated onto minimal medium containing various amino acids. What are the genotypes of each of the five colonies?



8. A petri plate with complete medium has six colonies growing on it after one of the conjugation experiments described earlier. The colonies are numbered, and the plate is used as a master to replicate onto plates of glucose-containing selective (minimal) medium with various combinations of additives. From the following data, which show the presence (+) or absence (−) of growth, give your best assessment of the genotypes of the six colonies.

	Colony					
On Minimal Medium +	**1**	**2**	**3**	**4**	**5**	**6**
Nothing	−	−	+	−	−	−
Xylose + arginine	+	−	+	+	−	−
Xylose + histidine	−	−	+	−	−	−
Arginine + histidine	−	+	+	+	−	−
Galactose + histidine	−	−	+	−	−	+
Threonine + isoleucine + valine	−	−	+	−	+	−
Threonine + valine + lactose	−	−	+	−	−	−

VIRAL PHENOTYPES

9. Give possible genotypes of an *E. coli*-phage T1 system in which the phage cannot grow on the bacterium. Give genotypes for a T1 phage that can grow on the bacterium.

SEXUAL PROCESSES IN BACTERIA AND BACTERIOPHAGES

10. What is a plasmid? How does one integrate into a host's chromosome? How does it leave?

11. In conjugation experiments, one Hfr strain should carry a gene for some sort of sensitivity (e.g., azt^s or str^s) so that the Hfr donors can be eliminated on selective media after conjugation has taken place. Should this locus be near to or far from the origin of transfer point of the Hfr chromosome? What are the consequences of either alternative?

12. How does a geneticist doing interrupted mating experiments know that the locus for the drug-sensitivity allele, used to eliminate the Hfr bacteria after conjugation, has crossed into the F^- strain?

13. Diagram the step-by-step events required to integrate foreign DNA into a bacterial chromosome in each of the three processes outlined in the chapter (transformation, conjugation, transduction). Do the same for viral recombination. (*See also* TRANSDUCTION)

14. The DNA from a prototrophic strain of *E. coli* is isolated and used to transform an auxotrophic strain deficient in the synthesis of purines ($purB^-$), pyrimidines ($pyrC^-$), and the amino acid tryptophan (trp^-). Tryptophan was used as the marker to determine whether transformation had occurred (the selected marker). What are the gene order and the relative co-occurrence frequencies between loci, given these data:

$trp^+\ pyrC^+\ purB^+$	86
$trp^+\ pyrC^+\ purB^-$	4
$trp^+\ pyrC^-\ purB^+$	67
$trp^+\ pyrC^-\ purB^-$	14

15. Using the data in figure 7.16, draw a tentative map of the *E. coli* chromosome.

16. Three Hfr strains of *E. coli* (P4X, KL98, and Ra-2) are mated individually with an auxotrophic F^- strain using interrupted mating techniques. Using the following data, construct a map of the *E. coli* chromosome, including distances in minutes.

	Approximate Time of Entry		
Donor Loci	**Hfr P4X**	**Hfr KL98**	**Hfr Ra−2**
gal^+	11	67	70
thr^+	94	50	87
xyl^+	73	29	8
lac^+	2	58	79
his^+	38	94	43
ilv^+	77	33	4
$argG^+$	62	18	19

How many different petri plates and selective media are needed?

17. Design an experiment using interrupted mating and create a resulting possible data set that would correctly map five of the loci on the *E. coli* chromosome (fig. 7.27).

18. Lederberg and his colleagues (Nester, Schafer, and Lederberg, 1963, *Genetics* 48:529) determined gene order and relative distance between genes using three markers in the bacterium *Bacillus subtilis.* DNA from a prototrophic strain ($trp^+\ his^+\ tyr^+$) was used to transform the auxotroph. The seven classes of transformants, with their numbers, are tabulated as follows:

trp^+	trp^-	trp^-	trp^+	trp^+	trp^-	trp^+
his^-	his^+	his^-	his^+	his^-	his^+	his^+
tyr^-	tyr^-	tyr^+	tyr^-	tyr^+	tyr^+	tyr^+
2,600	418	685	1,180	107	3,660	11,940

Outline the techniques used to obtain these data. Taking the loci in pairs, calculate co-occurrences. Construct the most consistent linkage map of these loci.

19. In a transformation experiment, an $a^+\ b^+\ c^+$ strain is used as the donor and an $a^-\ b^-\ c^-$ strain as the recipient. One hundred a^+ transformants are selected and then replica-plated to determine whether b^+ and c^+ are present. What can you conclude about the relative positions of the genes, based on the listed genotypes?

$a^+\ b^-\ c^-$	21
$a^+\ b^-\ c^+$	69
$a^+\ b^+\ c^-$	3
$a^+\ b^+\ c^+$	7

20. In a transformation experiment, an $a^+\ b^+\ c^-$ strain is used as donor and an $a^-\ b^-\ c^+$ strain as recipient. If you select for a^+ transformants, the least frequent class is $a^+\ b^+\ c^+$. What is the order of the genes?

21. A mating between his^+, leu^+, thr^+, pro^+, str^s cells (Hfr) and his^-, leu^-, thr^-, pro^-, str^r cells (F^-) is allowed to continue for twenty-five minutes. The mating is stopped, and the genotypes of the recombinants are determined. What is the first gene to enter, and what is the probable gene order, based on the following data?

Genotype	**Number of Colonies**
his^+	0
leu^+	12
thr^+	27
pro^+	6

22. **a.** In a transformation experiment, the donor is $trp^+\ leu^+\ arg^+$, and the recipient is $trp^-\ leu^-\ arg^-$. The selection process is for trp^+ transformants, which are then further tested. Forty percent are $trp^+\ arg^+$; 5% are $trp^+\ leu^+$. In what two possible orders could the genes be arranged?

b. You can do only one more transformation to determine gene order. You must use the same donor and recipient, but you can change the selection procedure for the initial transformants. What should you do, and what results should you expect for each order you proposed in *a*?

23. DNA from a bacterial strain that is $a^+\ b^+\ c^+$ is used to transform a strain that is $a^-\ b^-\ c^-$. The numbers of each transformed genotype appear. What can we say about the relative position of the genes?

Genotype	**Number**
$a^+\ b^-\ c^-$	214
$a^-\ b^+\ c^-$	231
$a^-\ b^-\ c^+$	206
$a^+\ b^+\ c^-$	11
$a^+\ b^+\ c^+$	6
$a^+\ b^-\ c^+$	93
$a^-\ b^+\ c^+$	14

24. An Hfr strain that is $a^+ b^+ c^+ d^+ e^+$ is mated with an F^- strain that is $a^- b^- c^- d^- e^-$. The mating is interrupted every five minutes, and the genotypes of the F^- recombinants are determined. The results appear following. (A *plus* indicates appearance; a *minus* the lack of the locus.) Draw a map of the chromosome and indicate the position of the F factor, the direction of transfer, and the minutes between genes.

Time	*a*	*b*	*c*	*d*	*e*
5	−	−	−	−	−
10	+	−	−	−	−
15	+	−	−	−	−
20	+	−	−	−	−
25	+	−	−	−	−
30	+	−	−	+	−
35	+	−	−	+	−
40	+	+	−	+	−
45	+	+	−	+	−
50	+	+	−	+	−
55	+	+	−	+	−
60	+	+	−	+	−
65	+	+	+	+	−
70	+	+	+	+	−
75	+	+	+	+	+

25. A bacterial strain that is lys^+ his^+ val^+ is used as a donor, and lys^- his^- val^- as the recipient. Initial transformants are isolated on minimal medium + histidine + valine.

a. What genotypes will grow on this medium?

b. These colonies are replicated to minimal medium + histidine, and 75% of the original colonies grow. What genotypes will grow on this medium?

c. The original colonies are also replicated to minimal medium + valine, and 6% of the colonies grow. What genotypes will grow on this medium?

d. Finally, the original colonies are replicated to minimal medium. No colonies grow. From this information, what genotypes will grow on minimal medium + histidine and on minimal medium + valine?

e. Based on this information, which gene is closer to *lys*?

f. The original transformation is repeated, but the original plating is on minimal medium + lysine + histidine. Fifty colonies appear. These colonies are replicated to determine their genotypes, with these results:

val^+ his^+ lys^+	0
val^+ his^- lys^+	37
val^+ his^+ lys^-	3

Based on all the results, what is the most likely gene order?

LIFE CYCLES OF BACTERIOPHAGES

26. Define *prophage, lysate, lysogeny,* and *temperate phage.*

27. Outline an experiment to demonstrate that two phages do not undergo recombination until a bacterium is infected simultaneously with both.

28. Doermann (1953, *Cold Spr. Harb. Symp. Quant. Biol.* 18:3) mapped three loci of phage T4: minute, rapid lysis, and turbid. He infected *E. coli* cells with both the triple mutant (*m r tu*) and the wild-type (m^+ r^+ tu^+) and obtained the following data:

m	m^+	*m*	*m*	m^+	m^+	*m*	m^+
r	*r*	r^+	*r*	r^+	*r*	r^+	r^+
tu	*tu*	*tu*	tu^+	*tu*	tu^+	tu^+	tu^+
3,467	474	162	853	965	172	520	3,729

What is the linkage relationship among these loci? In your answer include gene order, relative distance, and coefficient of coincidence.

29. Wild-type phage T4 (r^+) produce small, turbid plaques, whereas *r*II mutants produce large, clear plaques. Four *r*II mutants (*a–d*) are crossed. (Assume, for the purposes of this problem, that *a–d* are four closely linked loci. The actual structure of the *r*II region is presented in chapter 12. Here, assume that $a \times b$ means $a^- b^+ c^+ d^+ \times a^+ b^- c^+ d^+$.) These percentages of wild-type plaques are obtained in crosses:

$a \times b$	0.3
$a \times c$	1.0
$a \times d$	0.4
$b \times c$	0.7
$b \times d$	0.1
$c \times d$	0.6

Deduce a genetic map of these four mutants.

30. A phage cross is performed between $a^+ b^+ c^+$ and *a b c* phage. Based on these results, derive a complete map:

$a^+ b^+ c^+$	1,801
$a^+ b^+ c$	954
$a^+ b c^+$	371
$a^+ b c$	160
$a b^+ c^+$	178
$a b^+ c$	309
$a b c^+$	879
$a b c$	1,850
	6,502

31. The *r*II mutants of T4 phage will grow and produce large plaques on strain B; *r*II mutants will not grow on strain K12. Certain crosses are performed in strain B. (As with question 29, assume that the three mutants are of three separate loci in the *r*II region.) By diluting and plating on strain B, it is determined that each experiment generates about 250×10^7 phage. By dilution, approximately 1/10,000 of the progeny are plated on K12 to generate these wild-type recombinants (plaques on K12):

1×2	50
1×3	25
2×3	75

Draw a map of these three mutants (1, 2, and 3) and indicate the distances between them.

TRANSDUCTION

32. Define and illustrate *specialized* and *generalized transduction.*

33. In *E. coli,* the three loci *ara, leu,* and *ilvH* are within 1/2-minute map distance apart. To determine the exact order and relative distance, the prototroph (ara^+ leu^+ $ilvH^+$) was infected with transducing phage P1. The lysate was used to infect the auxotroph (ara^- leu^- $ilvH^-$). The ara^+ classes of transductants were selected to produce the following data:

ara^+	ara^+	ara^+	ara^+
leu^-	leu^+	leu^-	leu^+
$ilvH^-$	$ilvH^-$	$ilvH^+$	$ilvH^+$
32	9	0	340

Outline the specific techniques used to isolate the various transduced classes. What is the gene order and what are the relative cotransduction frequencies between genes? Why do some classes occur so infrequently?

34. Consider this portion of an *E. coli* chromosome:

thr *ara leu*

Three *ara* loci, *ara*-1, *ara*-2, and *ara*-3, are located in the *ara* region. A mutant of each locus (*ara*-1^-, *ara*-2^-, and *ara*-3^-) was isolated, and their order with respect to *thr* and *leu* was analyzed by transduction. The donor was always thr^+ leu^+ and the recipient was always thr^- leu^-. Each *ara* mutant was used as a donor in one cross and as a recipient in another; ara^+ transductants were selected in each case. The ara^+ transductants were then scored for leu^+ and thr^+. Based on the following results, determine the order of the ara^- mutants with respect to *thr* and *leu.*

Cross	Recipient	Donor	Ratio: $\frac{thr^- \, ara^+ \, leu^+}{thr^+ \, ara^+ \, leu^-}$
1	*ara*-1^-	*ara*-2^-	48.5
2	*ara*-2^-	*ara*-1^-	2.4
3	*ara*-1^-	*ara*-3^-	4.0
4	*ara*-3^-	*ara*-1^-	19.1
5	*ara*-2^-	*ara*-3^-	1.5
6	*ara*-3^-	*ara*-2^-	25.5

35. An *E. coli* strain that is leu^+ thr^+ azi^r is used as a donor in a transduction of a strain that is leu^- thr^- azi^s. Either leu^+ or thr^+ transductants are selected and then scored for unselected markers. The results are obtained:

Selected Marker	Unselected Markers
leu^+	48% azi^r
leu^+	2% thr^+
thr^+	3% leu^+
thr^+	0% azi^r

What is the order of the three loci?

CRITICAL THINKING QUESTIONS

1. Consider the data from table 7.4. Is there another way to interpret the data other than coming from a circular bacterial chromosome?

2. Why might transformation have evolved, given that the bacterium is importing DNA from a dead organism?

Suggested Readings for chapter 7 are on page B-4.

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8

CYTOGENETICS

STUDY OBJECTIVES

1. To observe the nature and consequences of chromosomal breakage and reunion 178
2. To observe the nature and consequences of variation in chromosome numbers in human and nonhuman organisms 190

STUDY OUTLINE

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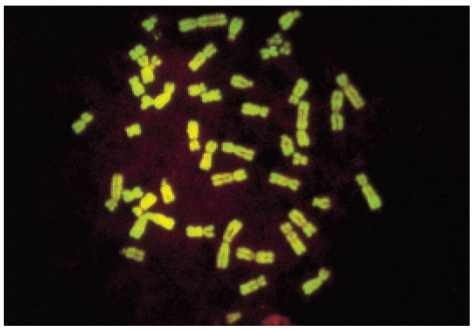

Chromosomes of an individual with trisomy 21, Down syndrome. (© Dr. Ram Verma /Phototake, NYC.)

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Our understanding of the chromosomal theory of genetics grew primarily through mapping loci, using techniques that require alternative allelic forms, or mutations, of these loci. Changes in the genetic material also occur at a much coarser level—the level of **cytogenetics,** which is a level visible under the light microscope. The word *cytogenetics* combines the words *cytology* and *genetics;* cytology is the study of cells. Cytogenetics is thus defined as the study of cells from the perspective of genetics. In practice, it is the study of changes in the gross structure and number of chromosomes in cells. In this chapter, we investigate how these alterations happen and what their consequences are to the organism.

VARIATION IN CHROMOSOMAL STRUCTURE

In general, chromosomes can break due to ionizing radiation, physical stress, or chemical compounds. When a break occurs in the chromosome before DNA replication, during the S phase of the cell cycle (see fig. 3.6), the break itself is replicated. After the S phase, any breaks that occur affect single chromatids.

Every break in a chromatid produces two ends. These ends have been described as "sticky," meaning simply that enzymatic processes of the cell tend to reunite them. Broken ends do not attach to the undamaged terminal ends of other chromosomes.(Normal chromosomal ends are capped with structures called *telomeres*—see chapter 15.) If broken ends are not brought together, they can remain broken. But, if broken chromatid ends are brought into apposition, they may rejoin in any of several ways. First, the two broken ends of a single chromatid can reunite. Second, the broken end of one chromatid can fuse with the broken end of another chromatid, resulting in an exchange of chromosomal material and a new combination of alleles. Multiple breaks can lead to a variety of alternative recombinations. These chromosomal aberrations have major genetic, evolutionary, and medical consequences. The types of breaks and reunions discussed in this chapter can be summarized as follows:

I. Noncentromeric breaks
   A. Single breaks
      1. Restitution
      2. Deletion
      3. Dicentric bridge
   B. Two breaks (same chromosome)
      1. Deletion
      2. Inversion
   C. Two breaks (nonhomologous chromosomes)

II. Centromeric breaks
   A. Fission
   B. Fusion

Single Breaks

If a chromosome breaks, the broken ends may rejoin. When the broken ends of a single chromatid rejoin (in a process called restitution), there is no consequence to the break. If they do not rejoin, the result is an **acentric fragment,** without a centromere, and a **centric fragment,** with a centromere. The centric fragment migrates normally during the division process because it has a centromere. The acentric fragment, however, is soon lost. It is subsequently excluded from the nuclei formed and eventually degrades. In other words, the viable, centric part of the chromosome has suffered a deletion. After mitosis, the daughter cell that receives the **deletion chromosome** may show several effects.

Pseudodominance is one possible effect. (This term was used in chapter 5 when we described alleles located on the X chromosome. With only one copy of the locus present, a recessive allele in males shows itself in the phenotype as if it were dominant—hence the term *pseudodominance.*) The normal chromosome homologous to the deletion chromosome has loci in the region, and recessive alleles show pseudodominance. A second possible effect is that, depending on the length of the deleted segment and the specific loci lost, the imbalance the deletion chromosome creates in the daughter cell may be lethal. If the deletion occurs before or during meiosis, it may be observed under the microscope. We discuss this event later in the chapter.

A single break can have yet another effect. Occasionally, the two centric fragments of a single chromosome may join, forming a two-centromere, or **dicentric, chromosome** and leaving the two acentric fragments to join or, alternatively, remain as two fragments (fig. 8.1). The acentric fragments are lost, as mentioned before. Because the centromeres are on sister chromatids, the dicentric fragment is pulled to opposite ends of a mitotic cell forming a bridge there; or, if meiosis is occurring, the dicentric fragment is pulled apart during the second meiotic division. The ultimate fate of this bridge is breakage as the spindle fibers pull the centromeres to opposite poles (or possibly exclusion from a new nucleus if the bridge is not broken).

The dicentric chromosome does not necessarily break in the middle, and subsequent processes exacerbate the imbalance created by an off-center break: duplications occur on one strand, whereas more deletions occur on the other (fig. 8.2). In addition, the "sticky" ends produced on both fragments increase the likelihood of repeating this **breakage-fusion-bridge cycle** in each generation. The great imbalances resulting from the duplications and deletions usually cause the cell line to die within several generations.

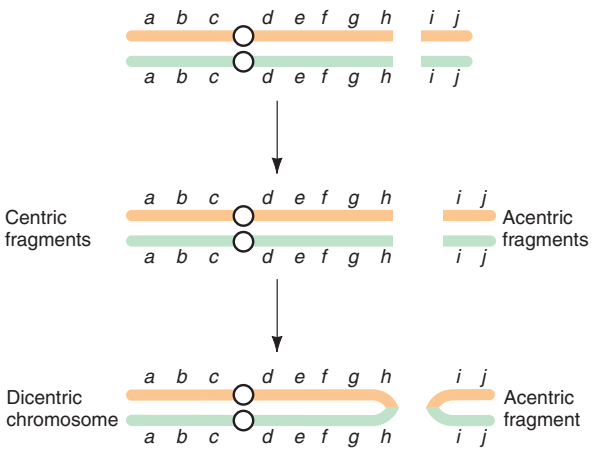


Figure 8.1 Chromosomal break with subsequent reunion to form a dicentric chromosome and an acentric fragment.

Two Breaks in the Same Chromosome

Deletion

Figure 8.3 shows two of the possible results when two breaks occur in the same chromosome. One alternative is a reunion that omits an acentric fragment, which is then lost. The centric piece, missing the acentric fragment (*e-f-g* in fig. 8.3), is a deletion chromosome. An organism having this chromosome and a normal homologue will have, during meiosis, a bulge in the tetrad if the deleted section is large enough (fig. 8.4). The bulge also appears in the paired, polytene giant salivary gland chromosomes of *Drosophila.* (Note that when a bulge like that illustrated in figure 8.4 is seen in paired chromosomes, it indicates that one chromosome has a piece that is missing in the other. In our example, the bulge resulted from a deletion in one chromosome; it could also result from an insertion of a piece in the other chromosome.)

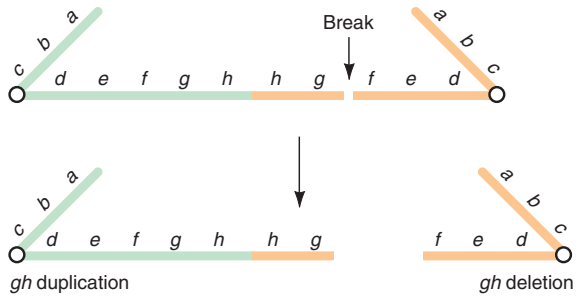


Figure 8.2 Breakage of a dicentric bridge causes duplications and additional deficiencies.

Inversion

Two breaks in the same chromosome can also lead to **inversion,** in which the middle section is reattached but in the inverted configuration (see fig. 8.3). An inversion has several interesting properties. To begin with, fruit flies homozygous for an inversion show new linkage relations when their chromosomes are mapped. One outcome of this new linkage arrangement is the possibility of a **position effect,** a change in the expression of a gene due to a changed linkage arrangement. Position effects are either stable, as in *Bar* eye of *Drosophila* (to be discussed), or variegated, as with *Drosophila* eye color. A normal female fly that is heterozygous (X^wX^+) has red eyes. If, however, the white locus is moved through an inversion so that it comes to lie next to heterochromatin (fig. 8.5), the fly shows a **variegation**—patches of the eye are white. This is presumably caused by a spread of the tight coiling of the heterochromatin, "turning off" the expression of the locus. In a heterozygote, if the turned-off allele is the wild-type, the cell will express the normally recessive white-eye allele. Depending on what happens in each cell, patches of red and white eye color result.

When synapsis occurs in an inversion heterozygote, either at meiosis or in the *Drosophila* salivary gland during endomitosis, a loop often forms to accommodate the point-for-point pairing process (figs. 8.6 and 8.7). An outcome of this looping tendency is **crossover suppression.** That is, an inversion heterozygote shows very little recombination of alleles within the inverted region. The reason is usually not that crossing over is actually

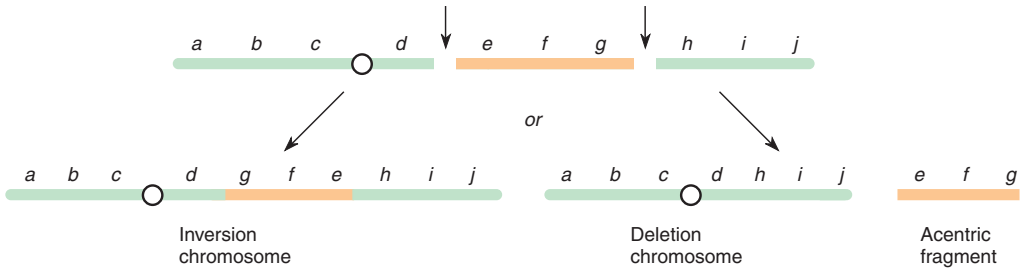


Figure 8.3 Two possible consequences of a double break (*top arrows*) in the same chromosome.

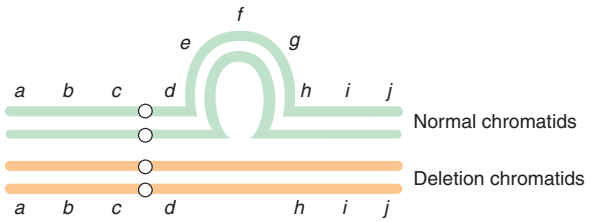


Figure 8.4 A bulge can occur in a meiotic tetrad if a large deletion has occurred.

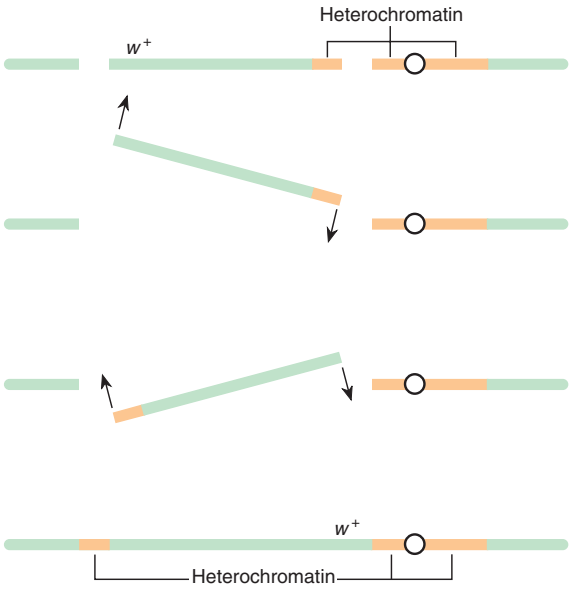


Figure 8.5 An inversion in the X chromosome of *Drosophila* produces a variegation in eye color in a female if her other chromosome is normal and carries the white-eye allele (X^w).

suppressed, but rather that the products of recombination within a loop are usually lost. (Suppression can also occur in small inversions where loops don't form.) Figure 8.8 shows a crossover within a loop. The two nonsister chromatids not involved in a crossover in the loop will end up in normal gametes (carrying either the normal chromosome or the intact inverted chromosome). The products of the crossover, rather than being a simple recombination of alleles, are a dicentric and an acentric chromatid. The acentric chromatid is not incorporated into a gamete nucleus, whereas the dicentric chromatid begins a breakage-fusion-bridge cycle that creates a genetic imbalance in the gametes. The gametes thus carry chromosomes with duplications and deficiencies.

The inversion pictured in figure 8.8 is a **paracentric inversion,** one in which the centromere is outside the inversion loop. A **pericentric inversion** is one in which the inverted section contains the centromere. It, too, suppresses crossovers, but for slightly different reasons (fig. 8.9). All four chromatid products of a single crossover within the loop have centromeres and are thus incorporated into the nuclei of gametes. However, the two recombinant chromatids are unbalanced—they both have duplications and deficiencies. One has a duplication for

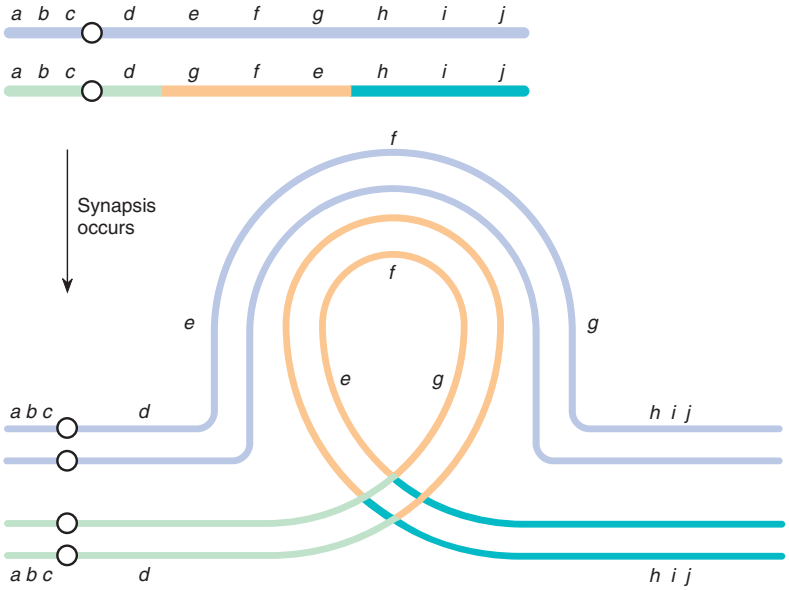


Figure 8.6 Tetrad at meiosis showing the loop characteristic of an inversion heterozygote.

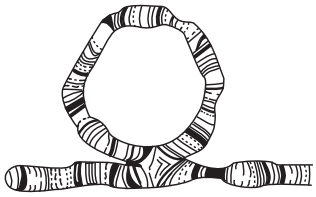


Figure 8.7 A *Drosophila* heterozygous for an inversion will show a loop in the salivary gland chromosomes. (Compare with figure 8.6.)

a-b-c-d and is deficient for *h-i-j,* whereas the other is the reciprocal—deficient for *a-b-c-d* and duplicated for *h-i-j* (in fig. 8.9). These duplication-deletion gametes tend to form inviable zygotes. The result, as with the paracentric inversion, is the apparent suppression of crossing over.

Results of Inversion

Crossing over within inversion loops results in **semisterility.** Almost all gametes that contain dicentric or imbalanced chromosomes form inviable zygotes. Thus, a certain proportion of the progeny of inversion heterozygotes are not viable.

Inversions have several evolutionary ramifications. Those alleles originally together in the noninversion chromosome and those found together within the inversion loop tend to stay together because of the low rate of successful recombination within the inverted region. If several loci affect the same trait, the alleles are referred to as a **supergene.** Until careful genetic analysis is done, the loci in a supergene could be mistaken for a single locus; they affect the same trait and are inherited apparently as a single unit. Examples include shell color and pattern in land snails and mimicry in butterflies (see chapter 21). Supergenes can be beneficial when they involve favorable gene combinations. However, at the same time, their inversion structure prevents the formation of new complexes. Supergenes, therefore, have evolutionary advantages and disadvantages. Chapter 21 discusses these evolutionary topics in more detail.

Sometimes the inversion process produces a record of the evolutionary history of a group of species. As species evolve, inversions can occur on preexisting inversions. This leads to very complex arrangements of loci. We can readily study these patterns in Diptera by noting the changed patterns of bands in salivary gland chromosomes. Since certain arrangements can only come about by a specific sequence of inversions, it is possible to know which species evolved from which. The same series of events can occur within the same species (box 8.1).

In summary then, inversions result in suppressed crossing over, semisterility, variegation position effects, and new linkage arrangements. All of these events have evolutionary consequences.

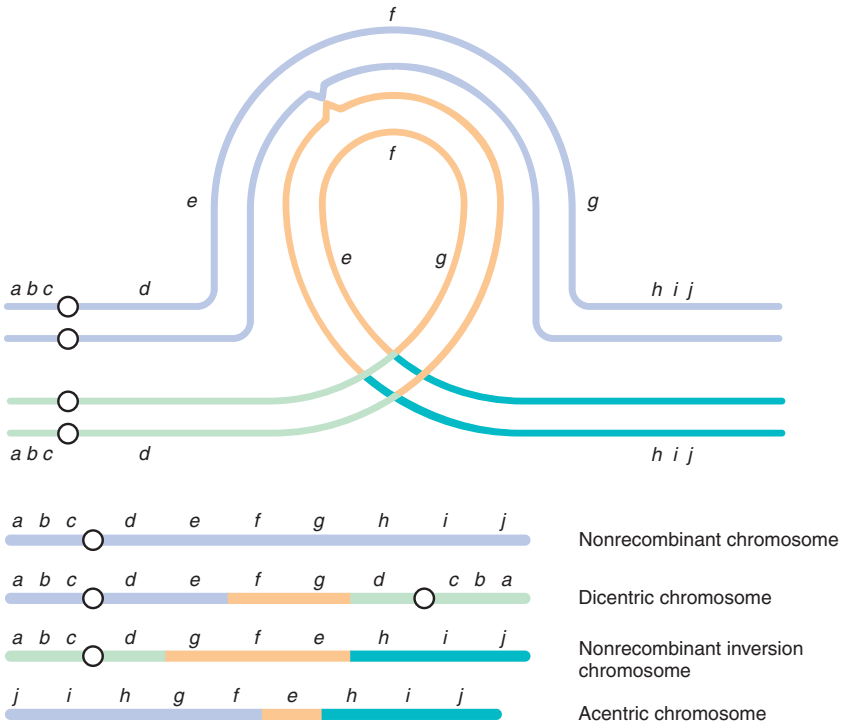


Figure 8.8 Consequences of a crossover in the loop region of a paracentric inversion heterozygote.

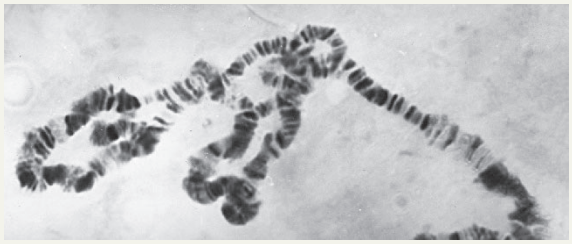
BOX 8.1

Experimental Methods

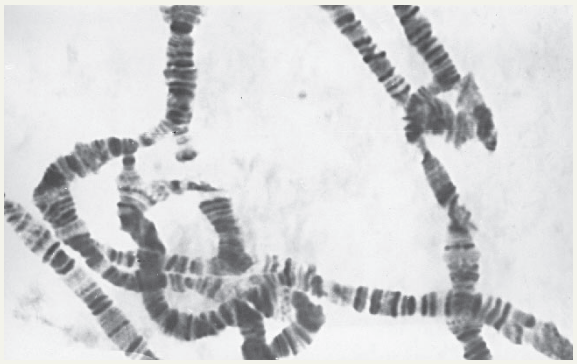
A Case History of the Use of Inversions to Determine Evolutionary Sequence

In 1966, David Futch published a study of the chromosomes of a fruit fly, *Drosophila ananassae,* an organism widely distributed throughout the tropical Pacific. The study was designed to determine something about the species status of various melanic forms of the fly. In the course of his work, Futch looked at the salivary gland chromosomes of flies from twelve different localities. He discovered twelve paracentric inversions, three pericentric inversions, and one translocation. Because of the precise banding patterns of these chromosomes, it was possible to determine the breakage points for each inversion.

Observation of several populations that have had sequential changes in their chromosomes makes it possible to determine the sequence of successive changes. Once one knows the sequence of changes in different populations of *Drosophila ananassae,* along with the geographic locations of the populations, it is possible to determine the history of the way the flies colonized these tropical islands. *D. ananassae* is particularly suited to this type of work because it is believed to be a recent invader to most of the Pacific Islands that it occupies. It is of interest to know about the spread of this species as an adjunct to studies of human migration in the Pacific Islands



(b)



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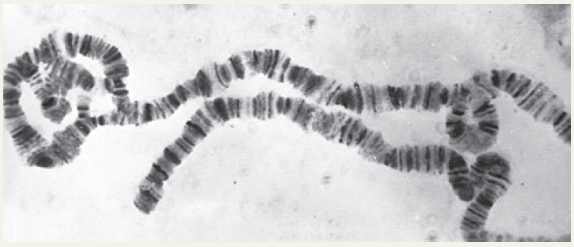


(a)

Figure 1 Photomicrographs of the left arm of chromosome 2 (2L) from larval *Drosophila ananassae* heterozygous for various complex gene arrangements. (*a*) Pairing when heterozygous for standard gene sequence and overlapping inversions (2LC; 2LD) and inversion 2LB (Standard × Tutuila light). (*b*) Pairing when heterozygous for standard gene sequence and single inversion 2LC and overlapping inversions (2LE; 2LB: Standard × New Guinea). (*c*) Pairing when heterozygous for overlapping inversions (2LD; 2LE; 2LF: Tutuila light × New Guinea). (From David G. Futch, "A study of speciation in South Pacific populations of *Drosophila ananassae,*" in Marshall R. Wheeler, ed., *Studies in Genetics,* no. 6615 [Austin: University of Texas Press, 1966]. Reproduced by permission.)

Two Breaks in Nonhomologous Chromosomes

Breaks can occur simultaneously in two nonhomologous chromosomes. Reunion can then take place in various ways. The most interesting case occurs when the ends of two nonhomologous chromosomes are translocated to each other in a **reciprocal translocation** (fig. 8.10). The organism in which this has happened, a reciprocal translocation heterozygote, has all the genetic material of the normal homozygote. Two outcomes of a reciprocal translocation, like those of an inversion, are new linkage arrangements in a homozygote—an organism with translocated chromosomes only—and variegation position effects.

During synapsis, either at meiosis or endomitosis, a point-for-point pairing in the translocation heterozygote

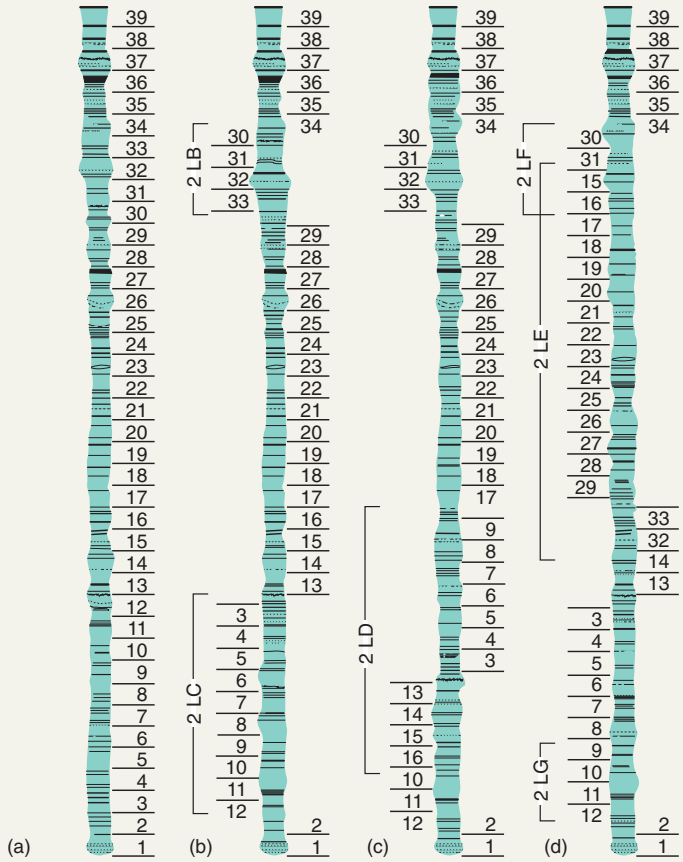


Figure 2 Chromosomal maps of 2L. (*a*) Standard gene sequence. (*b*) Ponape: breakpoints of 2LC and 2LB are indicated and the segments are shown inverted. (*c*) Tutuila light: breakpoints of 2LD are indicated. 2LC and 2LB are inverted. 2LD, which overlaps 2LC, is also shown inverted. (*d*) New Guinea: breakpoints of 2LE and 2LG are indicated. 2LC, 2LB, and 2LE are shown inverted. Note: only the breakpoints of 2LF and 2LG are shown; neither of these is inverted in the map. (From David G. Futch, "A study of speciation in South Pacific populations of *Drosophila ananassae,*" in Marshall R. Wheeler, ed., *Studies in Genetics,* no. 6615 [Austin: University of Texas Press, 1966]. Reproduced by permission.)

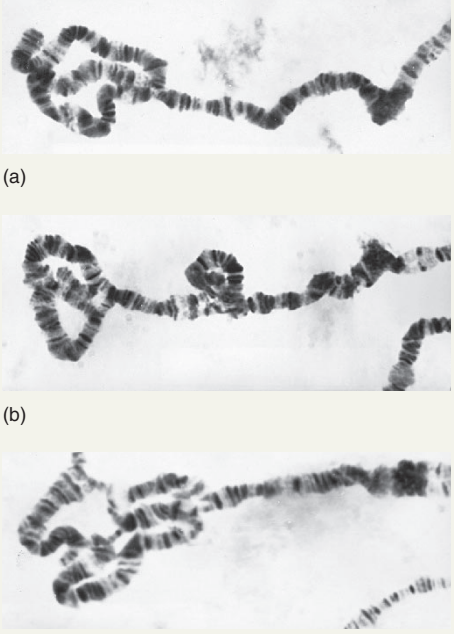


Figure 3 Photomicrographs of the right arm of chromosome 2 (2R) from larvae heterozygous for various complex gene arrangements. (*a*) Pairing when heterozygous for standard gene sequence and overlapping inversions (2RA; 2RB: Standard × Tutuila light). (*b*) Pairing when heterozygous for standard gene sequence and overlapping inversions (2RA; 2RC) and inversion 2RD (Standard × New Guinea). (*c*) Pairing when heterozygous for overlapping inversions 2RB, 2RC, and 2RD. Inversion 2RA is homozygous (Tutuila light × New Guinea). (From David G. Futch, "A study of speciation in South Pacific populations of *Drosophila ananassae,*" in Marshall R. Wheeler, ed., *Studies in Genetics,* no. 6615 [Austin: University of Texas Press, 1966]. Reproduced by permission.)

continued

can be accomplished by the formation of a cross-shaped figure (fig. 8.10). Such a figure is diagnostic of a reciprocal translocation. A single crossover in a reciprocal translocation heterozygote will not produce chromatids that are further imbalanced, as it does in an inversion heterozygote. However, reciprocal translocation heterozygotes do produce nonviable progeny. Problems can arise when centromeres separate at the first meiotic division.

Segregation After Translocation

Since two homologous pairs of chromosomes are involved, we have to keep track of the independent

BOX 8.1 CONTINUED

because *D. ananassae* is commensal with people.

Some of Futch's results are shown in figures 1–4, which diagram the left and right arms of the fly's second chromosome, as well as the synaptic patterns. We can see vividly the sequence of change in which one inversion occurs after a previous inversion has already taken place. In figures 2 and 4, the standard (*a*) gave rise to (*b*), which then gave rise independently to (*c*) and (*d*). The standard is from Majuro in the Marshall Islands and is believed to be in the ancestral group of the species. Ponape is the home of (*b*), (*c*) is from Tutuila (eastern Samoa), and (*d*) is from New Guinea. Thus, the sequence is Majuro to Ponape, and from there the same stock was transferred to Tutuila and New Guinea. This type of analysis has been useful in the *Drosophila* group throughout its range but especially in the Pacific Island populations and in the southwestern United States.

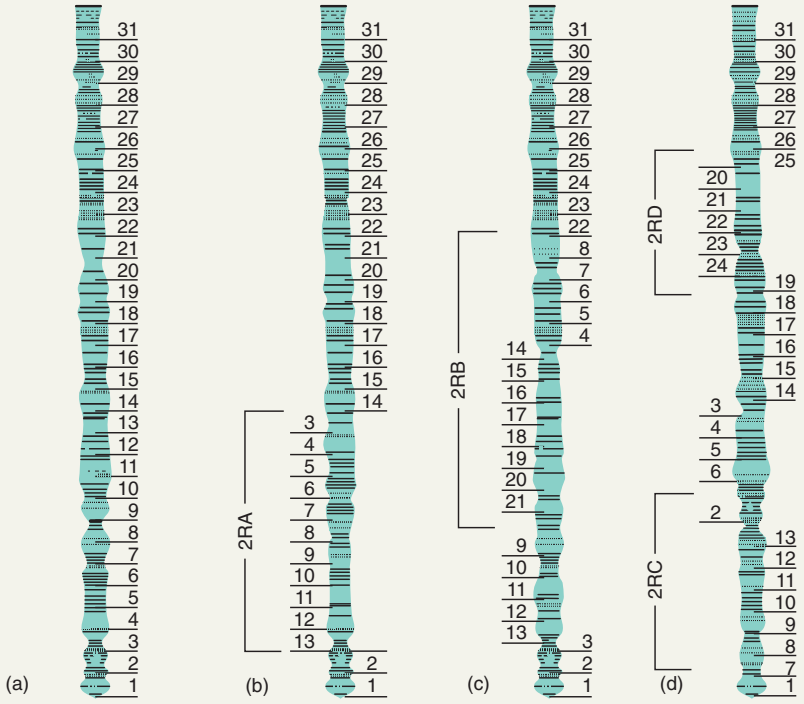


Figure 4 Chromosomal maps of 2R. (*a*) Standard gene sequence. (*b*) Ponape: breakpoints of 2RA are indicated and the segment is shown inverted. (*c*) Tutuila light: breakpoints of 2RB are indicated. 2RA is inverted and 2RB, which overlaps it, is also shown inverted. (*d*) New Guinea: breakpoints of 2RC and 2RD are indicated. 2RA is inverted; 2RC, which overlaps 2RA, and 2RD are shown inverted. (From David G. Futch, "A study of speciation in South Pacific populations of *Drosophila ananassae,*" in Marshall R. Wheeler, ed., *Studies in Genetics,* no. 6615 [Austin: University of Texas Press, 1966]. Reproduced by permission.)

segregation of the centromeres of the two tetrads. There are two common possibilities and one that occurs less often (fig. 8.11). The first, called **alternate segregation,** occurs when the first centromere assorts with the fourth centromere, leaving the second and third centromeres to go to the opposite pole. The result will be balanced gametes, one with normal chromosomes and the other with a reciprocal translocation. Also likely is the **adjacent-1** type of segregation, in which the first and third centromeres segregate together in the opposite direction from the second and fourth centromeres. Here, both types of gametes are unbalanced, carrying duplica-

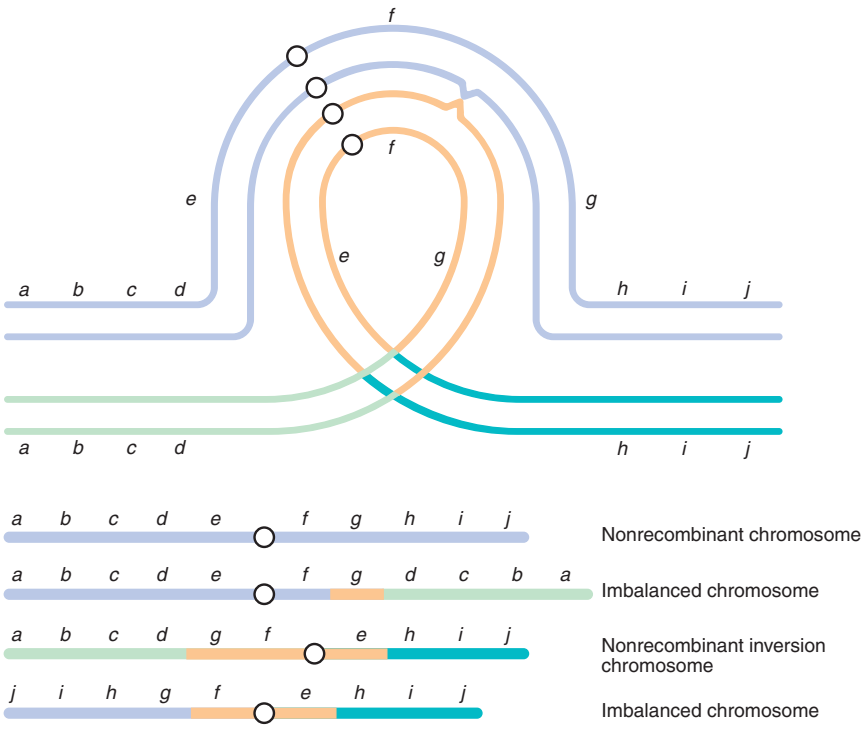


Figure 8.9 Consequences of a crossover in the loop region of a pericentric inversion heterozygote.

tions and deficiencies that are usually lethal. Since adjacent-1 segregation occurs at a relatively high frequency, a significant amount of sterility results from the translocation (as much as 50%).

An **adjacent-2** type of segregation (fig. 8.11), in which homologous centromeres go to the same pole (first with second, third with fourth), is a third possibility. This can result when the cross-shaped double tetrad opens into a circle in late prophase I. In the German cockroach, adjacent-2 patterns have been observed in 10 to 25% of meioses, depending upon which chromosomes are involved.

In summary, then, reciprocal translocations result in new linkage arrangements, variegated position effects, a cross-shaped figure during synapsis, and semisterility.

Centromeric Breaks

Another interesting variant of the simple reciprocal translocation occurs when two acrocentric chromosomes join at or very near their centromeres. The process, called a **Robertsonian fusion** after cytologist W. Robertson, produces a decrease in the number of chromosomes, although virtually the same amount of genetic material is maintained. Often, closely related species undergo Robertsonian fusions and end up with markedly different chromosome numbers without any significant difference in the quantity of their genetic material. Therefore, cytologists frequently count the number of chromosomal arms rather than the number of chromosomes to get a more accurate picture of species affinities. The number of arms is referred to as the **fundamental number,** or **NF** (French: *nombre fondamentale*). In a similar fashion, **centromeric fission** increases the chromosome number without changing the fundamental number.

Duplications

Duplications of chromosomal segments can occur, as we have just seen, by the breakage-fusion-bridge cycle or by crossovers within the loop of an inversion. There is another way that duplications arise in small adjacent regions of a chromosome. We illustrate this with a particularly interesting example, the *Bar* eye phenotype in *Drosophila* (fig. 8.12). The wild-type fruit fly has about 800 facets in each eye. The *Bar* (*B*) homozygote has about 70 (a range of 20–120 facets). Another allele, *Doublebar* (*BB:* sometimes referred to as *Ultrabar,* B^U), brings the facet number of the eye down to about 45 when heterozygous and to about 25 when homozygous. Around 1920, researchers

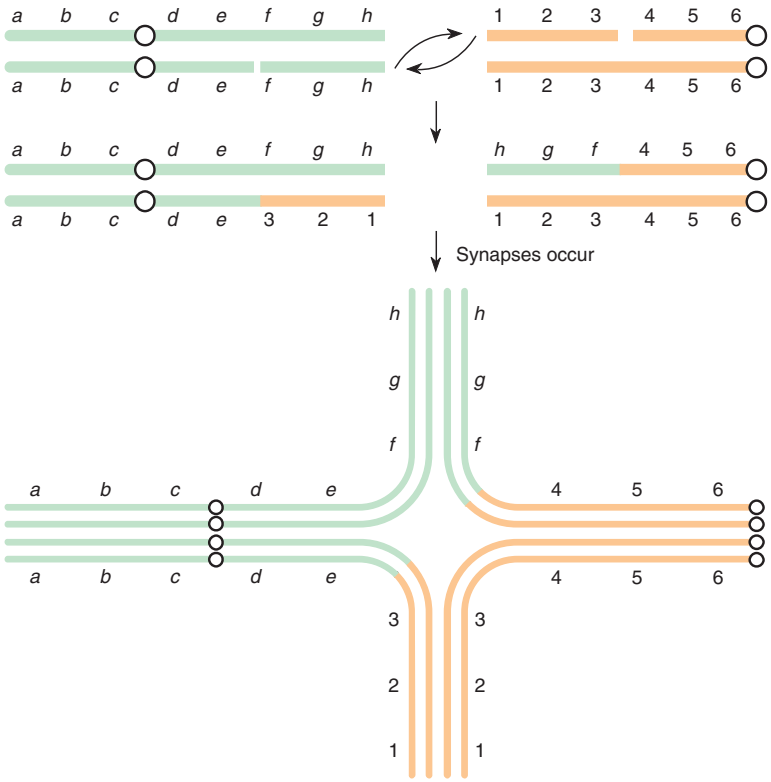


Figure 8.10 A reciprocal translocation heterozygote forms after breaks occur in nonhomologous chromosomes. Synapsis at meiosis forms a cross-shaped figure.

showed that about one progeny in 1,600 from homozygous *Bar* females is *Doublebar.* This is much more frequent than we expect from mutation.

Alfred Sturtevant found that in every *Doublebar* fly, a crossover had occurred between loci on either side of the *Bar* locus. He suggested that the change to *Doublebar* was due to **unequal crossing over** rather than to a simple mutation of one allele to another (fig. 8.13). If the homologous chromosomes do not line up exactly during synapsis, a crossover produces an unequal distribution of chromosomal material. Later, an analysis of the banding pattern of the salivary glands confirmed Sturtevant's hypothesis. It was found that *Bar* is a duplication of several bands in the 16A region of the X chromosome (fig. 8.14). *Doublebar* is a triplication of the segment.

A position effect also occurs in the *Bar* system. A *Bar* homozygote (*B/B*) and a *Doublebar*/wild-type heterozygote (BB/B^+) both have four copies of the 16A region. It would therefore be reasonable to expect that both genotypes would produce the same phenotype. However, the *Bar* homozygote has about seventy facets in each eye, whereas the heterozygote only has about forty-five. Thus, not only the amount of genetic material, but also its configuration, determines the extent of the phenotype. *Bar* eye was the first position effect discovered.

Chromosomal Rearrangements in Human Beings

Several human syndromes and abnormalities are the result of chromosomal rearrangements, including deletions and translocations. The most common are described here. Keep three points in mind as you read. First, all of these disorders are rare. Second, the deletion syndromes are often caused by a balanced translocation in one of the parents. And third, about one in five hundred live births contains a balanced rearrangement of some kind, either a reciprocal translocation or inversion.

Fragile-X Syndrome

The most common cause of inherited mental retardation is the **fragile-X syndrome.** It occurs in about one in every 1,250 males and about one in every 2,000 females. Symptoms include mental retardation, altered speech patterns, and other physical attributes. The condition is

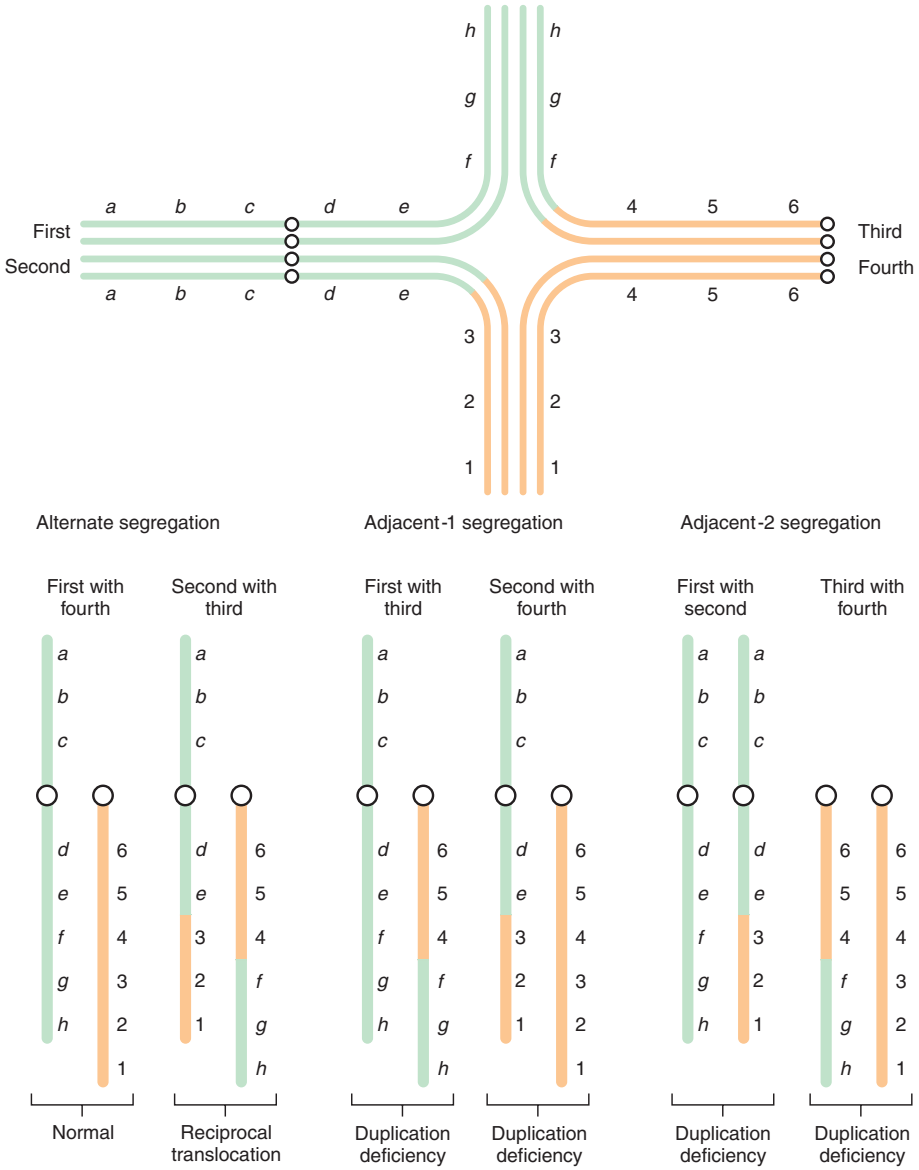


Figure 8.11 Three possible results of chromatid separation during meiosis in a reciprocal translocation heterozygote.

called the fragile-X syndrome because it is related to a region at the X chromosome tip that breaks more frequently than other chromosomal regions. However, the break is not required for the syndrome to occur, and the fragile-X chromosome is usually identified by the lack of chromatin condensation at the site; in fact, under the microscope, it appears that the tip of the chromosome is being held in place by a thread (fig. 8.15). The gene responsible for the syndrome is called *FMR-1,* for fragile-X mental retardation-1.

Fragile-X syndrome has a highly unusual pattern of inheritance: the chance of inheriting the disease increases through generations. This is so unusual a pattern that it was termed the Sherman Paradox. Approximately 20% of males with the fragile-X chromosome do not have symptoms but have grandchildren who do have the symptoms. The daughters of the symptomatic males also don't have symptoms, but obviously, they have another X chromosome to mask the symptoms. As generations proceed, the percentage of affected sons of

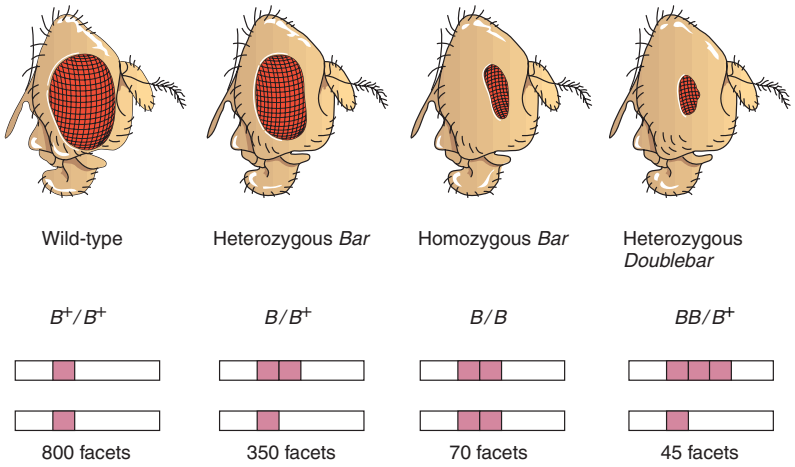


Figure 8.12 *Bar* eye in *Drosophila* females.

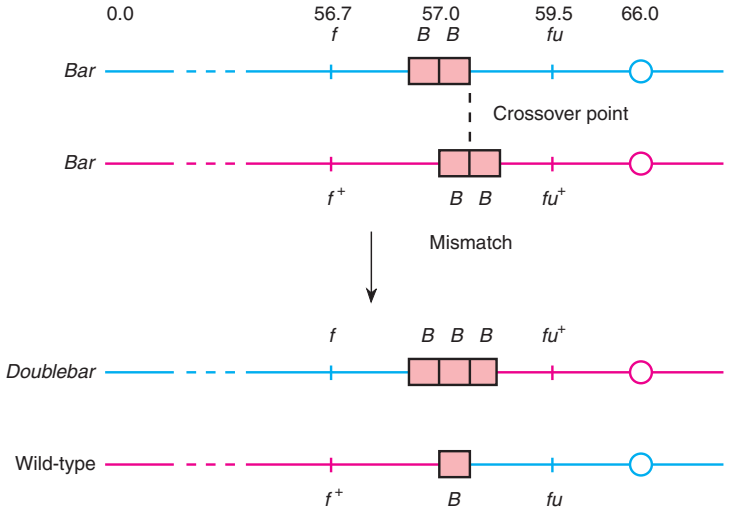


Figure 8.13 Unequal crossing over in a female *Bar*-eyed *Drosophila* homozygote as a result of improper pairing. A *Doublebar* chromosome (and concomitant wild-type chromosome) is produced by a crossover between forked (*f*) and fused (*fu*), two flanking loci.

carrier mothers increases. Molecular techniques, discussed in chapter 13, revealed the odd nature of this syndrome.

Basically, the *FMR-1* gene normally has between 6 and 50 copies of a three-nucleotide repeat, CCG. Chromosomes that have the fragile-site appearance have between 230 and 2,000 copies of the repeat. The number of repeats is very unstable; when carrier women transmit the chromosome, the number of repeats usually goes up. Repeat numbers above 230 inactivate the gene and thus cause the syndrome in men, who have only one copy of the X chromosome. The function of the gene is not currently known. This unusual form of inheritance, with unstable repeats in a gene, seems to be the mechanism in several other diseases as well, including muscular dystrophy and Huntington disease. We will discuss other unusual modes of inheritance in chapter 17.

Cri du Chat Syndrome, 46,XX or XY,5p–

The syndrome known as *cri du chat* (French: cry of the cat) is so called because of the catlike cry that about half the affected infants make. Microcephaly (an abnormally small head), congenital heart disease, and severe mental retardation are also common symptoms. This disorder arises from a deletion in chromosome 5 (fig. 8.16); most

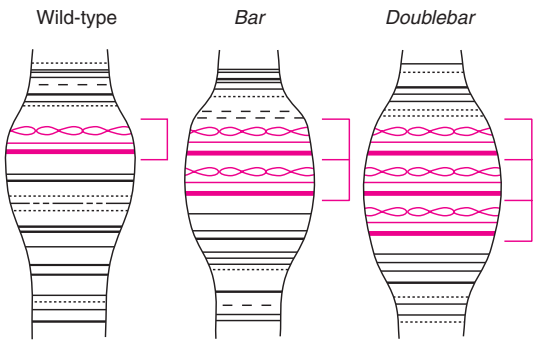


Figure 8.14 *Bar* region of the X chromosome of *Drosophila.*

Figure 8.15 Human metaphase chromosomes with the fragile-X site indicated by an arrow. (From Ian Craig, "Methylation and the Fragile X," *Nature* [1991] 349:742. Copyright © 1991 Macmillan Magazines, Ltd.)

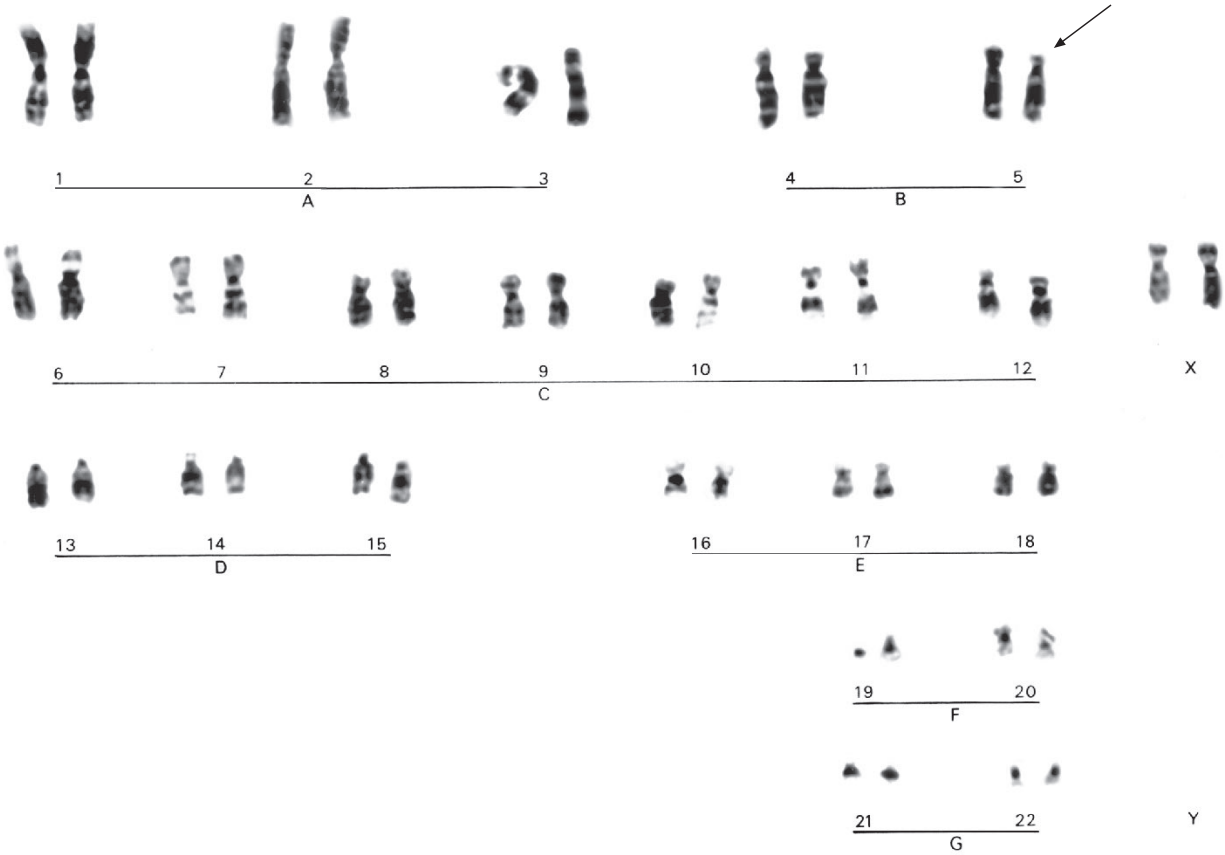


Figure 8.16 Karyotype of individual with cri du chat syndrome, due to a partial deletion of the short arm of chromosome 5 (5p–; *arrow*). (Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

other deletions studied (4p−, 13q−, 18p−, 18q−) also result in microcephaly and severe mental retardation. The rarity of viable deletion heterozygotes is consistent with the fact that viable monosomics (having a single chromosome of a pair) are rare. An individual heterozygous for a deletion is, in effect, monosomic for the deleted region of the chromosome. Evidently, monosomy or heterozygosity for larger deleted regions of a chromosome is generally lethal in human beings.

VARIATION IN CHROMOSOME NUMBER

Anomalies of chromosome number occur as either **euploidy** or **aneuploidy.** Euploidy involves changes in whole *sets* of chromosomes; aneuploidy involves changes in chromosome *number* by additions or deletions of less than a whole set.

Aneuploidy

An explanation for the terminology of aneuploid change appears in table 8.1. A diploid cell missing a single chromosome is **monosomic.** A cell missing both copies of that chromosome is **nullisomic.** A cell missing two nonhomologous chromosomes is a double monosomic. A similar terminology exists for extra chromosomes. For example, a diploid cell with an extra chromosome is **trisomic.** Aneuploidy results from nondisjunction in meiosis or by chromosomal lagging whereby one chromosome moves more slowly than the others during anaphase, is excluded from the telophase nucleus, and is thus lost. Here, nondisjunction is illustrated using the sex chromosomes in XY organisms such as human beings or fruit flies. Four examples are shown (fig. 8.17): nondisjunction in either the male or female at either the first or second meiotic divisions. Figure 8.18 shows the types of zygotes that can result when these nondisjunctional gametes fuse with normal gametes. All of the offspring produced are chromosomally abnormal. The names and kinds of these imbalances in human beings are detailed later in this chapter.

Bridges first showed the occurrence of nondisjunction in *Drosophila* in 1916 with crosses involving the white-eye locus. When a white-eyed female was crossed with a wild-type male, typically the daughters were wild-type and the sons were white-eyed. However, occasionally (one or two per thousand), a white-eyed daughter or a wild-type son appeared. This could be explained most easily by a nondisjunctional event in the white-eyed females, where X^wX^w and 0 eggs (without sex chromosomes) were formed. Under this hypothesis, if a Y-bearing sperm fertilized an X^wX^w egg, the offspring would be an X^wX^wY white-eyed daughter. If a normal X^+-bearing sperm fertilized the egg without sex chromosomes, the result would be an X^+0 wild-type son. Subsequently, these exceptional individuals were found by cytological examination to have precisely the predicted chromosomes (XXY daughters and X0 sons). The other types produced by this nondisjunctional event are the XX egg fertilized by an X-bearing sperm and the 0 egg fertilized by the Y-bearing sperm. The XXX zygotes are genotypically $X^wX^wX^+$, or wild-type daughters (which usually die), and Y0 flies (which always die).

Mosaicism

Rarely, an individual is made up of several cell lines, each with different chromosome numbers. These individuals are referred to as **mosaics** or **chimeras,** depending on the sources of the cell lines. Such conditions can be the result of nondisjunction or chromosomal lagging during mitosis in the zygote or in nuclei in the early embryo (mosaic). This is demonstrated, again for sex chromosomes, in figure 8.19. A lagging chromosome is shown in figure 8.20; in the figure, the X chromosome is lost in one

Table 8.1 **Partial List of Terms to Describe Aneuploidy, Using *Drosophila* as an Example (Eight Chromosomes: X, X, 2, 2, 3, 3, 4, 4)**

Type	Formula	Number of Chromosomes	Example
Normal	$2n$	8	X, X, 2, 2, 3, 3, 4, 4
Monosomic	$2n - 1$	7	X, X, 2, 2, 3, 4, 4
Nullisomic	$2n - 2$	6	X, X, 2, 2, 4, 4
Double monosomic	$2n - 1 - 1$	6	X, X, 2, 3, 4, 4
Trisomic	$2n + 1$	9	X, X, 2, 2, 3, 3, 4, 4, 4
Tetrasomic	$2n + 2$	10	X, X, 2, 2, 3, 3, 3, 3, 4, 4
Double trisomic	$2n + 1 + 1$	10	X, X, 2, 2, 2, 3, 3, 3, 4, 4

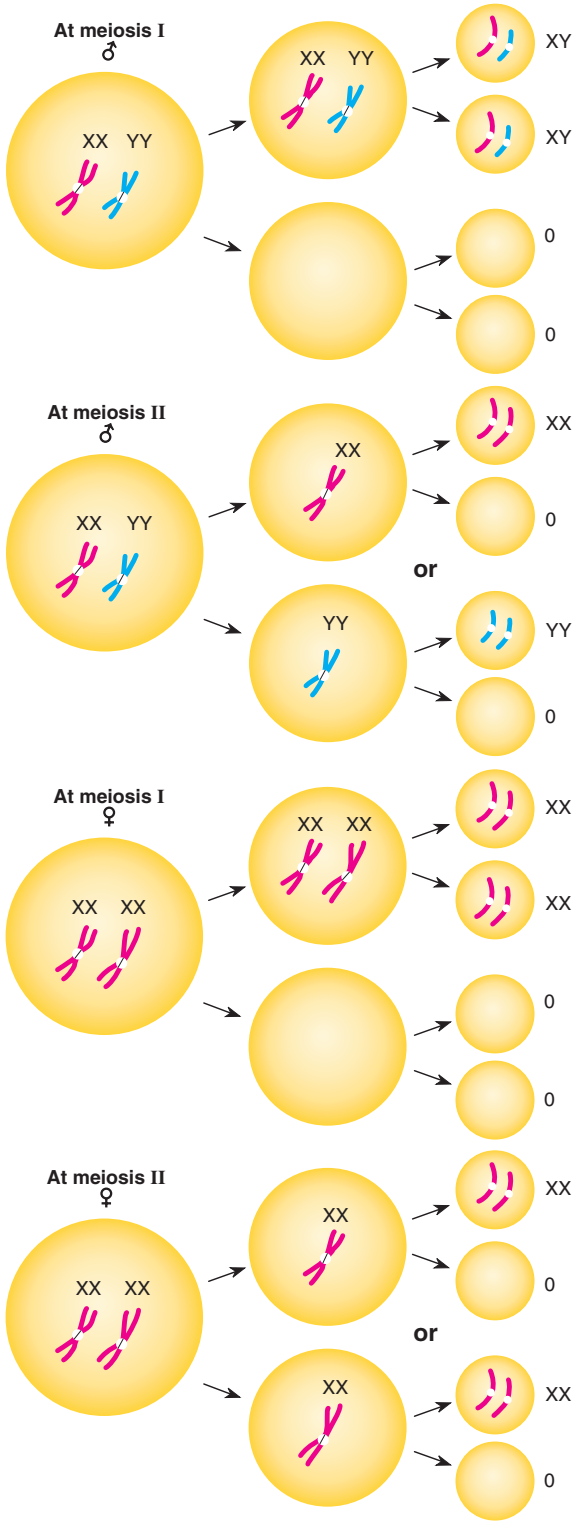


Figure 8.17 Nondisjunction of the sex chromosomes in *Drosophila* or human beings. "0" refers to the lack of sex chromosomes.

of the dividing somatic cells, resulting in an XX cell line and an X0 cell line. In *Drosophila,* if this chromosomal lagging occurs early in development, an organism that is part male (X0) and part female (XX) develops. Figure 8.21 shows a fruit fly in which chromosomal lagging has occurred at the one-cell stage, causing the fly to be half male and half female. A mosaic of this type, involving male and female phenotypes, has a special name—**gynandromorph.** (A **hermaphrodite** is an individual, not necessarily mosaic, with both male and female reproductive organs.) Many sex-chromosomal mosaics are known in humans, including XX/X, XY/X, XX/XY, and XXX/X. At least one case is known of a human XX/XY chimera that resulted from the fusion of two zygotes, one

Nondisjunction

Normal	♂ XY	♂ XX	♂ YY	♂ 0	♀ XX	♀ 0
♀X	XXY	XXX	XYY	X0		
♂X					XXX	X0
♂Y					XXY	Y0

Figure 8.18 Results of fusion of a nondisjunction gamete (*top*) with a normal gamete (*side*).

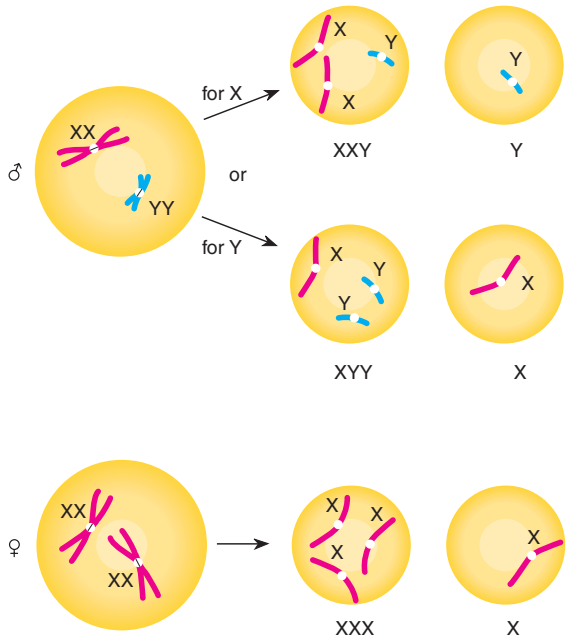


Figure 8.19 Mitotic nondisjunction of the sex chromosomes.

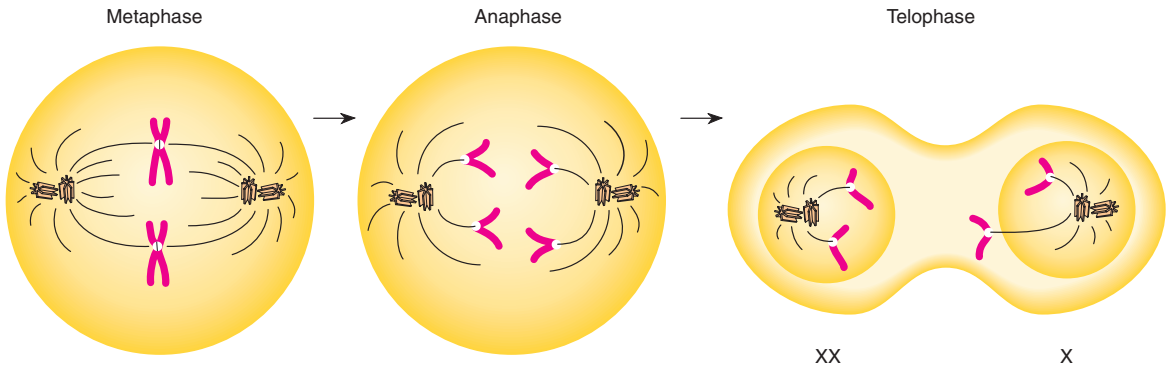


Figure 8.20 Chromosomal lagging at mitosis in the X chromosomes of a female *Drosophila.*

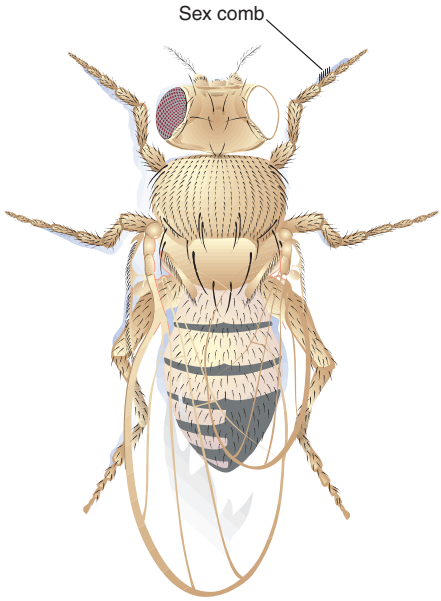


Figure 8.21 *Drosophila* gynandromorph. The *left side* is wild-type XX female; the *right side* is X0 male, hemizygous for white eye and miniature wing.

formed by a sperm fertilizing an ovum and the other formed by a second sperm fertilizing a polar body of that ovum.

Aneuploidy in Human Beings

Approximately 50% of spontaneous abortions (miscarriages) among women in the United States involve fetuses with some chromosomal abnormality; about half of these are autosomal trisomics. About one in 160 live human births has some sort of chromosomal anomaly; most are balanced translocations, autosomal trisomics, or sex-chromosomal aneuploids.

In the standard system of nomenclature, a normal human chromosome complement is 46,XX for a female and 46,XY for a male. The total chromosome number appears first, then the description of the sex chromosomes, and, finally, a description of autosomes if some autosomal anomaly is evident. For example, a male with an extra X chromosome would be 47,XXY. A female with a single X chromosome would be 45,X. Since all the autosomes are numbered, we describe their changes by referring to their addition (+) or deletion (−). For example, a female with trisomy 21 would be 47,XX,+21. The short arm of a chromosome is designated p, the longer arm, q. When a change in part of the chromosome occurs, a + after the arm indicates an increase in the length of that arm, whereas a minus sign (−) indicates a decrease in its length. For example, a translocation (t) that transfers part of the short arm of chromosome 9 to the short arm of chromosome 18 would be 46,XX, t(9p−;18p+). The semicolon indicates that both chromosomes kept their centromeres.

Following are descriptions of viable human aneuploids who survive long enough after birth to have a named syndrome.

Trisomy 21 (Down Syndrome), 47,XX or XY,+21

Down syndrome (figs. 8.22 and 8.23) affects about one in seven hundred live births. Most affected individuals are mildly to moderately mentally retarded and have congenital heart defects and a very high (1/100) risk of acute leukemia. They are usually short and have a broad, short skull; hyperflexibility of joints; and excess skin on the back of the neck. The physician John Langdon Down first described this syndrome in 1866. (Modern convention is to avoid the possessive form of a name in referring to a syndrome.) Down syndrome was the first human syndrome attributed to a chromosomal disorder; Jérôme Lejeune, a physician in Paris, published this finding in

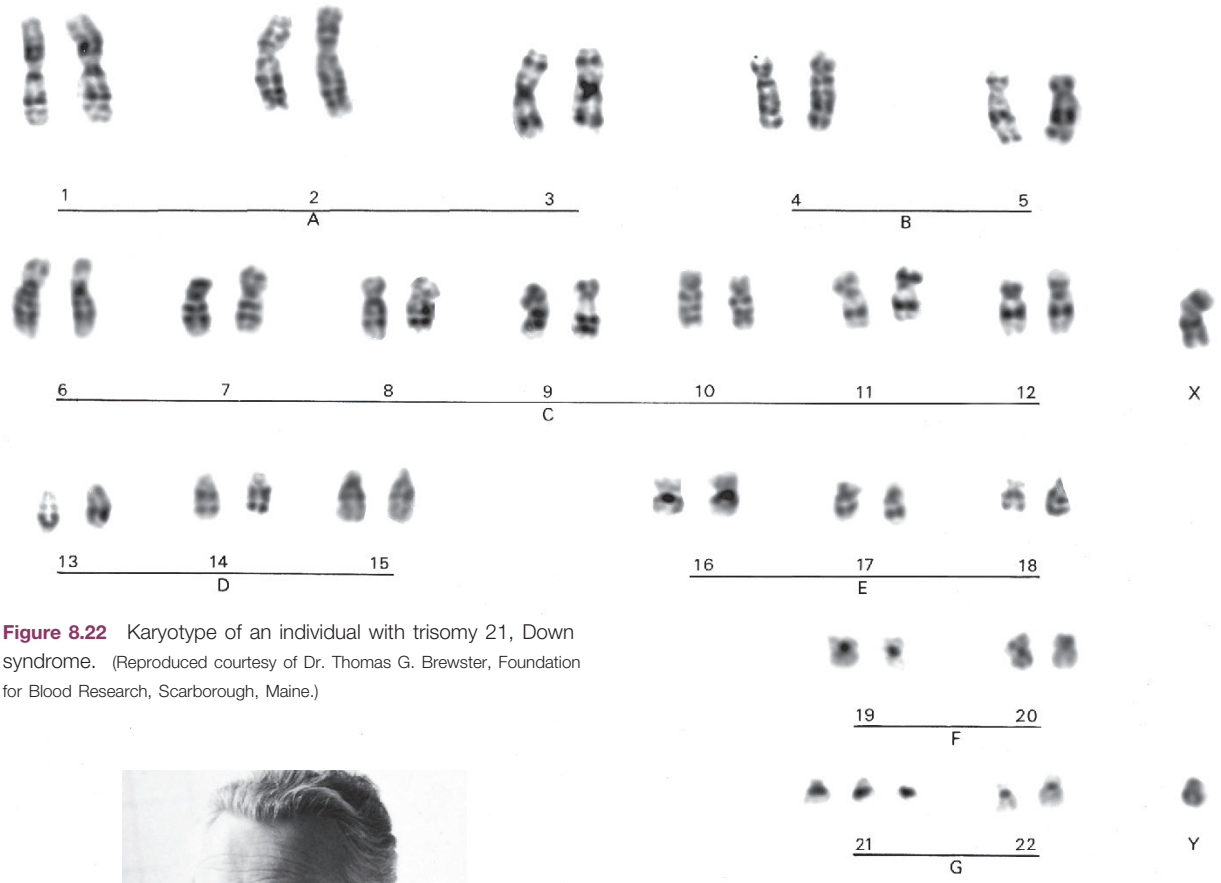


Figure 8.22 Karyotype of an individual with trisomy 21, Down syndrome. (Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

Jérôme Lejeune (1926–1994). (Courtesy of Dr. Jérôme Lejeune, Institut de Progenese, Paris.)

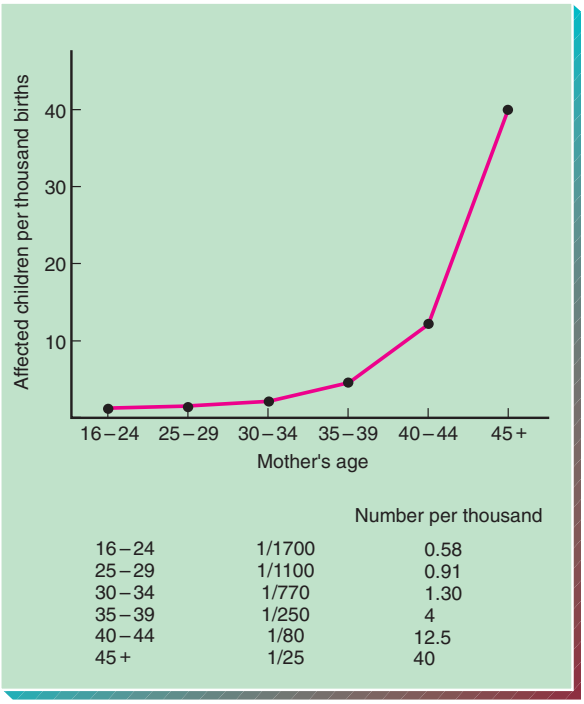
1959. An interesting aspect of this syndrome is its increased incidence among children of older mothers (fig. 8.24), a fact known more than twenty-five years before the discovery of the cause of the syndrome. Since the future ova are in prophase I of meiosis (dictyotene) since before the mother's birth, all ova are the same age as the female. Presumably, older ova are more susceptible to nondisjunction of chromosome 21.

Recently, techniques of molecular genetics (chapter 13) have been used to identify the origins of the three copies of chromosome 21 in a large sample of individuals with Down syndrome. As expected, the overwhelming majority of the extra copies of chromosome 21 (95%) were of maternal origin. About 5% of the cases of Down syndrome were of mitotic origin, occurring either in the gonad of one of the parents (evenly split between mothers and fathers) or possibly postzygotically in the fetus.

Familial Down Syndrome

Down syndrome (trisomy 21), as described, is usually the result of either a nondisjunctional event during gametogenesis or, rarely, a mitotic event. It is a function of maternal age and is not inherited. (Although about half the children of a person with trisomy 21 will have trisomy 21 because of aneuploid gamete production, the possibility that an unaffected relative of the person will have abnormal children is no greater than for a person of the same age chosen at random from the general population.) However, about 4% of those with Down syndrome have been found to have a translocation of chromosome 21,

Figure 8.23 Individual with trisomy 21. (© Hattie Young/SPL/Photo Researchers.)



		Number per thousand
16–24	1/1700	0.58
25–29	1/1100	0.91
30–34	1/770	1.30
35–39	1/250	4
40–44	1/80	12.5
45+	1/25	40

Figure 8.24 Increased risk of trisomy 21 attributed to the age of the mother. (From E. Hook, "Estimates of Maternal Age-Specific Risks of a Down-Syndrome Birth in Women age 34–41," *Lancet,* 2:33–34, Copyright © 1976 by The Lancet Ltd.)

usually associated with chromosome 14, 15, or 22. The translocational and nontranslocational types of Down syndrome have identical symptoms; however, a balanced translocation can be passed on to offspring (see fig. 8.11). Alternate segregation of centromeres in the translocation heterozygote produces either a normal gamete or one carrying the balanced translocation. Adjacent segregation causes partial trisomy for certain chromosomal parts. When this occurs for most of chromosome 21, Down syndrome results.

It is worth mentioning that aside from trisomy and translocation, Down syndrome can come about through mosaicism, as mentioned earlier, or a centromeric event. About 2% of individuals with Down syndrome are mosaic for cells with both two and three copies of chromosome 21. Some evidence suggests that the original zygotes were trisomic, but then a daughter cell lost one of the copies of chromosome 21. The severity of the symptoms in these individuals relates to the percentage of trisomic cells they possess. Mosaicism increases with maternal age, just as trisomy in general does. In extremely rare cases, Down syndrome is caused by an abnormal chromosome 21 that has, rather than a short and long arm, two identical long arms attached to the centromere. This type of chromosome, called an **isochromosome,** presumably occurs by an odd centromeric fission (fig. 8.25). Hence, a person with a normal chromosome 21 and an isochromosome 21 has three copies of the long arm of the chromosome and has Down syndrome.

Trisomy 18 (Edward Syndrome), 47,XX or XY,+18

Edward syndrome affects one in ten thousand live births (fig. 8.26). Most affected individuals are female, with 80 to 90% mortality by two years of age. The infant usually has an elfin appearance with small nose and mouth, a receding lower jaw, abnormal ears, and a lack of distal flexion creases on the fingers. The distal joints have limited motion, and the fingers display a characteristic posturing in which the little and index fingers overlap the middle two. The syndrome is usually accompanied by severe mental retardation.

Trisomy 13 (Patau Syndrome), 47,XX or XY,+13, and Other Trisomic Disorders

Patau syndrome affects one in twenty thousand live births. Diagnostic features are cleft palate, cleft lip, con-

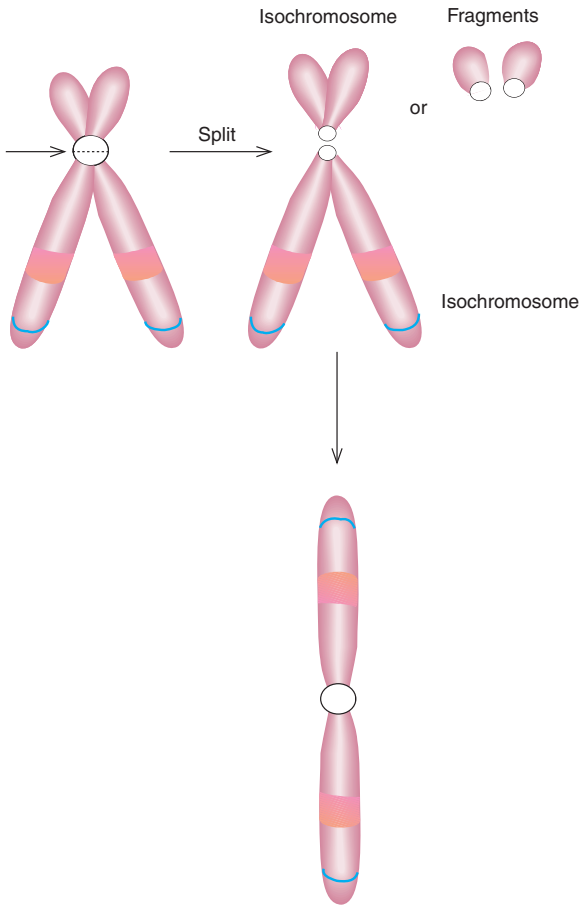


Figure 8.25 If the centromere of chromosome 21 breaks perpendicular to the normal division axis, it can form an isochromosome of the long arms and either an isochromosome of the short arms or two separate fragments. This can happen during anaphase of mitosis or meiosis II.

genital heart defects, polydactyly, and severe mental retardation. Mortality is very high in the first year of life.

Other autosomal trisomics are known but are extremely rare. These include trisomy 8 (47,XX or XY,+8) and cat's eye syndrome, a trisomy of an unidentified, small acrocentric chromosome (47,XX or XY,[+acrocentric]). Several aneuploids involving sex chromosomes are also known.

Turner Syndrome, 45,X

About one in ten thousand live female births is of an infant with Turner syndrome. This and 45,XX or XY,−21 and

Figure 8.26 Child with trisomy 18, Edward syndrome.
(Reproduced courtesy of Dr. Jérôme Lejeune, Institut de Progenese, Paris.)

45,XX or XY,−22 are the only nonmosaic, viable monosomics recorded in human beings (fig. 8.27), indicating the severe consequences monosomy has on all but the two smallest autosomes and a sex chromosome. Individuals with Turner syndrome usually have normal intelligence but underdeveloped ovaries, abnormal jaws, webbed necks, and shieldlike chests.

The symptoms of Turner syndrome have been logically deduced to be caused by a single dosage of genes that are normally present and active in two dosages. Thus, these genes would be located on both the X and Y chromosomes (pseudoautosomal) to provide two dosages in normal XY males and also be active in both X chromosomes in normal XX females. Therefore, they should be located on regions of the X chromosome that escape inactivation (see chapter 5). Studies of persons with small X-chromosomal deletions and molecular analyses of the X and Y chromosomes (outlined in chapter 13) have caused two genes to emerge as candidates: *ZFY* (on the Y chromosome, termed *ZFX* on the X chromosome) and *RPS4Y* (on the Y chromosome, termed *RPS4X* on the X chromosome). *ZFY* (zinc finger on the Y chromosome) was once believed to be the male-determining gene in mammals. *RPS4Y* encodes a ribosomal protein, one of the many proteins making up the ribosome.

It is interesting to note a dosage-compensation difference in people and mice, which have analogous genes termed *Zfx* and *Rps4x*. In mice, unlike in people, these genes are inactivated in the "Lyonized" X chromosome in females and have restricted activity in the Y

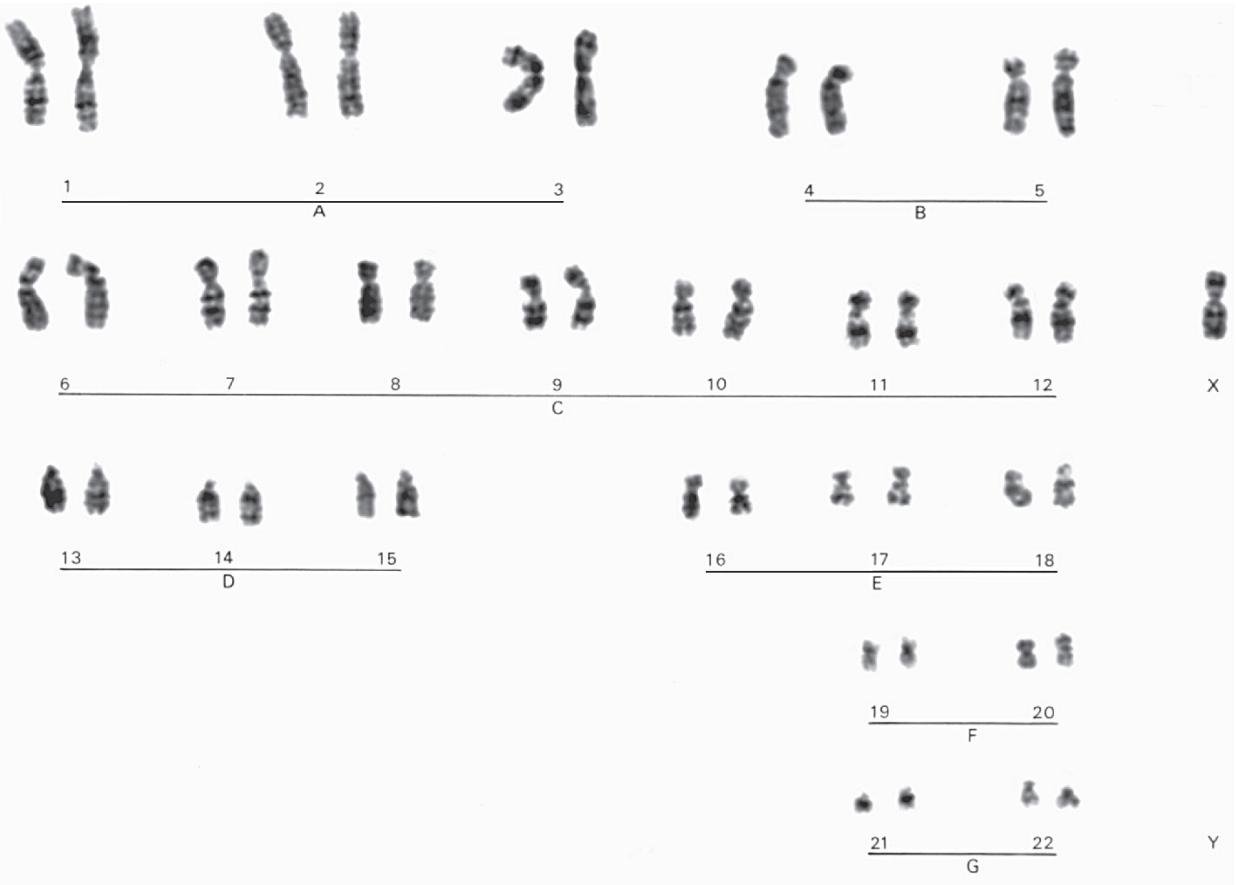


Figure 8.27 Karyotype of a person with Turner syndrome (X0). (Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

chromosome. Hence, mouse cells seem normally to have only one copy of these genes functioning in normal XY males and XX females. Therefore, we would predict that the X0 genotype in mice would produce few if any negative effects as compared with a human X0 genotype, since mouse cells of both sexes normally only have one functional copy of each gene. In fact, human Turner syndrome fetuses have a 99% prenatal mortality rate, but virtually no prenatal mortality affects mouse fetuses with the X0 genotype (born of XX mothers). This confirms our predictions and points to differences between people and mice in dosage-compensation mechanisms for specific genes.

XYY Karyotype, 47,XYY

About one in one thousand live male births is of an individual with an XYY karyotype. (We avoid the term *syndrome* here because XYY men have no clearly defined series of attributes, other than often being taller than normal.) Some controversy has surrounded this karyotype because it was once reported that it occurred in abundance in a group of mentally subnormal males in a prison hospital. Seven XYY males were found among 197 inmates, whereas only one in about two thousand control men were XYY. This study has subsequently been expanded and corroborated. Although it is now fairly well established that the incidence of XYY males in prison is about twentyfold higher than in society at large, the statistic is somewhat misleading: the overwhelming number of XYY men seem to lead normal lives. At most, about 4% of XYY men end up in penal or mental institutions, where they make up about 2% of the population.

There is some indication that the XYY men in prison had lower intelligence test levels. Thus, criminal tendency may be attributed to lower intelligence rather than a predisposition toward criminality caused by an extra Y chromosome. For the most part, expanded studies have indicated that XYY criminals do not commit violent crimes.

Research on this karyotype has produced its own problems. A research project at Harvard University on XYY males came under intense public pressure and was eventually terminated. The project, under the direction of Stanley Walzer (a psychiatrist) and Park Gerald (a geneticist), involved screening all newborn boys at the Boston Hospital for Women and following the development of those with chromosomal anomalies. The criticism of this work centered mainly on the necessity of informing parents that their sons had an XYY karyotype that might be associated with behavioral problems. Opponents of this work claimed that telling the parents could trigger a self-fulfilling prophecy; that is, parents who heard that their children were not normal and *might* cause trouble might then behave toward their children in a manner that would increase the probability that their children *would* cause trouble. The opponents claimed that the risks of this research outweighed the benefits. The project was terminated in 1975 primarily because of the harassment Walzer faced.

Klinefelter Syndrome, 47,XXY

The incidence of Klinefelter syndrome is about one in one thousand live births. Tall stature and infertility are common symptoms. Diagnosis is usually by buccal (cheek tissue) smear to ascertain the presence of a Barr body in a male, indicating an XXY karyotype. Some problems with behavior and speech development are associated with this syndrome.

Triple-X Female, 47,XXX, and Other Aneuploid Disorders of Sex Chromosomes

A triple-X female appears in about one in one thousand female live births. Fertility can be normal, but these individuals are usually mildly mentally retarded. Delayed growth, as well as congenital malformations, are also sometimes present. Other sex-chromosomal aneuploids, including XXXX, XXXXX, and XXXXY, are extremely rare. All seem to be characterized by mental retardation and growth deficiencies.

Euploidy

Euploid organisms have varying numbers of complete haploid chromosomal sets. We are already familiar with haploids (n) and diploids ($2n$). Organisms with higher numbers of sets, such as **triploids** ($3n$) and **tetraploids** ($4n$), are called polyploids. Three kinds of problems plague polyploids. First, the potential exists for a general imbalance in the organism due to the extra genetic material in each cell. For example, a triploid human fetus has about a one in a million chance to survive to birth, at which time death usually occurs due to problems in all organ systems. Second, if there is a chromosomal sex-determining mechanism, it may be disrupted by polyploidy. And third, meiosis produces unbalanced gametes in many polyploids.

If the polyploid has an odd number of sets of chromosomes, such as triploid ($3n$), two of the three homologues will tend to pair at prophase I of meiosis, producing a bivalent and a univalent. The bivalent separates normally, but the third chromosome goes independently to one of the poles. This separation results in a 50% chance of aneuploidy in each of the n-different chromosomes, rapidly decreasing the probability of a balanced gamete as n increases. Therefore, as n increases, so does the likelihood of sterility. An alternative to the bivalent-univalent type of synapsis is the formation of trivalents, which have similar problems (fig. 8.28). Even-numbered polyploids, such as tetraploids ($4n$), can do better during meiosis. If the centromeres segregate two by two in each of the n meiotic figures, balanced gametes can result. Often, however, the multiple copies of the chromosomes form complex figures during synapsis, including monovalents, bivalents, trivalents, and quadrivalents, tending to result in aneuploid gametes and sterility.

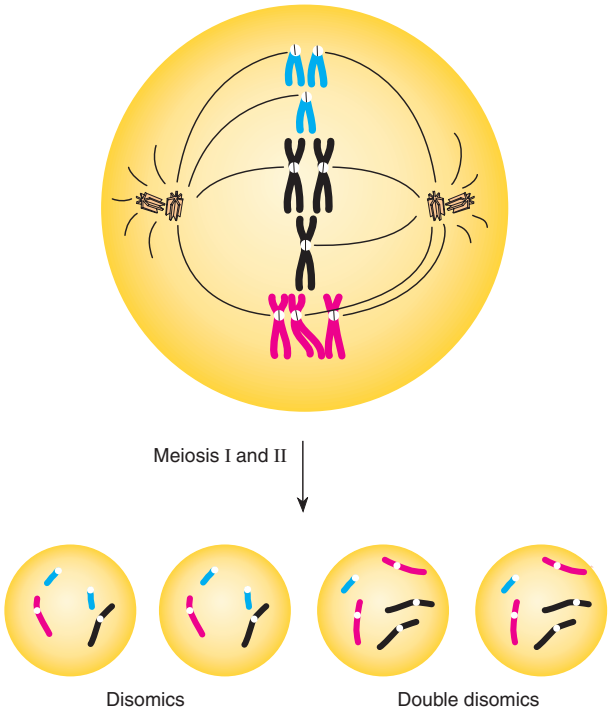


Figure 8.28 Meiosis in a triploid ($3n = 9$) and one possible resulting arrangement of gametes. The probability of a "normal" gamete is $(1/2)^n$ where n equals the haploid chromosome number. Here, $n = 3$ and $(1/2)^3 = 1/8$.

Some groups of organisms, primarily plants, have many polyploid members. An estimated 30 to 80% of all flowering plant species (angiosperms) are polyploids, as are 95% of ferns. (Polyploidy is apparently rare in gymnosperms and fungi.) For example, the genus of wheat, *Triticum,* has members with fourteen, twenty-eight, and forty-two chromosomes. Because the basic *Triticum* chromosome number is $n = 7$, these forms are $2n$, $4n$, and $6n$ species, respectively. Chrysanthemums have species of eighteen, thirty-six, fifty-four, seventy-two, and ninety chromosomes. With a basic number of $n = 9$, these species represent a $2n$, $4n$, $6n$, $8n$, and $10n$ series. In both these examples, the even-numbered polyploids are viable and fertile, but the odd-numbered polyploids are not.

Autopolyploidy

Polyploidy can come about in two different ways. In **autopolyploidy,** all of the chromosomes come from within the same species. In **allopolyploidy,** the chromosomes come from the hybridization of two different species (fig. 8.29). Autopolyploidy occurs in several different ways. The fusion of nonreduced gametes creates polyploidy. For example, if a diploid gamete fertilizes a normal haploid gamete, the result is a triploid. Similarly, if a diploid gamete fertilizes another diploid gamete, the result is a tetraploid. The equivalent of a nonreduced gamete comes about in meiosis if the parent cell is polyploid to begin with. For example, if one branch of a diploid plant is tetraploid, its flowers produce diploid gametes. These gametes are not the result of a failure to reduce chromosome numbers meiotically, but rather the result of successful meiotic reduction in a polyploid flower. The tetraploid tissue of the plant in this example can originate by the **somatic doubling** of diploid tissues.

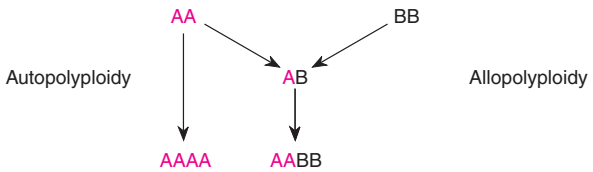


Figure 8.29 Autopolyploidy and allopolyploidy. If A and B are the haploid genomes of species A and B, respectively, then autopolyploidy produces a species with an AAAA karyotype, and allopolyploidy (with chromosome doubling) produces a species with AABB karyotype. If A represents seven chromosomes, then an AA diploid has fourteen chromosomes and an AAAA tetraploid has twenty-eight chromosomes. If B represents five chromosomes, then a BB diploid has ten chromosomes and an AABB allotetraploid has twenty-four chromosomes.

Somatic doubling can come about spontaneously or be caused by anything that disrupts the normal sequence of a nuclear division. For example, colchicine induces somatic doubling by inhibiting microtubule formation. This prevents the formation of a spindle and thus prevents the chromosomes from moving apart during either mitosis or meiosis. The result is a cell with double the chromosome number. Other chemicals, temperature shock, and physical shock can produce the same effect.

Allopolyploidy

Allopolyploidy comes about by cross-fertilization between two species. The resulting offspring have the sum of the reduced chromosome number of each parent species. If each chromosome set is distinctly different, the new organisms have difficulty in meiosis because no two chromosomes are sufficiently homologous to pair. Then every chromosome forms a univalent (unpaired) figure, and they separate independently during meiosis, producing aneuploid gametes. However, if an organism can survive by vegetative growth until somatic doubling takes place in gamete precursor cells ($2n \rightarrow 4n$), or alternatively, if the zygote was formed by two unreduced gametes ($2n + 2n$), the resulting offspring will be fully fertile because each chromosome has a pairing partner at meiosis. We can draw an example from the work of Russian geneticist G. D. Karpechenko.

In 1928, Karpechenko worked with the radish (*Raphanus sativus,* $2n = 18$, $n = 9$) and cabbage (*Brassica oleracea,* $2n = 18$, $n = 9$). When these two plants are crossed, an F_1 results with $n + n = 18$ (9 + 9). This plant, which is an allodiploid, has characteristics intermediate between the two parental species (fig. 8.30). If somatic doubling takes place, the chromosome number is doubled to thirty-six, and the plant becomes an allopolyploid (an allotetraploid of $4n$). Since each chromosome has a homologue, this allotetraploid is also referred to as an **amphidiploid.** If we did not know its past history, this plant would simply be classified as a diploid with $2n = 36$. In this case, the new amphidiploid cannot successfully breed with either parent because the offspring are sterile triploids. It is, therefore, a new species and has been named *Raphanobrassica.* As an agricultural experiment, however, it was not a success because it did not combine the best features of the cabbage and radish.

Polyploidy in Plants and Animals

Although polyploids in the animal kingdom are known (in some species of lizards, fish, invertebrates, and a

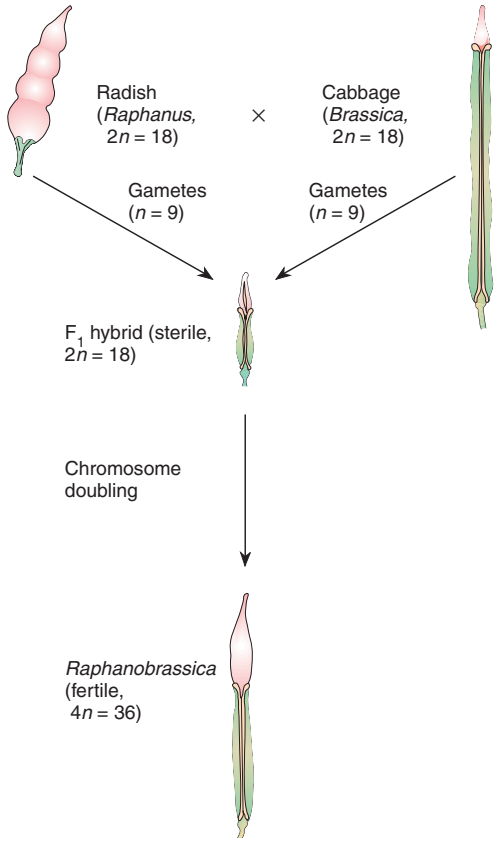


Figure 8.30 Hybridization of cabbage and radish, showing the resulting hybrid fruiting structures.

tetraploid mammal, the red viscacha rat), polyploidy as a successful evolutionary strategy is primarily a plant phenomenon. There are several reasons for this. To begin with, many more animals than plants have chromosomal sex-determining mechanisms. Polyploidy severely disrupts these mechanisms. For example, Bridges discovered a tetraploid female fruit fly, but it has not been possible to produce a tetraploid male. The tetraploid female's progeny were triploids and intersexes. A second reason why polyploidy is more common in plants is because plants can generally avoid the meiotic problems of polyploidy longer than most animals. Some plants can exist vegetatively, allowing more time for the rare somatic doubling event to occur that will produce an amphidiploid; animal life spans are more precisely defined, allowing less time for a somatic doubling. And third, many plants depend on the wind or insect pollinators to fertilize them and thus have more of an opportunity for hybridization. Many animals have relatively elaborate courting rituals that tend to restrict hybridization.

Polyploidy has been used in agriculture to produce "seedless" as well as "jumbo" varieties of crops. Seedless watermelon, for example, is a triploid. Its seeds are mostly sterile and do not develop. It is produced by growing seeds from the cross between a tetraploid variety and a diploid variety. Jumbo Macintosh apples are tetraploid.

SUMMARY

STUDY OBJECTIVE 1: To observe the nature and consequences of chromosomal breakage and reunion 178–190

Variation can occur in the structure and number of chromosomes in the cells of an organism. When chromosomes break, the ends become "sticky"; they tend to reunite with other broken ends. A single break can lead to deletions or the formation of acentric or dicentric chromosomes. Dicentrics tend to go through breakage-fusion-bridge cycles, which result in duplications and deficiencies.

Two breaks in the same chromosome can yield deletions and inversions. Variegation position effects, as well as new linkage arrangements, can result. Inversion heterozygotes produce loop figures during synapsis, which can form either at meiosis or in polytene chromosomes. Heterozygosity for an inversion suppresses crossovers; organisms that are heterozygotes are semisterile.

Reciprocal translocations can result from single breaks in nonhomologous chromosomes. These produce cross-shaped figures at synapsis and result in semisterility. The *Bar* eye phenotype of *Drosophila* is an example of a duplication that causes a position effect.

STUDY OBJECTIVE 2: To observe the nature and consequences of variation in chromosome numbers in human and nonhuman organisms 190–199

Changes in chromosome number can involve whole sets (euploidy) or partial sets (aneuploidy) of chromosomes. Aneuploidy usually results from nondisjunction or chromosomal lagging. Several medical syndromes, such as Down, Turner, and Klinefelter syndromes, and the XYY karyotype are caused by aneuploidy.

Polyploidy leads to difficulties in chromosomal sex-determining mechanisms, general chromosomal imbalance, and problems during meiotic segregation. It has been more successful in plants than in animals because plants generally lack chromosomal sex-determining mechanisms. Plants can also avoid meiotic problems by propagating vegetatively. In both animals and plants, even-numbered polyploids do better than odd-numbered polyploids because they have a better chance of producing balanced gametes during meiosis. Somatic doubling provides each chromosome in a hybrid organism with a homologue, and thus makes possible tetrad formation at meiosis. New species have arisen by polyploidy.

SOLVED PROBLEMS

PROBLEM 1: What are the consequences of an inversion?

Answer: In an inversion homozygote, the consequences are change in linkage arrangements, including new orders and map distances, and the possibility of position effects if a locus is placed into or near heterochromatin. In an inversion heterozygote, crossover suppression causes semisterility because zygotes that carry genic imbalances are lost. Inversion heterozygotes can be seen as meiotic loop structures or loops formed in endomitotic chromosomes such as those found in the salivary glands of fruit flies. In an evolutionary sense, inversions result in supergenes, locking together allelic combinations.

PROBLEM 2: What are the consequences of a monosomic chromosome in human beings?

Answer: In human beings, monosomy is rare, meaning that, with few exceptions, it is lethal. In fact, monosomics are also rare in spontaneous abortions, indicating that most monosomic fetuses are lost before the woman is aware of the pregnancy. The only monosomics known to be viable in human beings are Turner syndrome (45,X) and monosomics of chromosomes 21 and 22, the two smallest autosomal chromosomes.

PROBLEM 3: Ebony body (*e*) in flies is an autosomal recessive trait. A true-breeding ebony female (*ee*) is mated with a true-breeding wild-type male that has been irradiated. Among the wild-type progeny is a single ebony male. Explain this observation.

Answer: The cross is $ee \times e^+e^+$, and all F_1s should be e^+e (wild-type). The use of irradiation alerts us to the possibility of chromosomal breaks, as well as simple mutations. What type of chromosomal aberration would allow a recessive trait to appear unexpectedly? A deletion, which creates pseudodominance when there is no second allele, is a good possibility. The male in question could have gotten the ebony allele from its mother and no homologous allele from its father. Alternatively, the wild-type allele from the father could have mutated to an ebony allele.

EXERCISES AND PROBLEMS*

VARIATION IN CHROMOSOMAL STRUCTURE

1. What kind of figure is observed in meiosis of a reciprocal translocation homozygote?
2. Can a deletion result in the formation of a variegation position effect? If so, how?
3. Does crossover suppression occur in an inversion homozygote? Explain.
4. Which rearrangements of chromosomal structure cause semisterility?
5. What are the consequences of single crossovers during tetrad formation in a reciprocal translocation heterozygote?
6. Give the gametic complement, in terms of acentrics, dicentrics, duplications, and deficiencies, when a three-strand double crossover occurs within a paracentric inversion loop.
7. In studying a new sample of fruit flies, a geneticist noted phenotypic variegation, semisterility, and the nonlinkage of previously linked genes. What probably caused this, and what cytological evidence would strengthen your hypothesis?
8. In a second sample of flies, the geneticist found a position effect and semisterility. The linkage groups were correct, but the order was changed and crossing over was suppressed. What probably caused this, and what cytological evidence would strengthen your hypothesis?

* Answers to selected exercises and problems are on page A-9.

9. Diagram the results of alternate segregation for a three-strand double crossover between a centromere and the cross center in a reciprocal translocation heterozygote.

10. A heterozygous plant *A B C D E/a b c d e* is testcrossed with an *a b c d e/a b c d e* plant. Only the following progeny appear.

A B C D E/a b c d e
a b c d e/a b c d e
A b c d e/a b c d e
a B C D E/a b c d e
A B C D e/a b c d e
a b c d E/a b c d e

What is unusual about the results? How can you explain them?

11. White eye color in *Drosophila* is an X-linked recessive trait. A wild-type male is irradiated and mated with a white-eyed female. Among the progeny is a white-eyed female.

a. Why is this result unexpected, and how could you explain it?

b. What type of progeny would you expect if this white-eyed female is crossed with a normal, nonirradiated male?

12. You are trying to locate an enzyme-producing gene in *Drosophila,* which you know is located on the third chromosome. You have five strains with deletions for different regions of the third chromosome (a slash — / — indicates a deleted region):

```
Normal   0   10   20   30   40   50   60   map units
Strain A ////// ______________________________
Strain B  ___/////////////////_______________
Strain C  _____________/////////////_________
Strain D  ________________/////////////_____
Strain E  ______________________/////////////
```

You cross each strain with wild-type flies and measure the amount of enzyme in the F_1 progeny. The results appear as follows. In what region is the gene located?

Strain Crossed	Percentage of Wild-Type Enzyme Produced in F_1 Progeny
A	100
B	45
C	54
D	98
E	101

13. Consider the following table, which shows the number of viable progeny produced by a plant under standard conditions. Provide an explanation for the results.

P_1:	Strain A × Strain A	Strain B × Strain B	Strain A × Strain B
F_1:	765	750	775
F_2:	712	783	416

14. The map position for three X-linked recessive genes in *Drosophila* (*v,* vermilion eyes; *m,* miniature wings; and *s,* sable body) is:

v	*m*	*s*
33.0	36.1	43.0

A wild-type male is X-rayed and mated to a vermilion, miniature, sable female. Among the progeny is a single vermilion-eyed, long-winged, tan-bodied female. The following shows the progeny when this female is mated with a *v m s* hemizygous male.

Females	Males
87 vermilion, miniature, sable	89 vermilion, miniature, sable
93 vermilion	1 vermilion

Explain these results by drawing a genetic map.

15. In *Drosophila,* recessive genes clot (*ct*) and black body (*b*) are located at 16.5 and 48.5 map units, respectively, on the second chromosome. In one cross, wild-type females that are ct^+ b^+/ct *b* are mated with *ct b/ct b* males. They produce these progeny:

wild-type	1,250
clot, black	1,200
black	30
clot	20

What is unusual about the results? How can you explain them?

16. You have four strains of *Drosophila* (1–4) that were isolated from different geographic regions. You compare the banding patterns of the second chromosome and obtain these results (each letter corresponds to a band):

(1) m n r q p o s t u v
(2) m n o p q r s t u v
(3) m n r q t s u p o v
(4) m n r q t s o p u v

If (3) is presumed to be the ancestral strain, in what order did the other strains arise?

17. In *Drosophila,* the recessive gene for white eyes is located near the tip of the X chromosome. A wild-type male is irradiated and mated with a white-eyed female. Among the progeny is one red-eyed male. How can you explain the red-eyed male, and how could you test your hypothesis?

VARIATION IN CHROMOSOME NUMBER

18. Is a tetraploid more likely to show irregularities in meiosis or mitosis? Explain. What about these processes in a triploid?

19. How many chromosomes would a human tetraploid have? How many chromosomes would a human monosomic have?

20. Do autopolyploids or allopolyploids experience more difficulties during meiosis? Do amphidiploids have more or less trouble than auto- or allopolyploids?

21. If a diploid species of $2n = 16$ hybridizes with one of $2n = 12$, and the resulting hybrid doubles its chromosome number to produce an allotetraploid (amphidiploid), how many chromosomes will it have? How many chromosomes will an allotetraploid have if both parent species had $2n = 20$?

22. If nondisjunction of the sex chromosomes occurs in a female at the second meiotic division, what type of eggs will arise?

23. How might an X0/XYY human mosaic arise? An XX/XXY mosaic? How might a trisomy 21 individual arise?

24. Plant species P has $2n = 18$, and species U has $2n = 14$. A fertile hybrid is found. How many chromosomes does it have?

25. A woman with normal vision whose father was color-blind mates with a man with normal vision. They have a color-blind daughter with Turner syndrome. In which parent did nondisjunction occur?

26. A color-blind man mates with a woman with normal vision whose father was color-blind. They have a color-blind son with Klinefelter syndrome. In which parent did nondisjunction occur?

27. Describe a genetic event that can produce an XYY man.

28. Chromosomal analysis of a spontaneously aborted fetus revealed that the fetus was 92,XXYY. Propose an explanation to account for this unusual karyotype.

CRITICAL THINKING QUESTIONS

1. Various species in the grass genus *Bromus* have chromosome numbers of 14, 28, 42, 56, 70, 84, 98, and 112. What can you tell about the genetic relationships among these species and how they might have arisen?

2. There was a humorous television commercial in which someone accidentally discovered the desirability of combining chocolate and peanut butter. Could this combination be achieved by crossing peanut and cocoa plants?

Suggested Readings for chapter 8 are on page B-4.

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9

CHEMISTRY OF THE GENE

STUDY OBJECTIVES

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2. To examine the structure of DNA, the genetic material 211
3. To investigate the way in which DNA replicates 220

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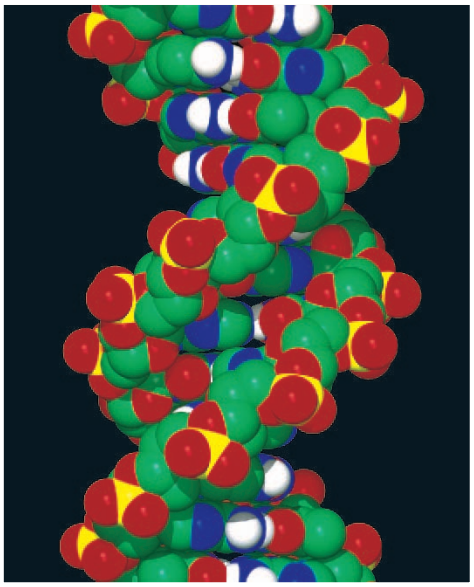
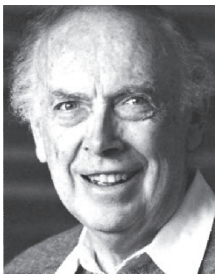
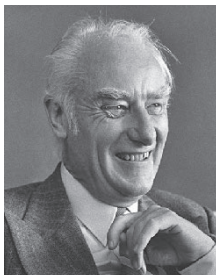


A computer-generated image of deoxyribonucleic acid, DNA. (© Professor K. Seddon & Dr. T. Evans/ Queen's University Belfast/SPL/Photo Researchers.)

In 1953, James Watson and Francis Crick published a two-page paper in the journal *Nature* entitled "Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid." It began as follows: "We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest." This paper, which first put forth the correct model of DNA structure, is a milestone in the modern era of molecular genetics, compared by some to the work of Mendel and Darwin (box 9.1). (Watson, Crick, and X-ray crystallographer Maurice Wilkins won Nobel Prizes for this work; Rosalind Franklin, also an X-ray crystallographer, was acknowledged, posthumously, to have played a major role in the discovery of the structure of DNA.) Once the structure of the genetic material had been determined, an understanding of its method of replication and its functioning quickly followed.



James D. Watson (1928–). (Cold Spring Harbor Laboratory Research Library Archives. Margot Bennet, photographer.)



Francis Crick (1916–). (Reproduced by permission of Herb Weitman, Washington University, St. Louis, Missouri.)

Maurice H. F. Wilkins (1916–). (Courtesy of Dr. Maurice H. F. Wilkins and Biophysics Department, King's College, London.)

IN SEARCH OF THE GENETIC MATERIAL

This chapter begins a sequence of nine chapters on the molecular structure of the genetic material, its replication, its expression, and the control of its expression. In this chapter, we look at the evidence that DNA is the genetic material, the chemistry of DNA, and the way in which DNA replicates, including the general enzymatic processes. We look first at prokaryotic, then at eukaryotic, DNA replication. Note that we concentrate on the molecular structure of DNA because, generally, structure reveals function: molecules have shapes that define how they work.

Required Properties of a Genetic Material

We begin with a look at the properties that a genetic material must have and review the evidence that nucleic acids make up the genetic material. To comprise the genes, DNA must carry the information to control the synthesis of the enzymes and proteins within a cell or organism; self-replicate with high fidelity, yet show a low level of mutation; and be located in the chromosomes.

Control of the Proteins

The growth, development, and functioning of a cell are controlled by the proteins within it, primarily its enzymes. Thus, the nature of a cell's phenotype is controlled by the protein synthesis within that cell. The genetic material must therefore determine the need for and effective amounts of the enzymes in a cell. For example, given inorganic salts and glucose, an *E. coli* cell can synthesize, through its enzyme-controlled biochemical pathways, all of the compounds it needs for growth, survival, and reproduction. In contrast, a mammalian red blood cell primarily produces hemoglobin.

At this point we need to review some basic information regarding enzymes. An enzyme is a protein that acts as a catalyst for a specific metabolic process without itself being markedly altered by the reaction. Most reactions that enzymes catalyze could occur anyway, but only under conditions too extreme to take place within living systems. For example, many oxidations occur naturally at high temperatures. Enzymes allow these reactions to occur within the cell by lowering the **free energy of activation ($\Delta G^{\ddagger}$)** of a particular reaction. In other words, an enzyme allows a reaction to take place without needing the boost in energy that heat usually supplies (fig. 9.1).

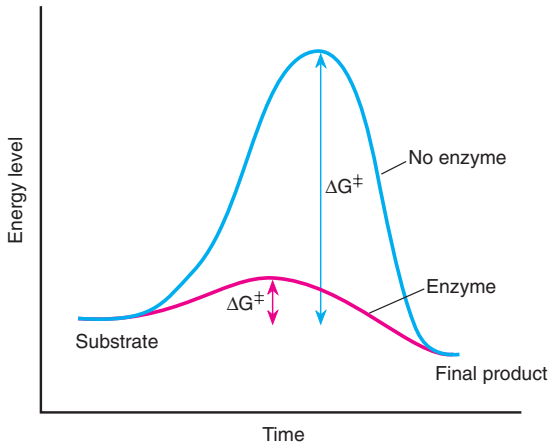


Figure 9.1 An enzyme lowers the free energy of activation ($\Delta G^{\ddagger}$) for a particular reaction.

BOX 9.1

Historical Perspectives

Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey.[1] They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram [fig. 1]). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′, 5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration,' the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3.4 A in the *z*-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

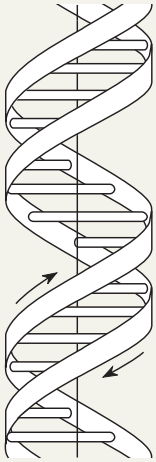


Figure 1 This figure is purely diagrammatic. The two *ribbons* symbolize the two phosphate-sugar chains, and the *horizontal rods* represent the pairs of bases holding the chains together. The *vertical line* marks the fiber axis. (Reprinted with permission from *Nature,* Vol. 171, No. 4356. Watson and Crick, "Molecular Structure of Nucleic Acids," pp. 737–738. Copyright © 1953 Macmillan Magazines Limited.)

Most metabolic processes, such as the biosynthesis or degradation of molecules, occur in pathways, with enzyme facilitating each step in the pathway (see chapter 2). The metabolic pathway for the conversion of threonine into isoleucine (two amino acids) appears in figure 9.2. Each reaction product in the pathway is altered by an enzyme that converts it to the next product. The enzyme threonine dehydratase, for example, converts threonine into α-ketobutyric acid. Enzymes are composed of folded polymers of amino acids. The average protein is three hundred to five hundred amino acids long; only twenty naturally occurring amino acids are used in constructing these proteins. The sequence of amino acids determines the final structure of an enzyme. (We discuss the structure of proteins in more detail in chapter 11.) The genetic material determines the sequence of the amino acids.

The three-dimensional structure of enzymes permits them to perform their function. An enzyme combines with its substrate or substrates (the molecules it works on) at a part of the enzyme called the **active site** (fig. 9.3). The substrates "fit" into the active site, which has a shape that allows only the specific substrates to enter. This view of the way an enzyme interacts with its substrates is called the *lock-and-key model* of enzyme functioning.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical *z*-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations), it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid. It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereo-chemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their coworkers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. Watson
F. H. C. Crick

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 2.

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1. Pauling, L., and Corey, R. B., *Nature,* 171, 346 (1953); *Proc. U.S. Nat. Acad. Sci.,* 39, 84 (1953).
2. Furberg, S., *Acta Chem. Scand.,* 6, 634 (1952).
3. Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta,* 9, 402 (1952).
4. Wyatt, G. R., *J. Gen. Physiol.,* 36, 201 (1952).
5. Astbury, W. T., *Symp. Soc. Exp. Biol. 1, Nucleic Acid* 66 (Camb. Univ. Press, 1947).
6. Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys.* Acta, 10, 192 (1953).

When the substrates are in their proper position in the active site of the enzyme, the particular reaction that the enzyme catalyzes takes place. The reaction products then separate from the enzyme and leave it free to repeat the process. Enzymes can work at phenomenal speeds. Some can catalyze as many as a million reactions per minute.

Not all of the cell's proteins function as catalysts. Some are structural proteins, such as keratin, the main component of hair. Other proteins are regulatory—they control the rate at which other enzymes work. Still others are involved in different functions; albumins, for example, help regulate the osmotic pressure of blood.

Replication

The genetic material must be capable of precisely directing its own replication so that every daughter cell receives an exact copy. Some **mutability,** or the ability to change, is also required, because we know that the genetic material has changed, or evolved, over the history of life on earth. In their 1953 paper, Watson and Crick had already worked out the replication process based on the structure of DNA. The fidelity of the replication process is so great that the error rate is only about one in a billion.

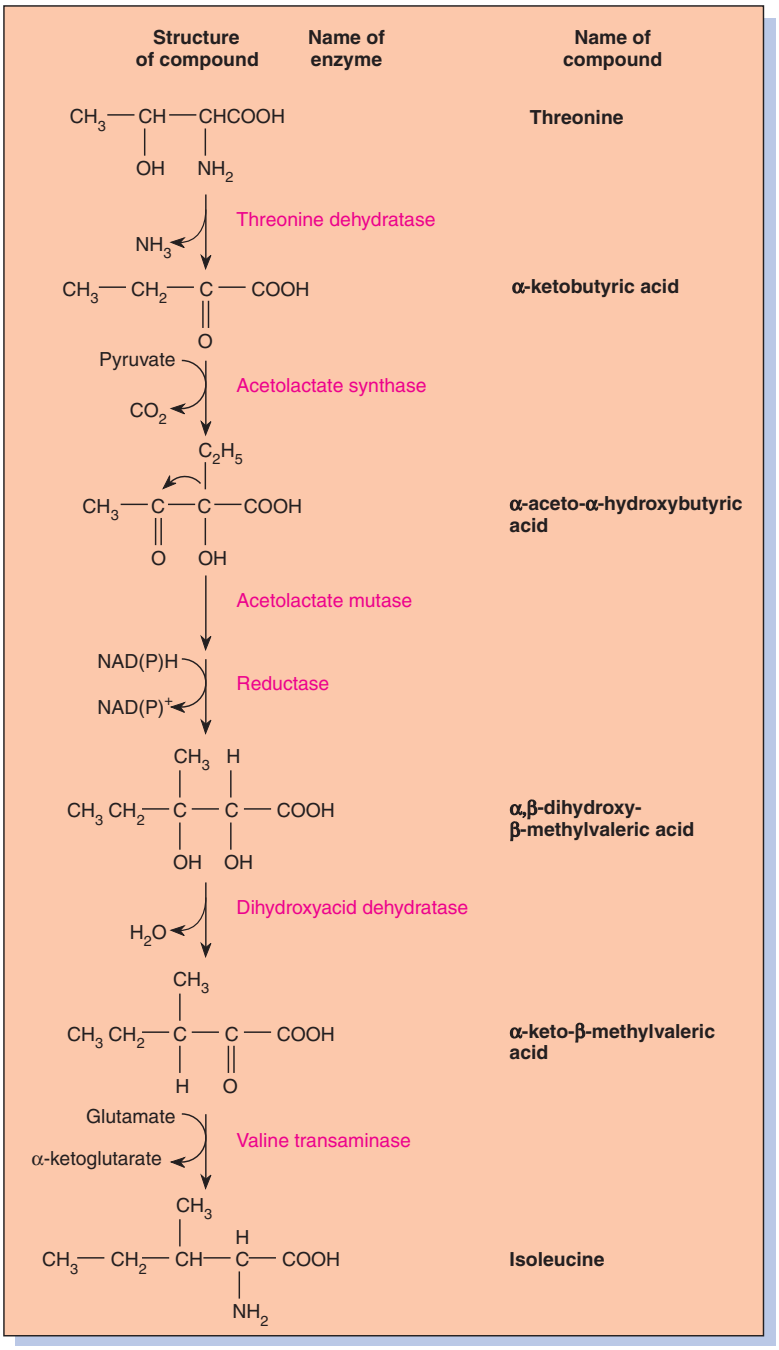


Figure 9.2 Metabolic pathway of conversion of the amino acid threonine into isoleucine.

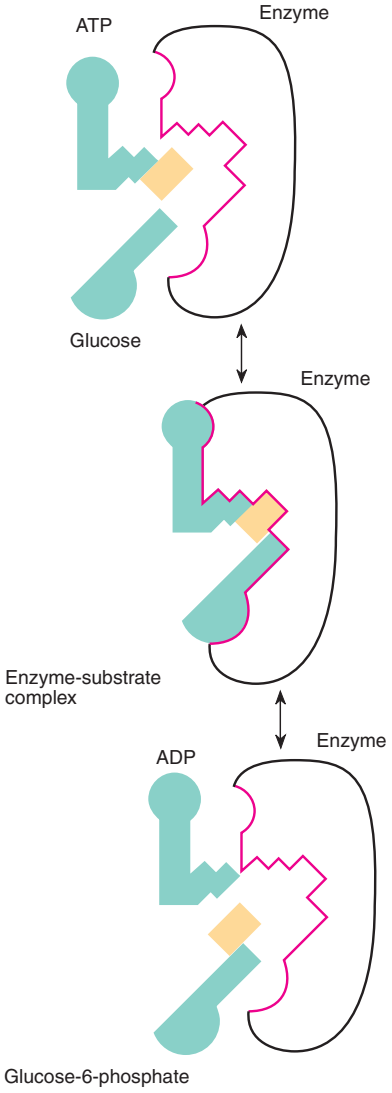
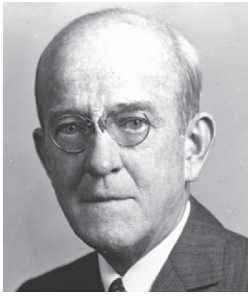


Figure 9.3 The active site of an enzyme recognizes a specific substance. In this case, ATP plus glucose is converted into ADP and glucose-6-phosphate by the enzyme hexokinase. The active site is diagrammed in *red*. The terminal phosphate group of ATP is *tan*.

Location

It has been known since the turn of the century that genes, the discrete functional units of genetic material, are located in chromosomes within the nuclei of eukaryotic cells: the way chromosomes behave during the cellular division stages of mitosis and meiosis mimics the behavior of genes. Thus, the genetic material in eukaryotes must be a part of the chromosomes.

For a long time, proteins were considered the most probable genetic material because they have the neces-



Oswald T. Avery (1877–1955). (Courtesy of the National Academy of Sciences.)

sary molecular complexity. The twenty naturally occurring amino acids can be combined in an almost unlimited variety, creating thousands and thousands of different proteins. The first proof that the genetic material is deoxyribonucleic acid (DNA) came in 1944 from Oswald Avery and his colleagues. The Watson and Crick model in 1953 ended a period when many thought DNA was the genetic material, but its structure was unknown.

Evidence for DNA as the Genetic Material

Transformation

In 1928, F. Griffith reported that heat-killed bacteria of one type could "transform" living bacteria of a different type. Griffith demonstrated this transformation using two strains of the bacterium *Streptococcus pneumoniae.* One strain (S) produced smooth colonies on media in a petri plate because the cells had polysaccharide capsules. It caused a fatal bacteremia (bacterial infection) in mice. Another strain (R), which lacked polysaccharide capsules, produced rough colonies on petri plates (fig. 9.4); it did not have a pathological effect on mice. Bacteria of the rough strain are engulfed by the mice's white blood cells; bacteria of the virulent smooth strain survive because their polysaccharide coating protects them.

Griffith found that neither heat-killed S-type nor live R-type cells, by themselves, caused bacteremia in mice. However, if he injected a mixture of live R-type and heat-killed S-type cells into mice, the mice developed a bacteremia identical to that caused by living S-type cells (fig. 9.5). Thus, something in the heat-killed S cells transformed the R-type bacteria into S-type cells.

In 1944, Oswald Avery and two of his associates, C. MacLeod and M. McCarty, reported the nature of the transforming substance. Avery and his colleagues did their work **in vitro** (literally, in glass), using colony morphology on culture media rather than bacteremia in mice as evidence of transformation. They ruled out proteins, carbohydrates, and lipids by their extraction procedure, by the chemical analysis of the transforming material, and by demonstrating that the only enzymes that destroyed the transforming ability were enzymes that destroyed DNA. This study provided the first experimental evidence that DNA was the genetic material: DNA transformed R-type bacteria into S-type bacteria.

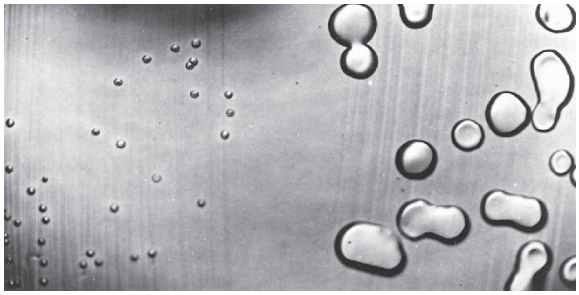
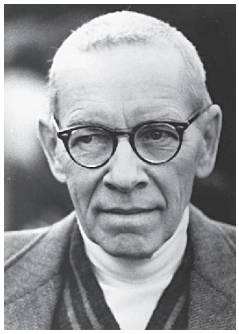


Figure 9.4 Petri plate with smooth and rough colonies of *Streptococcus pneumoniae.* R (rough) strain colonies appear on the *left* and S (smooth) colonies on the *right* on the same agar. Magnification 3.5×. (O. T. Avery, C. M. Macleod, and M. McCarty, "Studies on the chemical nature of the substance inducing transformation of pneumococcal types." Reproduced from the *Journal of Experimental Medicine* 79 (1944):137–58, fig. 1 by copyright permission of the Rockefeller University Press. Reproduced by permission. Photograph made by Mr. Joseph B. Haulenbeek.)

Phage Labeling

Valuable information about the nature of the genetic material has also come from viruses. Of particular value are studies of bacterial viruses—the bacteriophages, or phages. Since phages consist only of nucleic acid surrounded by protein, they lend themselves nicely to the determination of whether the protein or the nucleic acid is the genetic material.

A. D. Hershey and M. Chase published, in 1952, the results of research that supported the notion that DNA is the genetic material and, in the process, helped to



A. D. Hershey (1908–1997). (Courtesy of Dr. A. D. Hershey.)

Martha Chase. (Courtesy of Cold Springs Harbor Laboratory Archives.)

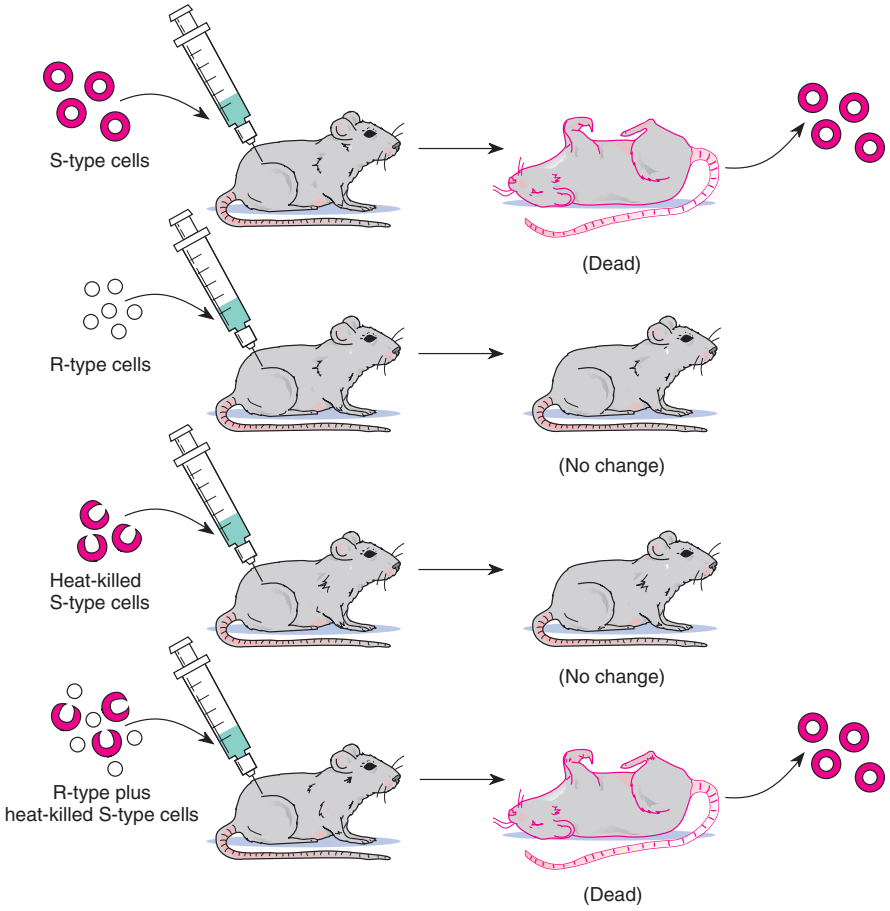


Figure 9.5 Griffith's experiment with *Streptococcus.* S-type cells will kill mice; so will heat-killed S-type cells injected with live R-type cells. S-type cells are recovered from dead mice in both cases.

explain the nature of the viral infection process. Since all nucleic acids contain phosphorus, whereas proteins do not, and since most proteins contain sulfur (in the amino acids cysteine and methionine), whereas nucleic acids do not, Hershey and Chase designed an experiment using radioactive isotopes of sulfur and phosphorus to keep separate track of the viral proteins and nucleic acids during the infection process. They used the T2 bacteriophage and the bacterium *Escherichia coli.* The phages were labeled by having them infect bacteria growing in culture medium containing the radioactive isotopes ^{35}S or ^{32}P. Hershey and Chase then proceeded to identify the material injected into the cell by phages attached to the bacterial wall.

When ^{32}P-labeled phages were mixed with unlabeled *E. coli* cells, Hershey and Chase found that the ^{32}P label entered the bacterial cells and that the next generation of phages that burst from the infected cells carried a significant amount of the ^{32}P label. When ^{35}S-labeled phages were mixed with unlabeled *E. coli,* the researchers found that the ^{35}S label stayed outside the bacteria for the most part. Hershey and Chase thus demonstrated that the outer protein coat of a phage does not enter the bacterium it infects, whereas the phage's inner material, consisting of DNA, does enter the bacterial cell (fig. 9.6). Since the DNA is responsible for the production of the new phages during the infection process, the DNA, not the protein, must be the genetic material.

RNA as Genetic Material

In some viruses, RNA (ribonucleic acid) is the genetic material. The tobacco mosaic virus that infects tobacco plants consists only of RNA and protein. The single, long RNA molecule is packaged within a rodlike structure formed by over two thousand copies of a single protein. No DNA is present in tobacco mosaic virus particles (fig. 9.7*a*). In 1955, H. Fraenkel-Conrat and R. Williams

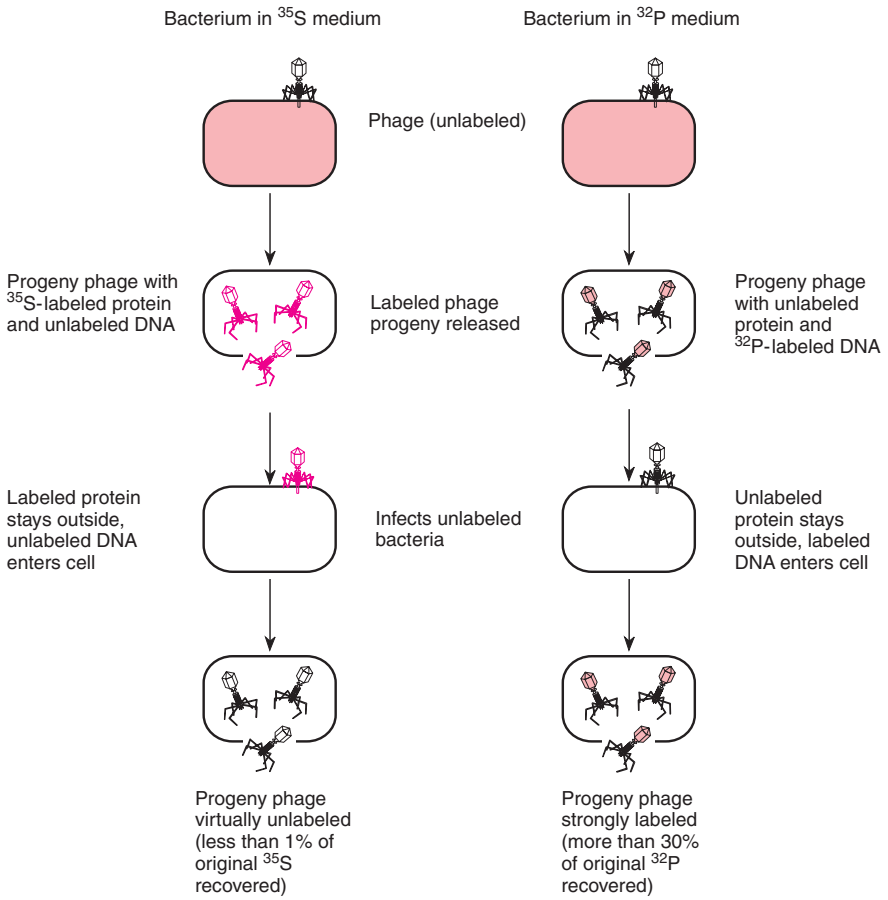


Figure 9.6 The Hershey and Chase experiments using ^{35}S-labeled and ^{32}P-labeled T2 bacteriophages. The nucleic acid label (^{32}P) enters the *E. coli* bacteria during infection; the protein label (^{35}S) does not.

showed that a virus can be separated, in vitro, into its component parts and reconstituted as a viable virus. This finding led Fraenkel-Conrat and B. Singer to reconstitute tobacco mosaic virus with parts from different strains (fig. 9.7*b*). For example, they combined the RNA from the common tobacco mosaic virus with the protein from the masked (M) strain of tobacco mosaic virus. They then made the reciprocal combination of common-type protein and M-type RNA. In both cases, the tobacco mosaic virus produced during the process of infection was the type associated with the RNA, not with the protein. Thus, it was the nucleic acid (RNA in this case) that was the genetic material. Subsequently, scientists rubbed pure tobacco mosaic virus RNA into plant leaves. Normal infection and a new generation of typical, protein-coated tobacco mosaic virus resulted, confirming RNA as the genetic material for this virus.

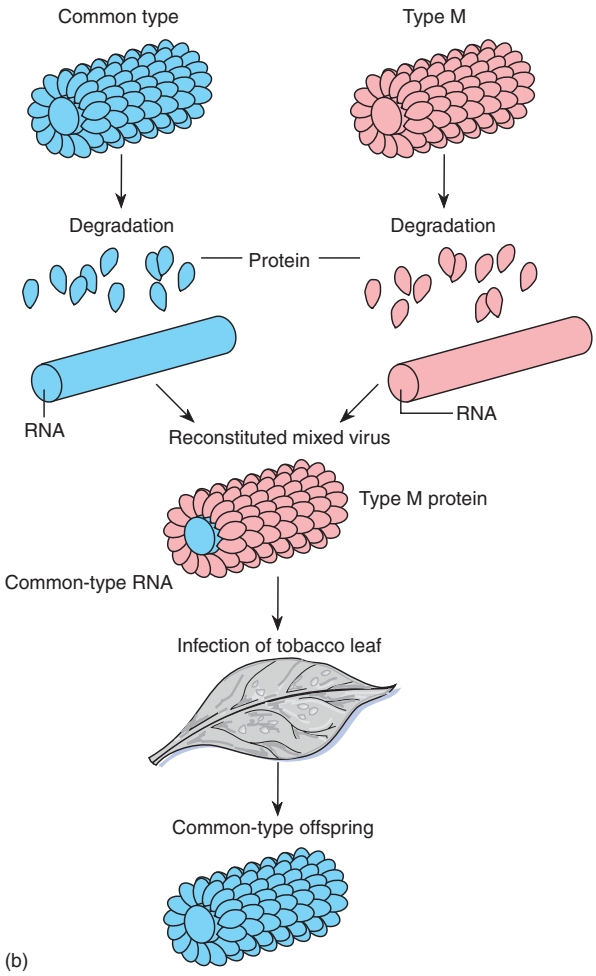
We thus conclude that DNA is the genetic material. In the few viruses that do not have DNA, RNA serves as the genetic material. The only exception to these statements is one type of disease that is transmitted by a protein without accompanying DNA or RNA (box 9.2).

CHEMISTRY OF NUCLEIC ACIDS

Having identified the genetic material as the nucleic acid DNA (or RNA), we proceed to examine the chemical structure of these molecules. Their structure will tell us a good deal about how they function.

Nucleic acids are made by joining **nucleotides** in a repetitive way into long, chainlike polymers. Nucleotides are made of three components: phosphate, sugar, and a nitrogenous base (table 9.1 and fig. 9.8). When incorporated into a nucleic acid, a nucleotide contains one of each of the three components. But, when free in the cell

(a)



(b)

Figure 9.7 (*a*) Electron micrograph of tobacco mosaic virus. Magnification 37,428×. (*b*) Reconstitution experiment of Fraenkel-Conrat and Singer. The nucleic acid (RNA), not the protein component of the virus, controls inheritance.
([*a*] © Biology Media/Photo Researchers, Inc.)

Table 9.1 **Components of Nucleic Acids**

	Phosphate	Sugar	Base: Purines	Base: Pyrimidines
DNA	Present	Deoxyribose	Guanine Adenine	Cytosine Thymine
RNA	Present	Ribose	Guanine Adenine	Cytosine Uracil

pool, nucleotides usually occur as triphosphates. The energy held in the extra phosphates is used, among other purposes, to synthesize the polymer. A **nucleoside** is a sugar-base compound. Nucleotides are therefore nucleoside phosphates (fig. 9.9). (Note that ATP, adenosine triphosphate, the energy currency of the cell, is a nucleoside triphosphate.)

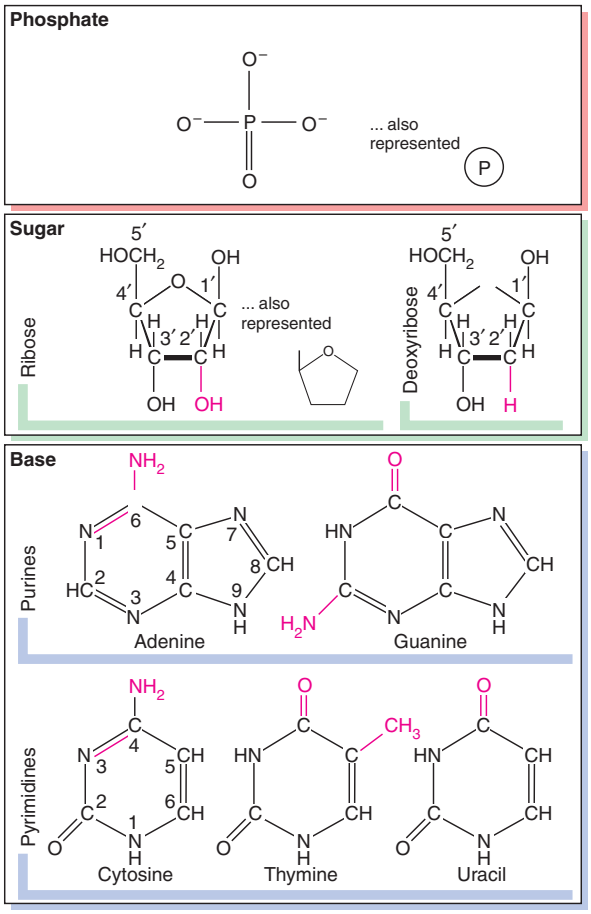


Figure 9.8 Components of nucleic acids: phosphate, sugars, and bases. Primes are used to number the ring positions in the sugars to differentiate them from the ring positions in the bases.

BOX 9.2

Biomedical Applications

Prions: The Biological Equivalent of Ice-Nine

Without exception, the genetic material is either DNA or RNA; it is RNA only in a few viruses. Since virtually all transmissible diseases are of bacterial or viral origin, this means that transmissible diseases are also caused by organisms with DNA or RNA as their genetic material. However, in one interesting situation, a transmissible disease appears to be caused by an agent without genetic material. Four human neurological diseases and six similar animal diseases are caused, we believe, by proteins without DNA or RNA. (Two conditions in yeast are probably caused in a similar way.) The human diseases are kuru, Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, and a recently discovered fatal familial insomnia. The animal diseases are scrapie (sheep and goats), four encephalopathies (bovine, feline, ungulate, and mink), and chronic wasting disease (deer and elk). All of these diseases are extremely slow to develop, all are fatal, and all are believed to be caused either by the ingestion of a protein from an infected individual or from a mutation of the normal gene. None of the diseases as yet has a cure, and the mechanism of action is not completely understood.

The diseases appear to be caused by a protein, similar to one normally produced in the brain of healthy individuals. The term **prion** (taken from *pr*oteinaceous *i*nfectious particle) has been given to these agents by Stanley Prusiner at the University of California in San Francisco, a 1997 Nobel laureate. He, along with colleagues, isolated the prion protein (PrP) and recently located the gene that codes for the protein on the short arm of chromosome 20. In addition to the infective form, a familial (inherited) form of these diseases can result from a mutation of the gene that codes for the prion protein active in normal individuals (probably at least all mammals). The normal protein is termed PrP^{C}, and the mutated form is referred to as PrP^{Sc}. Normally, PrP^{C} is a glycoprotein found on the membrane surface of the cells of the brain and some other tissues.

Although no cures exist for these diseases, kuru, at least, seems to be almost eradicated. It was found only among people in part of New Guinea who practiced cannibalism. Once the people stopped this practice, the spread of the disease also ceased; kuru does not seem to be generated to any major extent by mutation. By controlling feeding practices, it is believed, bovine spongiform encephalopathy will also disappear. In the past, cows were fed protein supplements contaminated by material from infected animals.

In England, a recent epidemic of bovine spongiform encephalopathy (BSE, or mad cow disease) peaked in 1992–1993, affecting over 160,000 cattle. At least fourteen cases of a variant of Creutzfeldt-Jakob disease in people in England and France were attributed to eating affected beef, creating a panic in England. With a change away from using animal matter in cattle feed and a culling of cattle herds, the epidemic has ended. However, new human cases may show up in the future owing to the long incubation period of this prion disease.

The obvious question is, how does a protein that does not appear to contain genetic material cause a transmissible disease when ingested? Prusiner has suggested several mechanisms that would allow an infective protein to induce copies of the normal protein to become infective. One of these mechanisms involves a cascade in which an infective PrP^{Sc} binds with a normal PrP^{C}, resulting in two infective PrP^{Sc} proteins. From this, one produces two, two produce four, four produce eight, and so on. As Nancy Touchette, writing in *The Journal of NIH Research,* pointed out, this is the way Kurt Vonnegut described the behavior of the mythical ice-nine in his 1963 book, *Cat's Cradle.* In this fictional account, a single seed caused all of the water on earth, by a chain reaction cascade, to form into a novel type of ice. We have not yet resorted to science fiction to answer the mystery of prion function; however, it seems reasonable to guess that an eventual understanding of the mechanism of prion function will provide us with a biological novelty.

The sugars differ only in the presence (ribose in RNA) or absence (deoxyribose in DNA) of an oxygen in the 2′ position. (The carbons of the sugars are numbered 1′ to 5′. The primes are used to avoid confusion with the numbering system of the bases; see fig. 9.8.) DNA and RNA both have four bases (two **purines** and two **pyrimidines**) in their nucleotide chains. Both molecules have the purines **adenine** and **guanine** and the pyrimidine **cytosine.** DNA has the pyrimidine **thymine;** RNA has the pyrimidine **uracil.** Thus, three of the nitrogenous bases are found in both DNA and RNA, whereas thymine is unique to DNA, and uracil is unique to RNA.

A nucleotide is formed in the cell when a base attaches to the 1′ carbon of the sugar and a phosphate attaches to

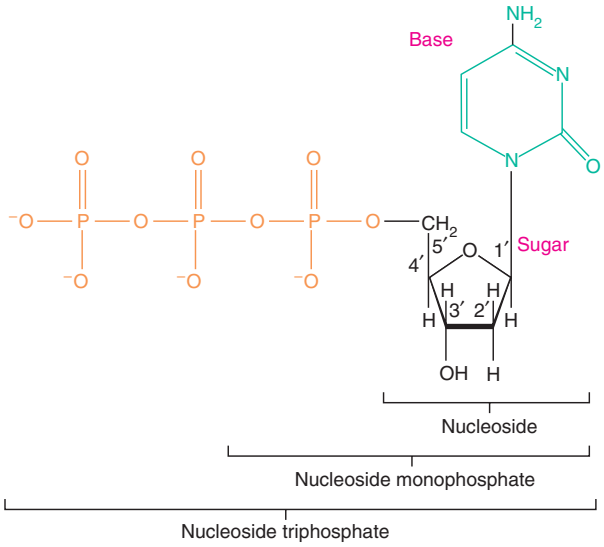


Figure 9.9 The structure of a nucleoside and two nucleotides: a nucleoside monophosphate and a nucleoside triphosphate.

the 5′ carbon of the same sugar (fig. 9.10); the nucleotide takes its name from the base (table 9.2). Nucleotides are linked together **(polymerized)** by the formation of a bond between the phosphate at the 5′ carbon of one nucleotide and the hydroxyl (OH) group at the 3′ carbon of an adjacent molecule. Very long strings of nucleotides can be polymerized by this **phosphodiester bonding** (fig. 9.11).

Biologically Active Structure

Although the identities of the nucleotides that polymerized to form a strand of DNA or RNA were known, the actual structures of these nucleic acids when they function as the genetic material remained unknown until 1953. The general feeling was that the biologically active structure of DNA was more complex than a single string of nucleotides linked together by phosphodiester bonds, and that several interacting strands were involved. In 1953, Linus Pauling, a Nobel laureate who had discovered the

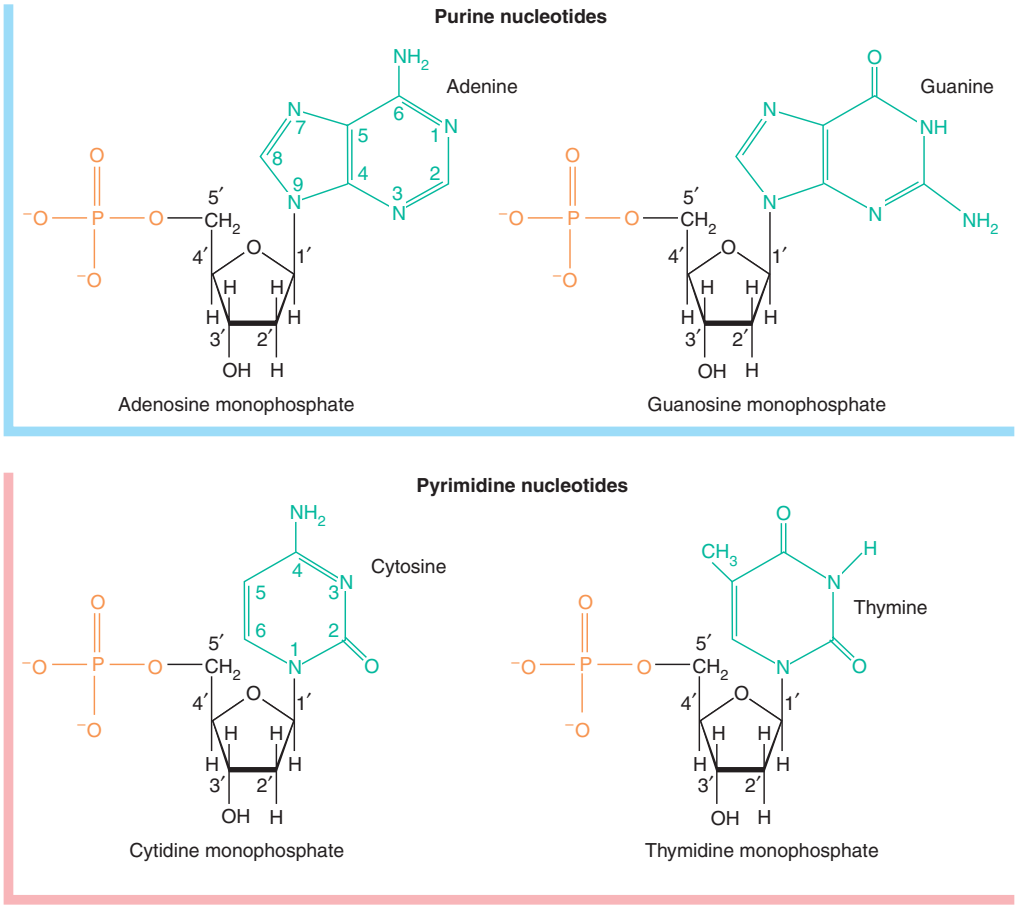


Figure 9.10 Structure of the four deoxyribose nucleotides.

Table 9.2 Nucleotide Nomenclature

Base	Nucleotide (nucleoside monophosphate)	Abbreviation					
		Monophosphate		Diphosphate		Triphosphate	
		Ribose	Deoxyribose	Ribose	Deoxyribose	Ribose	Deoxyribose
Guanine	Guanosine monophosphate	GMP		GDP		GTP	
	Deoxyguanosine monophosphate		dGMP		dGDP		dGTP
Adenine	Adenosine monophosphate	AMP		ADP		ATP	
	Deoxyadenosine monophosphate		dAMP		dADP		dATP
Cytosine	Cytidine monophosphate	CMP		CDP		CTP	
	Deoxycytidine monophosphate		dCMP		dCDP		dCTP
Thymine	Deoxythymidine monophosphate		dTMP		dTDP		dTTP
Uracil	Uridine monophosphate	UMP		UDP		UTP	

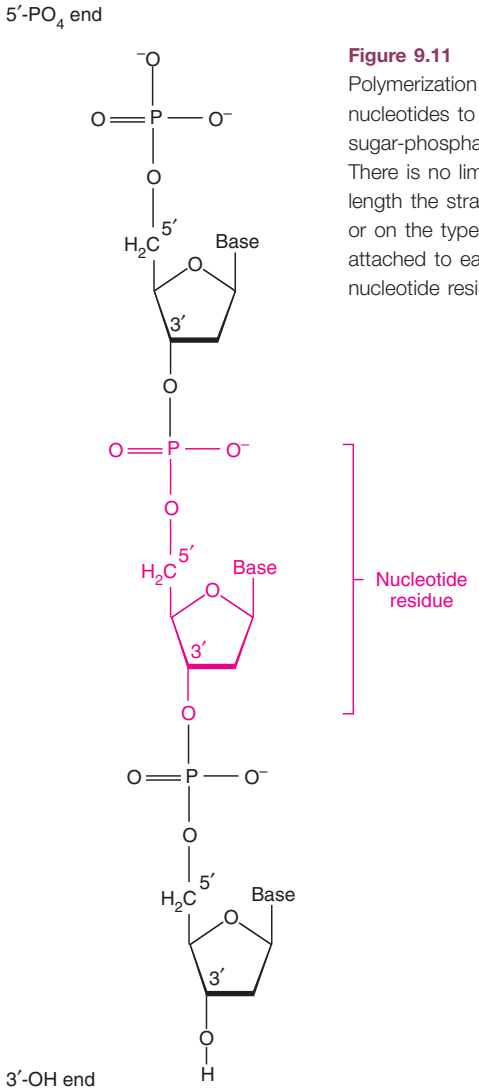


Figure 9.11 Polymerization of adjacent nucleotides to form a sugar-phosphate strand. There is no limit to the length the strand can be or on the type of base attached to each nucleotide residue.

helical structure of proteins, was investigating a three-stranded structure for the genetic material, whereas Watson and Crick had decided that a two-stranded structure was more consistent with available evidence. Three lines of evidence directed Watson and Crick: the chemical nature of the components of DNA, X-ray crystallography, and Chargaff's ratios.

DNA X-Ray Crystallography

All the time Watson and Crick were studying DNA structure, Maurice Wilkins, Rosalind Franklin, and their colleagues were using **X-ray crystallography** to analyze the structure of DNA. The molecules in a crystal are arranged in an orderly way, so that when a beam of X rays is aimed at the crystal, the beam scatteres in an orderly fashion. The scatter pattern can be recorded on photographic film or computer-controlled devices. The nature of this pattern depends on the structure of the crystal. The cross in the center of the photograph in figure 9.12 indicates that the molecule is a helix; the dark areas at the top and bottom come from the bases, stacked perpendicularly to the main axis of

Rosalind E. Franklin (1920–1958). (Courtesy of Cold Spring Harbor Laboratory.)

the molecule. This image of the DNA molecule stimulated Watson and Crick's understanding of its structure.

Chargaff's Ratios

Until Erwin Chargaff's work, scientists had labored under the erroneous **tetranucleotide hypothesis.** This hypothesis proposed that DNA was made up of equal quantities of the four bases; therefore, a subunit of this DNA consisted of one copy of each base. Chargaff carefully analyzed the base composition of DNA in various species (table 9.3). He found that although the relative amount of a given nucleotide differs among species, the amount of adenine equaled that of thymine and the amount of guanine equaled that of cytosine. That is, in the DNA of all the organisms studied, a 1:1 correspondence exists between the purine and pyrimidine bases. This is known as **Chargaff's rule.** Chargaff's observations disproved the tetranucleotide hypothesis; the four bases of DNA did not occur in a 1:1:1:1 ratio. His results gave insight to Watson and Crick in the development of their model.

The Watson-Crick Model

With the information available, Watson and Crick began constructing molecular models. They found that a possible structure for DNA was one in which two helices coiled around one another (a **double helix**), with the sugar-phosphate backbones on the outside and the bases on the inside. This structure would fit the dimensions X-ray crystallography had established for DNA if the bases from the two strands were opposite each other and formed "rungs" in a helical "ladder" (fig. 9.13). The diameter of the helix could only be kept constant at about 20 Å (10 angstrom units = 1 nanometer) if one purine and one pyrimidine base made up each rung. Two purines per rung would be too big, and two pyrimidines would be too small.

After further experimentation with models, Watson and Crick found that the hydrogen bonding necessary to form the rungs of their helical ladder could occur readily between certain base pairs, the pairs that Chargaff found in equal frequencies. (Hydrogen bonds are very weak bonds in which two electronegative atoms, such as O and N, share a hydrogen atom between them. They have 3 to 5% of the strength of a covalent bond.) Thermodynamically stable

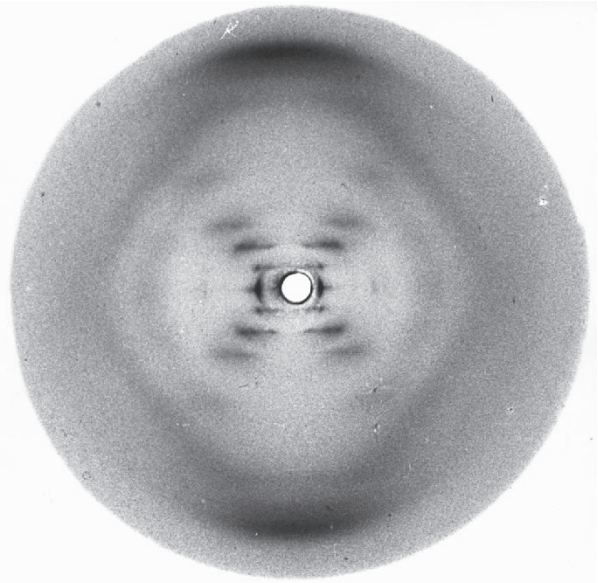
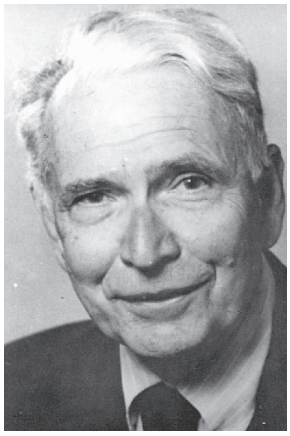


Figure 9.12 Scatter pattern of a beam of X rays passed through crystalline DNA. (Source: Reprinted by permission from R. E. Franklin and R. Gosling, "Molecular configuration in sodium thymonucleate," *Nature* 171:740–41. Copyright 1953 by Macmillan Journals Limited.)



Erwin Chargaff (1905–). (Courtesy of Dr. Erwin Chargaff.)

Table 9.3 **Percentage Base Composition of Some DNAs**

Species	Adenine	Thymine	Guanine	Cytosine
Human being (liver)	30.3	30.3	19.5	19.9
Mycobacterium tuberculosis	15.1	14.6	34.9	35.4
Sea urchin	32.8	32.1	17.7	18.4

Source: From E. Chargaff and J. Davidson, *The Nucleic Acids,* Academic Press, 1955.

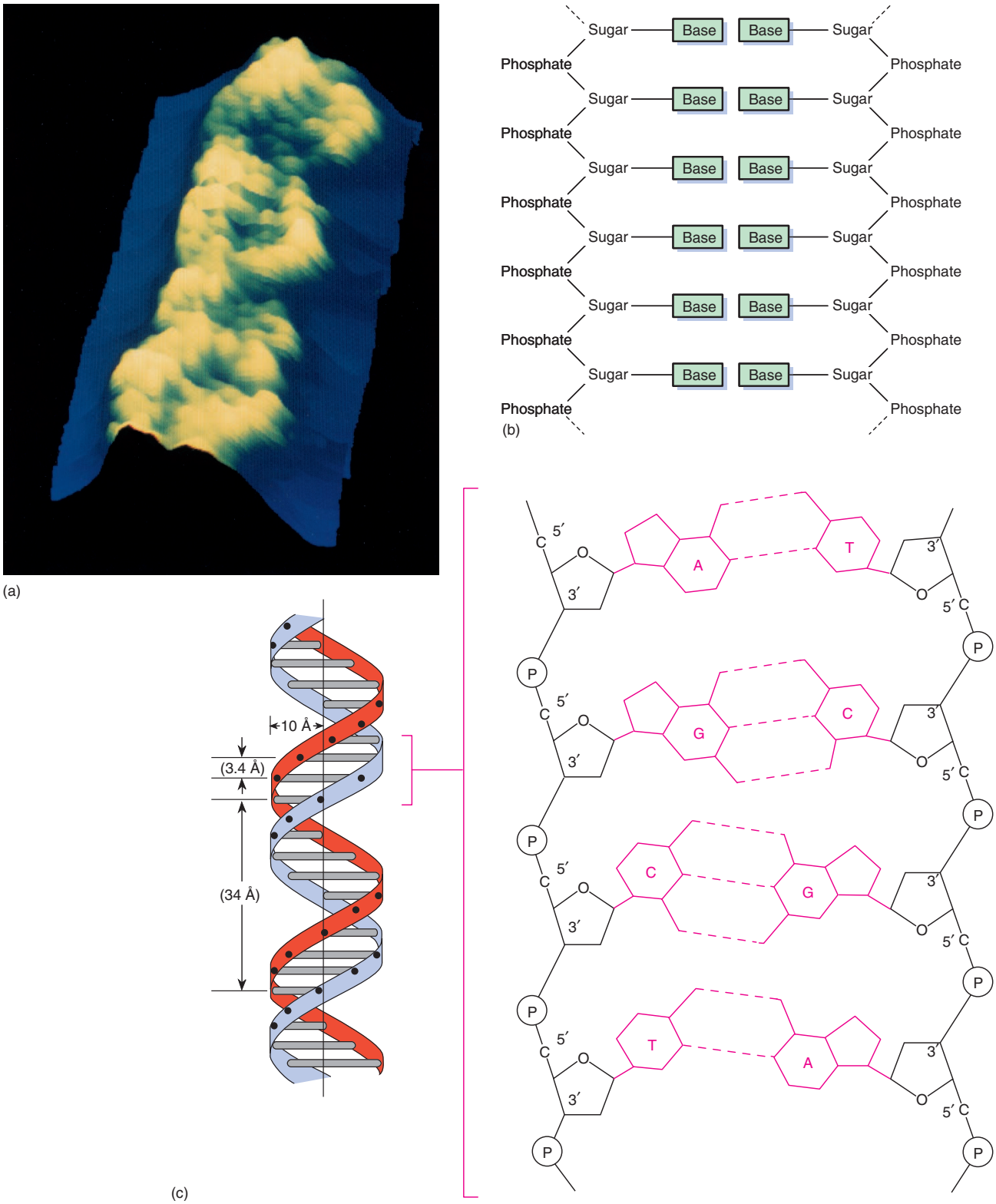


Figure 9.13 Double helical structure of DNA. (*a*) DNA magnified twenty-five million times by scanning tunneling microscopy. (*b*) Component parts. (*c*) Line drawing. ([*a*] © John D. Baldeschweiler.)

hydrogen bonding occurs between thymine and adenine and between cytosine and guanine (fig. 9.14). The relationship is one of **complementarity.** There are two hydrogen bonds between adenine and thymine and three between cytosine and guanine.

Another point about DNA structure relates to the **polarity** that exists in each strand. That is, one end of a DNA strand has a 5′ phosphate and the other end has a 3′ hydroxyl group. Watson and Crick found that hydrogen bonding would occur if the polarity of the two strands ran in opposite directions; that is, if the two strands were **antiparallel** (fig. 9.15).

DNA Denaturation

Denaturation studies indicated that the hydrogen bonding in DNA occurs in the way Watson and Crick suggested. Hydrogen bonds, although individually very weak, give structural stability to a molecule in large enough numbers. However, the hydrogen bonds can be broken and the DNA strands separated when the DNA molecule is heated in water. At a certain point, the thermal agitation overcomes the hydrogen bonding, and the molecule becomes **denatured** (or "melts"). It is logical that the more hydrogen bonds DNA contains the higher the temperature needed to denature it. It thus follows that since a G-C (guanine-cytosine) base pair has three hydrogen bonds to every two in an A-T (adenine-thymine) base pair, the higher the G-C content in a given molecule of DNA, the higher the temperature required to denature it. This relationship exists (fig. 9.16).

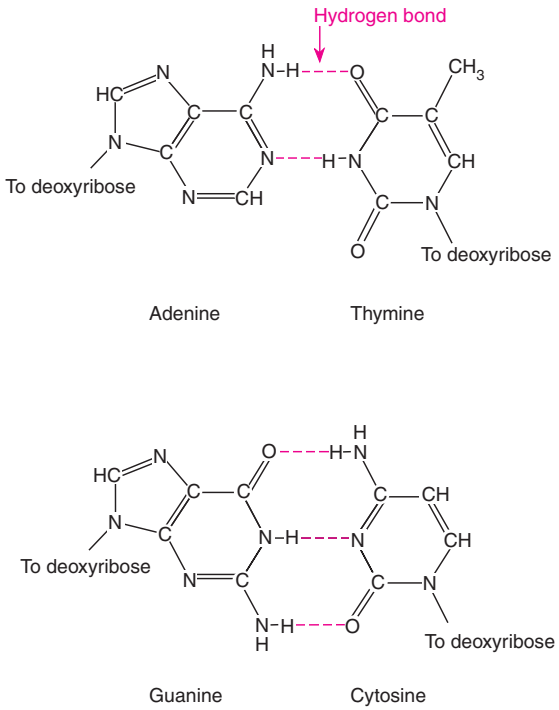


Figure 9.14 Hydrogen bonding between the nitrogenous bases in DNA.

Requirements of Genetic Material

Let us now return briefly to the requirements we have said a genetic material needs to meet: (1) control of protein synthesis, (2) self-replication, and (3) location on the chromosomes in the nucleus (in organisms with nuclei). Does DNA (or when DNA is absent, RNA) meet these requirements?

Control of Enzymes

In the next several chapters, we examine the details of protein synthesis. We will see that DNA does possess the complexity required to direct protein synthesis. Although complementarity restricts the base opposite a given base in a double helix, there are no restrictions on the sequence of bases on a given strand. Later, we will show that each sequence of three bases in DNA specifies a particular amino acid during protein synthesis. The **ge-**

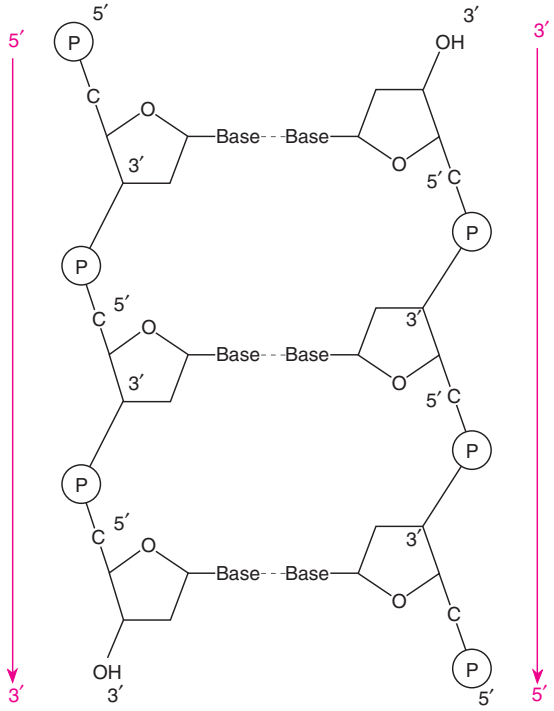


Figure 9.15 Polarity of the DNA strands. Polarity is established by the 3′ and 5′ carbons of a given sugar. For example, moving down the left strand, the polarity is 5′ → 3′ (read as *five-prime to three-prime*). Moving down the right strand, the polarity is 3′ → 5′.

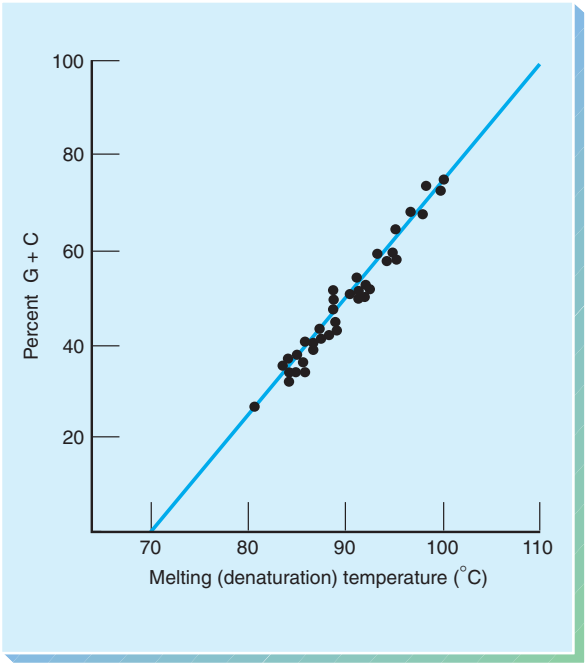


Figure 9.16 Relationship of the number of hydrogen bonds (G-C content) and the thermal stability of DNA from different sources. (From J. Marmur and P. Doty, Jr., "Relationship of the Number of Hydrogen Bonds and the Thermal Bonds and the Thermal Stability of DNA from Different Sources," *Journal of Molecular Biology,* 5:109–112. Copyright © 1962 Academic Press LTD.)

netic code gives the relationship of DNA bases to the amino acids in proteins.

Replication

Watson and Crick hinted in their 1953 paper how DNA might replicate. Their observation stemmed from the property of complementarity. Since the base sequence on one strand is complementary to the base sequence on the opposite strand, each strand could act as a template for a new double helix if the molecule simply "unzipped," allowing each strand to specify the sequence of bases on a new strand by complementarity (fig. 9.17). Mutability would occur due to mispairings, other errors in replication, or damage to the DNA.

Location

DNA must reside in the nucleus of eukaryotes, where the genes occur on chromosomes, or in the chromosomes of prokaryotes and viruses. In both prokaryotes and eukaryotes, the majority of the cell's DNA is in the chromosomes. And all viruses contain either DNA or RNA. Thus, DNA fulfills all the requirements of a genetic material. RNA can fulfill the same requirements in RNA viruses and viroids.

Alternative Forms of DNA

The form of DNA we have described so far is called **B DNA.** It is a right-handed helix: it turns in a clockwise manner when viewed down its axis. The bases are stacked almost exactly perpendicular to the main axis, with about ten base pairs per turn (34 Å; see fig. 9.13*c*). However, DNA can exist in other forms. If the water content increases to about 75%, the A form of DNA **(A DNA)** occurs. In this form, the bases tilt in regard to the axis, and there are more base pairs per turn. However, this and other known forms of DNA are relatively minor variations on the right-handed B form.

In 1979, Alexander Rich and his colleagues at MIT discovered a left-handed helix that they called **Z DNA** because its backbone formed a zigzag structure (fig. 9.18). Z DNA was found by X-ray crystallographic analysis of very small DNA molecules composed of repeating G-C sequences on one strand with the complementary C-G sequences on the other (alternating purines and pyrimidines). Z DNA looks like B DNA with each base rotated 180 degrees, resulting in a zigzag, left-handed structure (fig. 9.19). (The original configuration of the bases is referred to as the *anti* configuration; the rotated configuration is called the *syn* configuration.)

Originally, it was thought that Z DNA would not prove of interest to biologists because it required very high salt concentrations to become stable. However, it was found that Z DNA can be stabilized in physiologically normal conditions if methyl groups are added to the cytosines. Z DNA may be involved in regulating gene expression in eukaryotes. We return to this topic in chapter 16 (box 9.3).

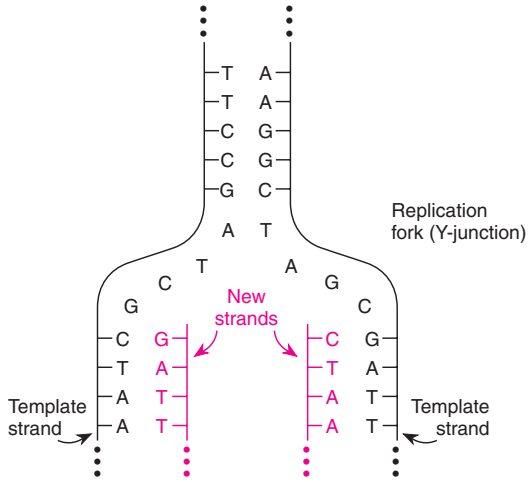


Figure 9.17 Complementarity provides a possible mechanism for accurate DNA replication. The parent duplex opens, and each strand becomes a template for a new duplex.

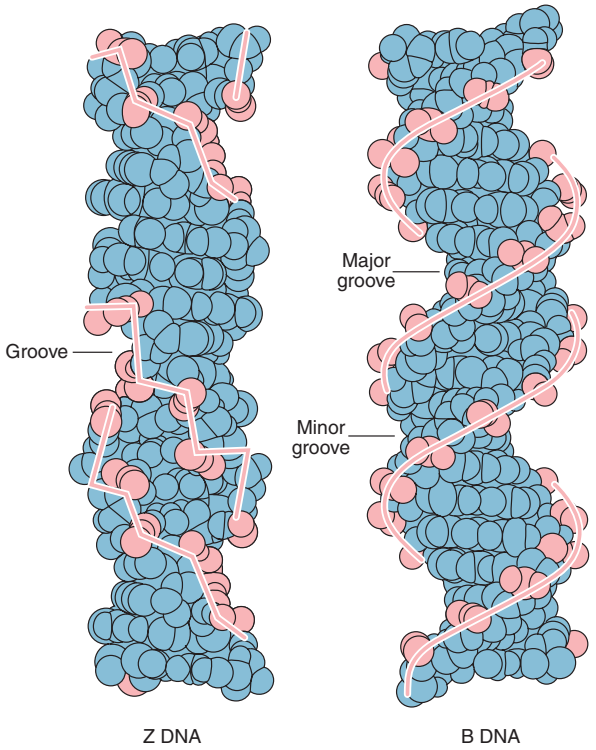


Figure 9.18 Z (*left*) and B (*right*) DNA. The *lines* connect phosphate groups. (Reproduced with permission from the *Annual Review of Biochemistry,* Volume 53, © 1984 by Annual Reviews, Inc.)

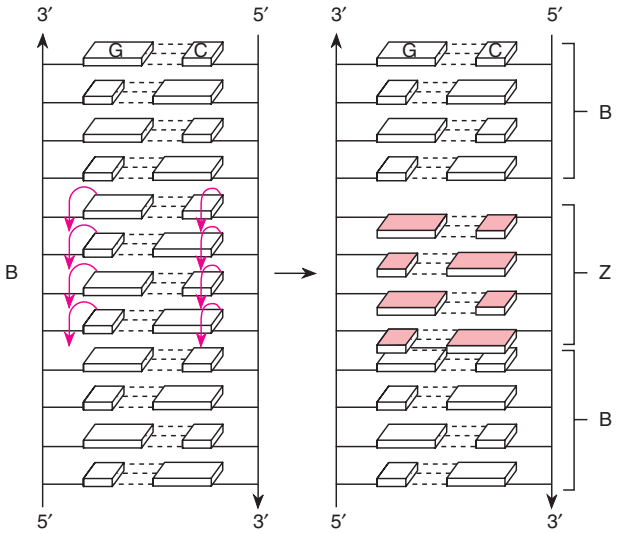


Figure 9.19 B DNA converts to Z DNA by the rotation of bases as indicated by the curved arrows. (Reproduced with permission from *Annual Review of Biochemistry,* Volume 53, © 1984 by Annual Reviews, Inc.)

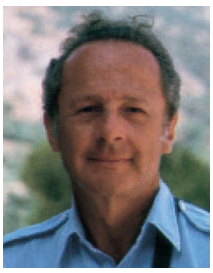
DNA REPLICATION—THE PROCESS

In their 1953 paper, Watson and Crick hinted that the replication of the double helix could take place as the DNA unwinds, so that each strand would form a new double helix by acting as a **template** for a newly synthesized strand (see fig. 9.17). For example, when a double helix is unwound at an adenine-thymine (A-T) base pair, one unwound strand would carry A and the other would carry T. During replication, the A in the template DNA would pair with a T in a newly replicated DNA strand, giving rise to another A-T base pair. Similarly, the T in the other template strand would pair with an A in the other newly replicated strand, giving rise to another A-T base pair. Thus, one A-T base pair in one double helix would result in two A-T base pairs in two double helices. This process would repeat at every base pair in the double helix of the DNA molecule.

This mechanism is called **semiconservative** replication because, although the entire double helix is not conserved in replication, each strand is. Every daughter DNA molecule has an intact template strand and a newly replicated strand. This is not the only way that replication could occur. The alternative methods are **conservative** and **dispersive.** In conservative replication, in which the whole original double helix acts as a template for a new one, one daughter molecule would consist of the original parental DNA, and the other daughter would be totally new DNA. In dispersive replication, some parts of the original double helix are conserved, and some parts are not. Daughter molecules would consist of part template and part newly synthesized DNA. In reality, the dispersive category is the all-inclusive "other" category, including any possibility other than conservative and semiconservative replication.

The Meselson and Stahl Experiment

In 1958, M. Meselson and F. Stahl reported the results of an experiment designed to determine the mode of DNA



Matthew Meselson (1930–). (Courtesy of Dr. Matthew Meselson. Photograph by Bud Gruce.)

Franklin W. Stahl (1929–). (Courtesy of Dr. Franklin W. Stahl.)

BOX 9.3

Biomedical Applications

Multiple-Stranded DNA

Under natural conditions, single-stranded RNA and double-stranded DNA are the rule. However, under laboratory conditions, it is possible to induce a third strand of DNA to interdigitate itself into the major groove of the double helix of normal DNA in a sequence-specific fashion. That is, the third strand of DNA will not just interdigitate anywhere, but will form a stable triplex at a specific sequence (fig. 1). The rules of binding are a little less precise than normal; not all sequences are recognized, and recognition can depend on surrounding sequences. However, a thymine in the third strand will usually recognize an adenine in an adenine-thymine base pair (T•A-T), and a cytosine in the third strand will recognize a guanine in a guanine-cytosine base pair (C+G-C).

Triple-stranded nucleotide chains were first created in 1957 by three scientists at the National Institutes of Health—Alexander Rich, David Davies, and Gary Felsenfeld—while they were creating artificial nucleic acids. At the time, triple-stranded DNA seemed like a laboratory curiosity. Now it seems of interest because it may have valuable uses both experimentally and clinically. (Rich apparently had the same experience in his codiscovery of Z DNA, which at first seemed like an oddity but now is the focus of some attention—see chapter 16.) Now, researchers are able to form triplexes in naturally occurring DNA. Two applications of this technology are actively being pursued.

Both applications arise because a single strand of DNA is capable of recognizing a relatively long sequence of the double-stranded DNA in a chromosome. Thus, it is possible to selectively locate a particular genic sequence. Once the third strand locates a particular sequence on a chromosome, two things can happen. First, triplex DNA formation can prevent a particular gene from expressing itself. By the same technique, triplex DNA can also be an abortifacient, a safe method for preventing the implantation of a fetus by preventing the expression of genes under the control of the hormone progesterone.

The second use of triplex DNA is to cut DNA at a specific place by adding a cleaving compound to both ends of the third strand of DNA. Once the third strand has interdigitated, it can then break the original double helix. For example, S. Strobel and P. Dervan at the California Institute of Technology have used a chemical complex containing iron attached to both 3′ and 5′ ends of the third strand of DNA. The addition of a third chemical then initiates the cleavage reaction. The cleavage of the original duplex can be of value in modern recombinant DNA technology (see chapter 13). Whether triplex DNA will ever be of value is not certain at this time. However, it seems to have good potential for therapeutic use and to help in studying and mapping the human genome.

More recently, four-stranded DNA molecules have been found, in which double helices of certain sequences interdigitate to form four-stranded structures. These may be of importance in the formation of crossover sites or in the structures at the ends of eukaryotic chromosomes (see chapter 15).

continued

replication. Some historians and philosophers of science consider this the most elegant scientific experiment ever designed. Meselson and Stahl grew *E. coli* in a medium containing a heavy isotope of nitrogen, ^{15}N. (The normal form of nitrogen is ^{14}N.) After growing for several generations on the ^{15}N medium, the DNA of *E. coli* was denser. The researchers determined the density of the strands using a technique known as **density-gradient centrifugation.** In this technique, a cesium chloride (CsCl) solution is spun in an ultracentrifuge at high speed for several hours. Eventually an equilibrium arises between centrifugal force and diffusion, so that a density gradient is established in the tube with an increasing concentration of CsCl from top to bottom. If DNA (or any other substance) is added, it concentrates and forms a band in the tube at the point where its density is the same as that of the CsCl. If several types of DNA with different densities are added, they form several bands. The bands are detectable under ultraviolet light at a wavelength of 260 nm (nanometers), which nucleic acids absorb strongly.

Meselson and Stahl transferred the bacteria with heavy (^{15}N) DNA to a medium containing only ^{14}N. The new DNA, replicated in the ^{14}N medium, was intermediate in density between light (^{14}N) and heavy (^{15}N) DNA, because the replication was semiconservative (fig. 9.20). If replication had been conservative, two bands would have appeared at the first generation of replication—an original ^{15}N DNA and a new ^{14}N double helix. And, throughout the experiment, if the method of replication had been conservative, the original DNA would have continued to show up as a ^{15}N band. (This, of course, did not happen.) If the method of replication had been dispersive, various multiple-banded patterns would have appeared, depending on the degree of dispersiveness. The results figure 9.20 shows are completely consistent with semiconservative replication and only with semiconservative replication.

BOX 9.3 CONTINUED

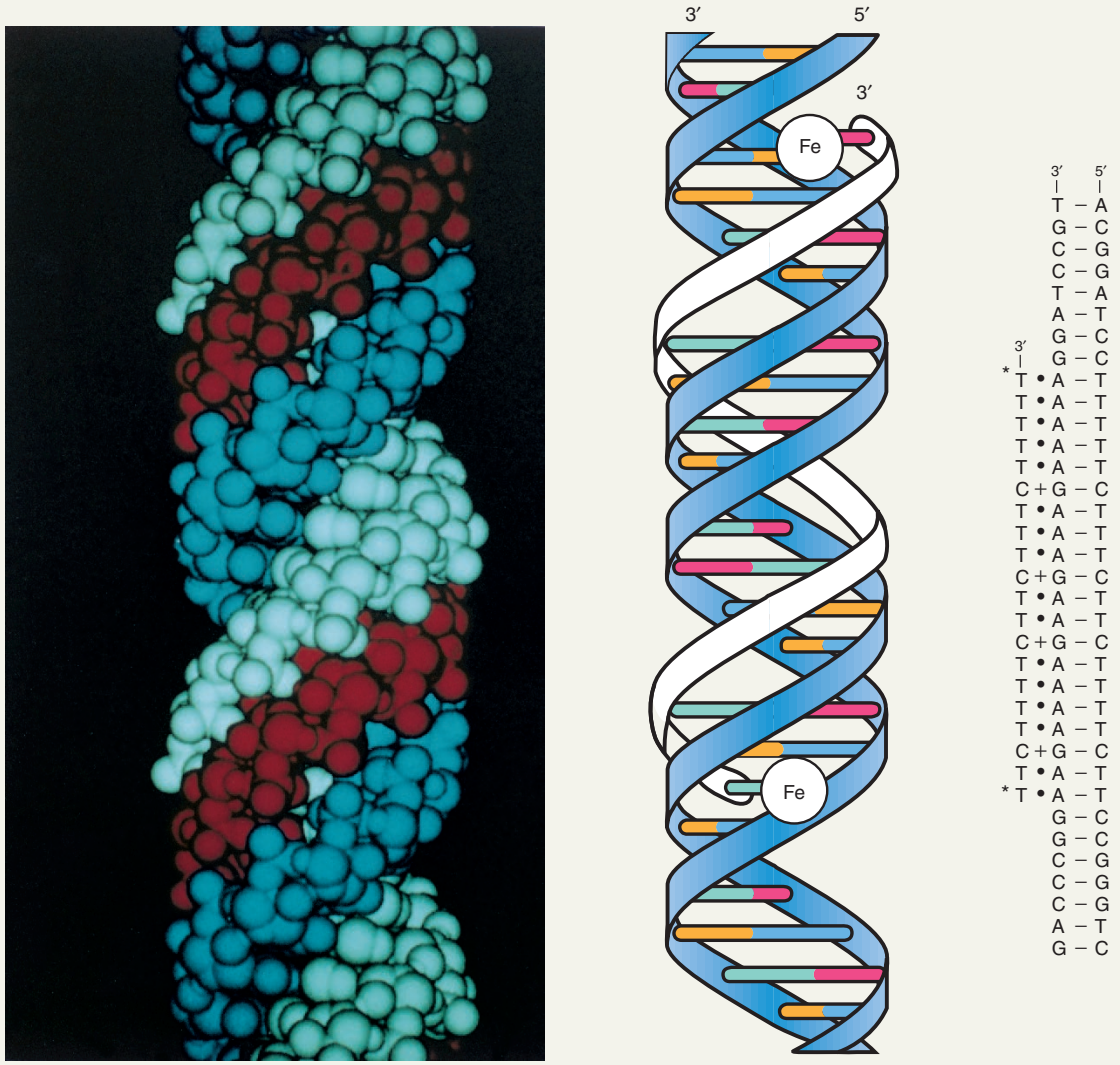


Figure 1 A triple helix of DNA. (*a*) Space-filling model of (*b*). (*c*) Sequence of third strand on *left* and double helix on *right*. The *asterisks* in (*c*) and the *Fe* symbols in (*b*) refer to the attached iron compound. (© Courtesy Dr. Michael E. Hogan, Triplex Pharmaceutical Corporation.)

Autoradiographic Demonstration of DNA Replication

In 1963, J. Cairns used **autoradiography** to verify the semiconservative method of replication photographically. This technique makes use of the fact that radioactive atoms expose photographic film. The visible silver grains on the film can then be counted to provide an estimate of the quantity of radioactive material present. Cairns grew *E. coli* bacteria in a medium containing radioactive thymine, a component of one of the DNA nucleotides. The radioactivity was in tritium (^{3}H). Cairns then carefully extracted the DNA from the bacteria and placed it on photographic emulsion for a period of time. He developed the emulsion to produce autoradiographs

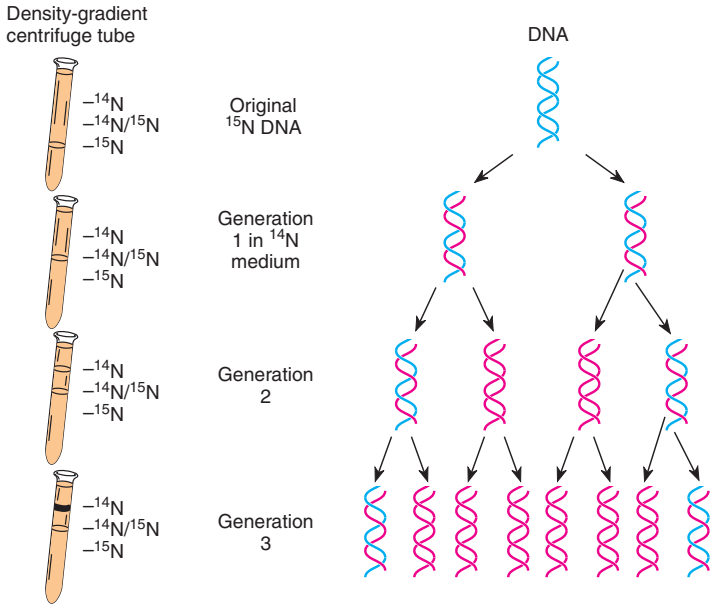
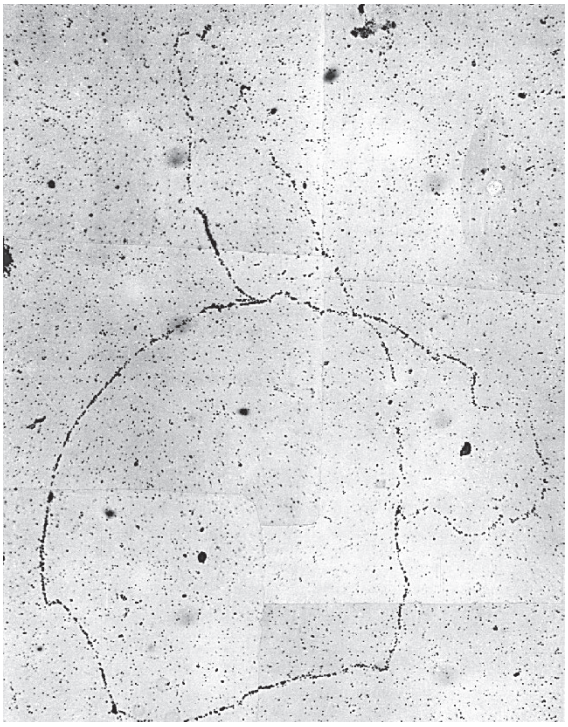


Figure 9.20 The Meselson and Stahl experiment to determine the mode of DNA replication. The bands in the centrifuge tube are visible under ultraviolet light. The pattern of bands (*left*) comes about from semiconservative DNA replication (*right*) of ^{15}N DNA (*blue*) replicating in a ^{14}N medium (*red*).

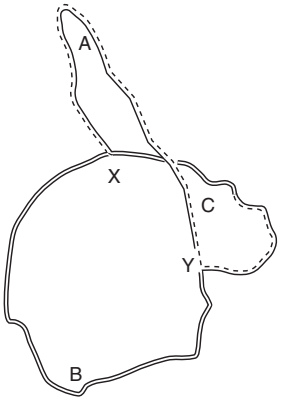


(a)

that he then examined under the electron microscope (fig. 9.21). Each grain of silver represents a radioactive decay. Interpretation of this autoradiograph reveals several points. The first, known at the time, is that the *E. coli* DNA is a circle. The second point is that the DNA is replicated while maintaining the integrity of the circle. That is, the circle does not appear to break during the process of DNA replication; an intermediate **theta structure** forms (topologically similar in shape to the Greek letter theta, θ). Third, replication of the DNA seems to be occurring at one or two moving **Y-junctions** in the circle, which further supports the semiconservative mode of replication. The DNA is unwound at a given point, and replication proceeds at a Y-junction, in a semiconservative manner, in one or both directions (see fig. 9.17).

Figure 9.22 diagrams the way in which the two Y-junctions move along the circle to the final step, forming two new circles. The steps by themselves do not support either a unidirectional or a bidirectional mode of replication. That is, a theta structure will develop if either one or both Y-junctions is active in replication. But with autoradiography, it is possible to determine whether new growth is occurring in only one or in both directions.

In some cases, radioactivity was not applied to the cell until DNA replication had already begun. In these cases, the radioactive label appeared after the theta structure



(b)

Figure 9.21 (*a*) Autoradiograph of *E. coli* DNA during replication. (*b*) Diagram has labels on the three segments, *A, B,* and *C,* created by the existence of two forks, *X* and *Y,* in the DNA. Forks are created when the circle opens for replication. Length of the chromosome is about 1,300 μm. ([*a*] From J. Cairns, "The chromosome of *E. coli*", *Cold Spring Harbor Symposia on Quantitative Biology,* 28. Copyright © 1963 by Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Reprinted by permission.)

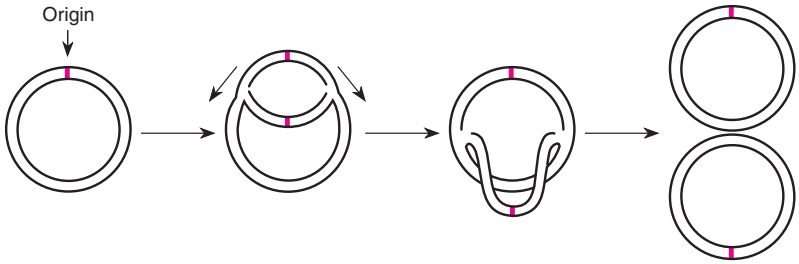


Figure 9.22 Observable stages in the DNA replication of a circular chromosome, assuming bidirectional DNA synthesis. The intermediate figures are called theta structures.

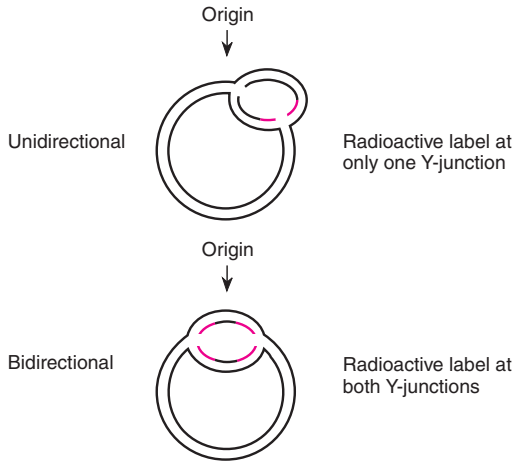
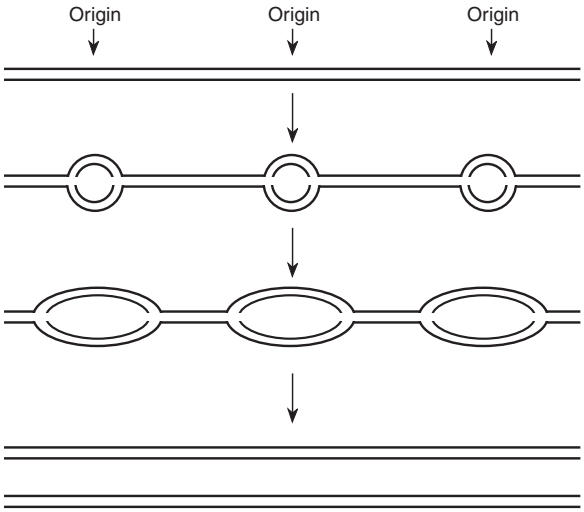


Figure 9.23 Radioactive labels distinguish unidirectional from bidirectional DNA replication. In these hypothetical experiments, DNA replication was allowed to begin, and then a radioactive label was added. After a short period of time, the process was stopped and the autoradiographs prepared. In bidirectional replication (the actual case), the label appears at both Y-junctions.



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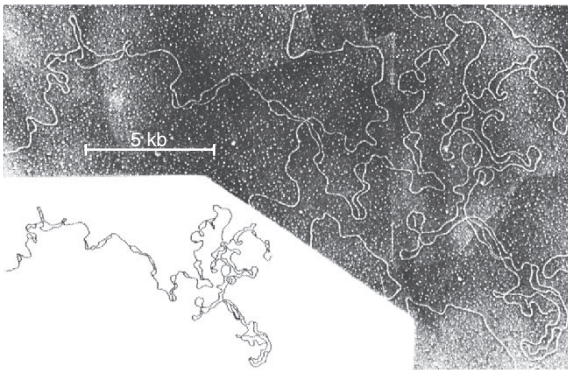


(b)

Figure 9.24 Replication bubbles. (*a*) Formation of bubbles (eyes) in eukaryotic DNA because of multiple DNA synthesis sites of origin. (*b*) Electron micrograph (and explanatory line drawing) of replicating *Drosophila* DNA showing these bubbles. ([*b*] H. Kreigstein and D. Hogness, "Mechanism of DNA replication in *Drosophila* chromosomes: Structure of replication forks and evidence for bidirectionality," *Proceeding of the National Academy of Sciences USA,* 71 (1974):135–39. Reproduced by permission.)

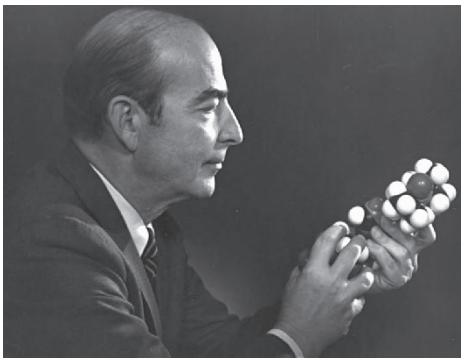
had already begun forming. Figure 9.23 illustrates hypothetical outcomes for either unidirectional or bidirectional replication. By counting silver grains in autoradiographs, Cairns found growth to be bidirectional. Both autoradiographic and genetic analysis have subsequently verified this finding.

In eukaryotes, the DNA molecules (chromosomes) are larger than in prokaryotes and are not circular; there are also usually multiple sites for the initiation of replication. Thus, each eukaryotic chromosome is composed of many replicating units, or **replicons**—stretches of DNA with a single origin of replication. In comparison, the *E. coli* chromosome is composed of only one replicon. In eukaryotes, these replicating units form "bubbles" (or "eyes") in the DNA during replication (fig. 9.24).

DNA REPLICATION—THE ENZYMOLOGY

Let us turn now to the details of the processes that take place during DNA replication. Like virtually all metabolic processes, DNA replication is under the control of enzymes. The evidence for the details we describe comes from physical, chemical, and biochemical studies of enzymes and nucleic acids and from the analysis of mutations that influence the replication processes. More recent techniques of recombinant DNA technology and nucleotide sequencing have allowed us to determine the nucleotide sequences of many of these key regions in DNA and RNA. We will look first at *E. coli.*

There are three major enzymes that will polymerize nucleotides into a growing strand of DNA in *E. coli.* These enzymes are **DNA polymerase** I, II, and III. DNA polymerase I, discovered by Arthur Kornberg, who subsequently won the Nobel Prize for his work, is primarily utilized in filling in small DNA segments during replication and repair processes. DNA polymerase II can serve as an alternative repair polymerase; it can also replicate DNA if the template is damaged. DNA polymerase III is the primary polymerase during normal DNA replication.



Arthur Kornberg (1918–). (Courtesy of Dr. Arthur Kornberg. Photograph by Karsh.)

In the simplest model of DNA replication, new nucleotides would be simultaneously added, according to the rules of complementarity, on both strands of newly synthesized DNA at the replication fork as the DNA opens up. But a problem exists, created by DNA's antiparallel nature; the two strands of a DNA double helix run in opposite directions. Going in one direction on the duplex, for example, one strand is a 5′ → 3′ strand, whereas the other is a 3′ → 5′ strand. These directions refer to the numbering of carbon atoms across the sugar. In figure 9.25, going from the bottom of the figure to the top, the left-hand strand is a 3′ → 5′ strand, and the right-hand strand is a 5′ → 3′ strand. Since DNA replication involves the formation of two new antiparallel strands with the old single strands as templates, one new strand would have to be replicated in the 5′ → 3′ direction and the other in the 3′ → 5′ direction.

However, all the known polymerase enzymes add nucleotides in only the 5′ → 3′ direction. That is, the polymerase catalyzes a bond between the first 5′-PO_4 group of a new nucleotide and the 3′-OH carbon of the last nucleotide in the newly synthesized strand (fig. 9.25). The polymerases cannot create the same bond with the 5′ phosphate of a nucleotide already in the DNA and the 3′ end of a new nucleotide. Thus, the simple model needs some revision.

Continuous and Discontinuous DNA Replication

Autoradiographic evidence leads us to believe that replication occurs simultaneously on both strands. **Continuous replication** is, of course, possible on the 3′ → 5′ template strand, which begins with the necessary 3′-OH **primer.** (Primer is double-stranded DNA—or, as we shall see, a DNA-RNA hybrid—continuing as single-stranded DNA template. The strand being synthesized has a 3′-OH available; fig. 9.26.) A **discontinuous** form of replication takes place on the complementary strand, where it occurs in short segments, moving backward, away from the Y-junction (fig. 9.27). These short segments, called **Okazaki fragments** after R. Okazaki, who first saw them, average about 1,500 nucleotides in prokaryotes and 150 in eukaryotes. The strand synthesized continuously is referred to as the **leading strand,** and the strand synthesized discontinuously is referred to as the **lagging strand.**

Once initiated, continuous DNA replication can proceed indefinitely. DNA polymerase III on the leading-strand template has what is called high **processivity:** once it attaches, it doesn't release until the entire strand is replicated. Discontinuous replication, however, requires the repetition of four steps: primer synthesis, elongation, primer removal with gap filling, and ligation.

Primer Synthesis and Elongation

To synthesize Okazaki fragments, a primer must be created *de novo* (Latin: from the beginning). None of the DNA polymerases can create that primer. Instead, **primase,** an RNA polymerase coded for by the *dnaG* gene, creates the primer, ten to twelve nucleotides, at the site

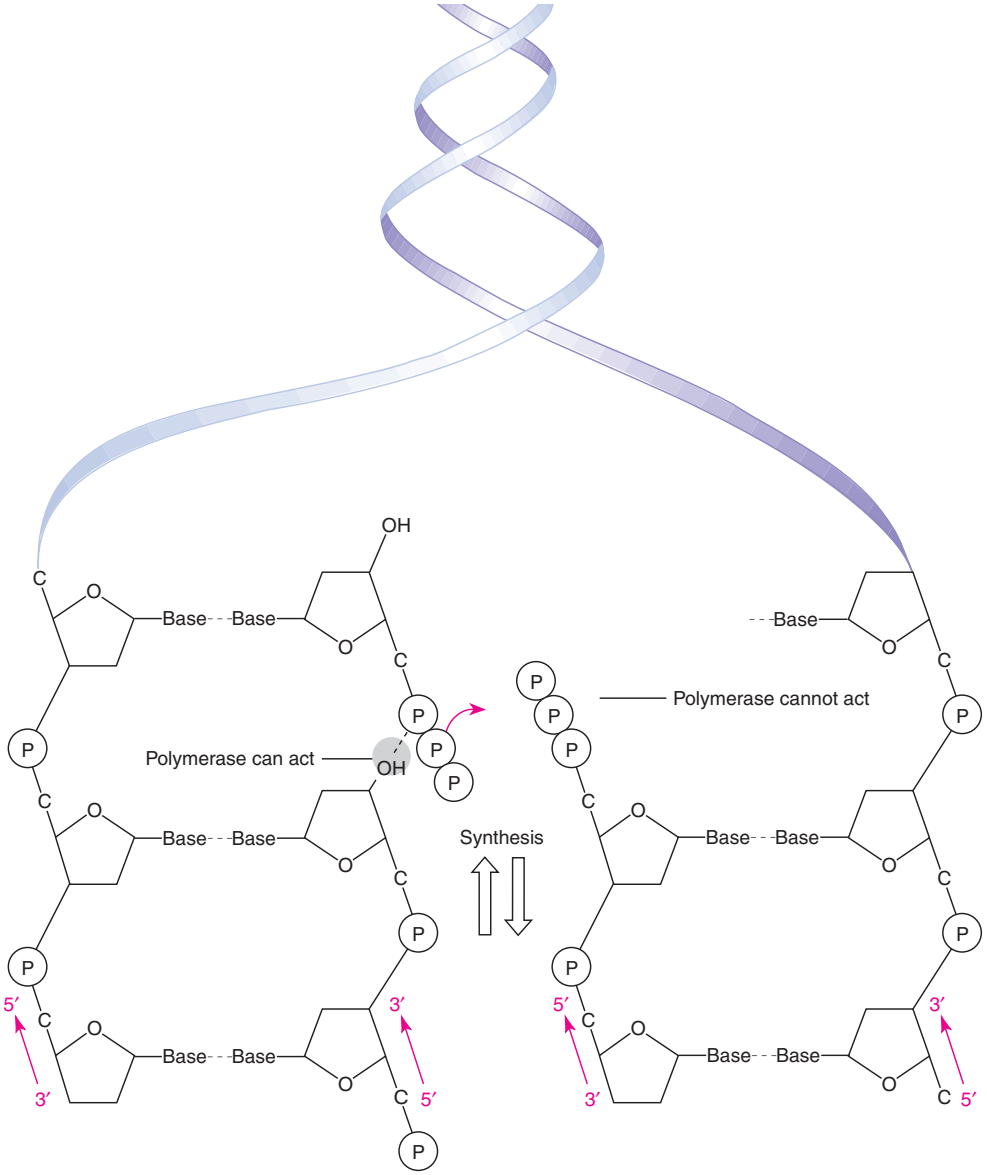


Figure 9.25 New nucleotides can be added to DNA only during replication in the 5′ → 3′ direction.

of Okazaki fragment initiation (fig. 9.28). The result is a short RNA primer that provides the free 3′-OH group that DNA polymerase III needs in order to synthesize the Okazaki fragment. DNA polymerase III continues until it reaches the primer RNA of the previously synthesized Okazaki fragment. At that point, it stops and releases from the DNA.

All three prokaryotic polymerases not only can add new nucleotides to a growing strand in the 5′ → 3′ direction, but also can remove nucleotides in the opposite 3′ → 5′ direction. This property is referred to as *3′ → 5′ exonuclease activity.* Enzymes that degrade nucleic acids are nucleases. They are classified as **exonucleases** if they remove nucleotides from the end of a nucleotide strand or as **endonucleases** if they can break the sugar-phosphate backbone in the middle of a nucleotide strand. At first glance, exonuclease activity seems like an extremely curious property for a polymerase to have—

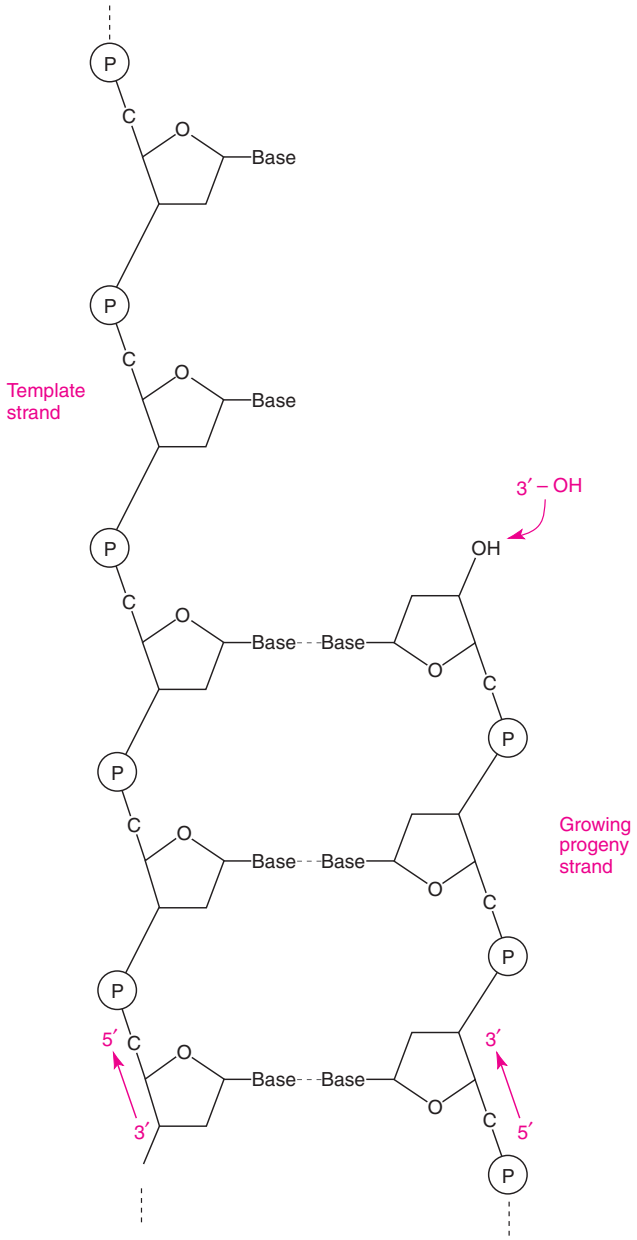


Figure 9.26 Primer configuration for DNA replication. A 3′-OH group must be available on the nascent progeny strand opposite a continuing single-stranded template.

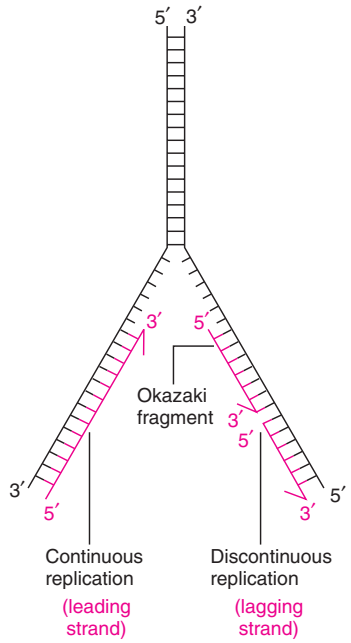


Figure 9.27 Discontinuous model of DNA replication. Lagging-strand replication requires Okazaki fragments to form going backward, away from the Y-junction.

curious unless we think about its ability to check complementarity. If the complementarity is improper, meaning that the wrong nucleotide has been inserted, the polymerase can remove the incorrect nucleotide, put in the proper one, and continue on its way. This is known as the **proofreading** function of DNA polymerase. In addition, exonuclease activity can remove the RNA primers of Okazaki fragments.

Primer Removal with Gap Filling

DNA polymerase I is a polymerase when it adds nucleotides, one at a time, and an exonuclease when it removes nucleotides one at a time. To complete the Okazaki fragment, DNA polymerase I acts in both capacities. (DNA polymerase I mutants cannot properly connect Okazaki fragments.) DNA polymerase I completes the Okazaki fragment by removing the previous RNA primer and replacing it with DNA nucleotides (fig. 9.29). When DNA polymerase I has completed its nuclease and polymerase activity, the two previous Okazaki fragments are almost complete. All that remains is for a single phosphodiester bond to form.

Ligation

DNA polymerase I cannot make the final bond to join two Okazaki fragments. The configuration needing completion is shown in figure 9.30. An enzyme, **DNA ligase,**

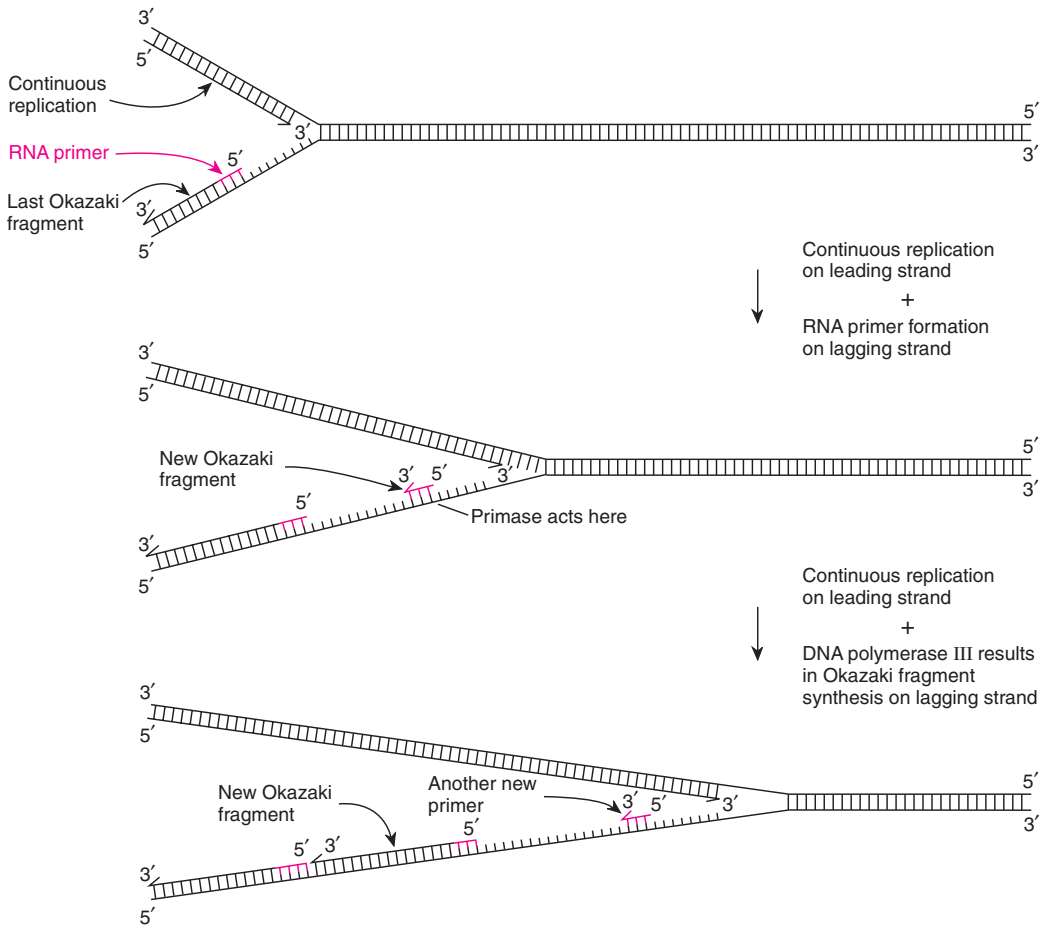


Figure 9.28 Primer formation and elongation create an Okazaki fragment during discontinuous DNA replication.

completes the task by making the final phosphodiester bond in an energy-requiring reaction.

A question of evolutionary interest is why RNA is used to prime DNA synthesis. Why not use DNA directly and avoid the exonuclease and resynthesis activity seen in figure 9.29? Probably, making use of RNA primers lowers the error rate of DNA replication. That is, priming is an inherently error-prone process since nucleotides are initially added without a stable primer configuration. To prevent long-term errors in the DNA, an RNA primer is put in that can later be recognized and removed. Resynthesis by polymerase I is in a much more stable primer configuration (a long primer) and thus makes very few errors.

Another question of evolutionary interest is why DNA synthesis cannot take place in the $3' \rightarrow 5'$ direction. Probably, the answer has to do with proofreading and the exonuclease removal of mismatched nucleotides. When an incorrect nucleotide is found and removed, the next nucleotide brought in, in the $5' \rightarrow 3'$ direction, has a triphosphate end available to provide the energy for its own incorporation (see fig. 9.25). Consider what would happen if the polymerase were capable of adding nucleotides in the opposite direction. The energy for the phosphodiester bond would be coming from the triphosphate already attached in the growing $3' \rightarrow 5'$ strand (see fig. 9.25). Then, if an error in complementarity were detected and the polymerase removed the most recently added nucleotide from the $3' \rightarrow 5'$ strand, the last nucleotide in the double helix would no longer have a triphosphate available to provide energy for the diester bond with the next nu-

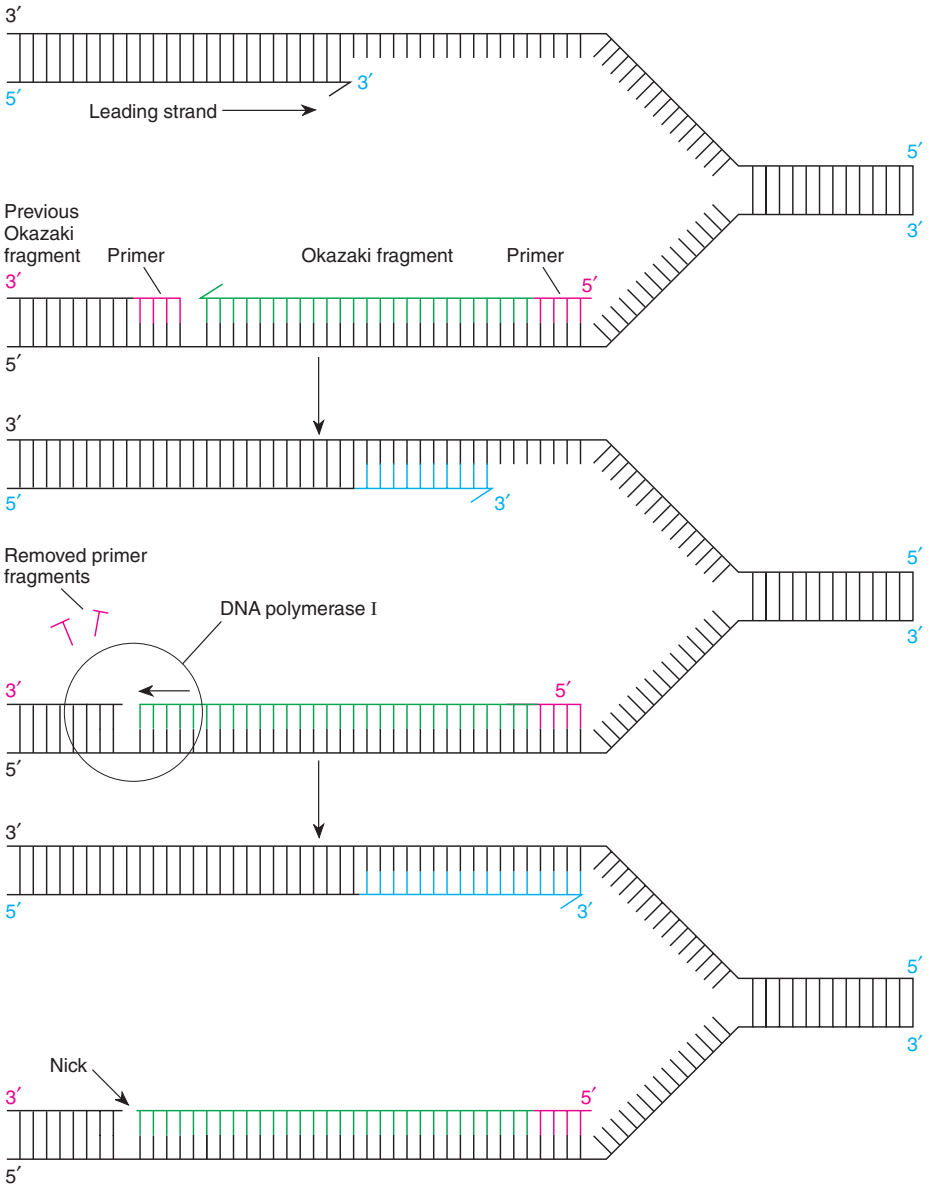


Figure 9.29 The completion of an Okazaki fragment requires that DNA polymerase I replace the RNA primer base by base with DNA nucleotides. A final nick in the DNA backbone remains (*arrow*).

cleotide. Continued polymerization would thus require additional enzymatic steps to provide the energy needed for the process to continue. This could stop or slow the process down considerably. As it is, the process incorporates about four hundred nucleotides per second with an error rate of about one incorrect pairing per 10^9 bases. (Other repair systems further improve this error rate—see chapter 12.)

The Origin of DNA Replication

Each replicon (e.g., the *E. coli* chromosome, or a segment of a eukaryotic chromosome with an origin of replication) must have a region where DNA replication initiates. In *E. coli,* this region is referred to as the genetic locus *oriC;* it occurs at map location 84 minutes (see fig. 7.27). For DNA replication to begin, several steps must occur.

First, the appropriate initiation proteins must recognize the specific origin site. Then the site must be opened and stabilized. And, finally, a replication fork must be initiated in both directions, involving continuous and discontinuous DNA replication. Although most of the proteins involved are known, there are still a few gaps in our knowledge.

OriC, the origin of replication in *E. coli,* is about 245 base pairs long and is recognized by **initiator proteins.** These proteins, the product of the *dnaA* locus, open up the double helix. (Other DNA-binding proteins are also involved here.) The initiator proteins then take part in the attachment of DNA **helicase,** the product of the *dnaB* gene, which unwinds DNA at the Y-junction. Helicase is then responsible for recruiting (binding) the rest of the proteins that form the replication initiation complex. First is primase, which creates RNA primers. Together, the helicase and primase comprise a **primosome,** attached to the lagging-strand template. As the primosomes move along, they create RNA primers that

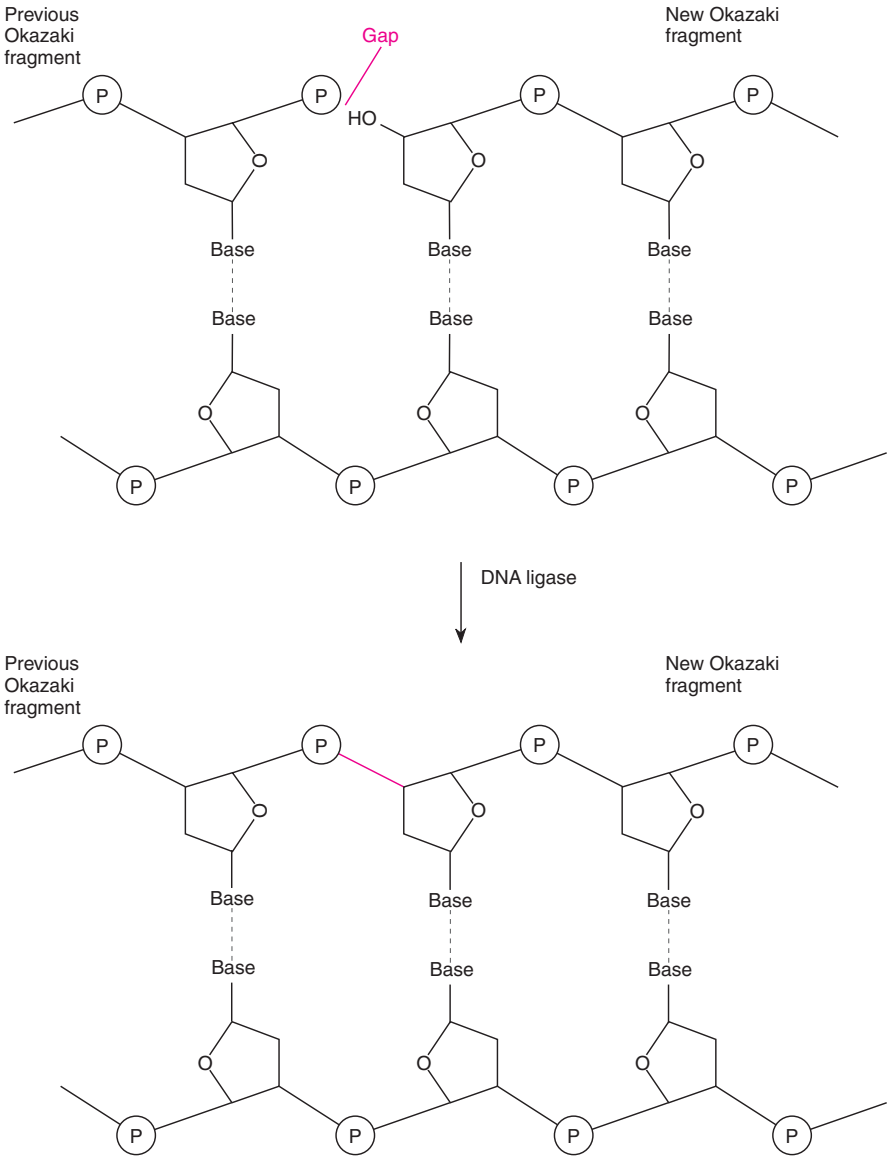


Figure 9.30 After DNA polymerase I removes the RNA primer to complete an Okazaki fragment, a final gap remains. DNA ligase closes it.

DNA polymerase III uses to initiate leading-strand synthesis. As primers are being laid down on the lagging-strand template, Okazaki fragment synthesis begins, and Y-junction activity then proceeds as outlined earlier (see figs. 9.28, 9.29, and 9.30).

DNA polymerase III **holoenzyme** is a very large protein composed of ten subunits (table 9.4). Three of the subunits, α, ε, and θ, form the polymerization core, with both 5′ → 3′ polymerase activity and 3′ → 5′ exonuclease activity. One subunit, the β subunit, is a "processivity clamp." As a dimer (two identical copies attached head to tail), the protein forms a "doughnut" around the DNA so it can move freely on the DNA. When it is attached to the core enzyme, the polymerase is held tightly to the DNA and shows high processivity (fig. 9.31): the leading strand is usually synthesized entirely without the enzyme leaving the template (fig. 9.32). The remaining subunits are involved in processivity control and replisome formation. They allow the polymerase to move off and on the DNA of the lagging-strand template as Okazaki fragments are completed (a process known as **polymerase cycling**).

Eukaryotes have evolved at least nine DNA polymerases, named DNA polymerase α, β, γ, δ, ε, ζ, η, θ, and ι. DNA polymerase δ seems to be the major replicating enzyme in eukaryotes, forming replisomes as in *E. coli.* In eukaryotes, the polymerase α-primase complex adds the Okazaki fragment primers, first adding an RNA primer and then a short length of DNA nucleotides. Polymerase ε may be involved in repair or in normal DNA replication, as is polymerase δ. DNA polymerase γ appears to replicate mitochondrial DNA. The remaining polymerases are probably involved in DNA repair, with polymerase β being the major repair polymerase, as polymerase I is in

Table 9.4 **Summary of the Enzymes Involved in DNA Replication in *E. coli***

Enzyme or Protein	Genetic Locus	Function
DNA polymerase I	*polA*	Gap filling and primer removal
DNA polymerase II	*polB*	Replicating damaged templates
DNA polymerase III		
α subunit	*dnaE*	Polymerization core; 5′ → 3′ polymerase
ε subunit	*dnaQ*	Polymerization core; 3′ → 5′ exonuclease
θ subunit	*holE*	Polymerization core
β subunit	*dnaN*	Processivity clamp (as a dimer)
τ subunit	*dnaX*	Preinitiation complex; dimerization of core
γ subunit	*dnaX*	Preinitiation complex; loads clamp
δ subunit	*holA*	Processivity core
δ′ subunit	*holB*	Processivity core
χ subunit	*holC*	Processivity core
ψ subunit	*holD*	Processivity core
Helicase	*dnaB*	Primosome; unwinds DNA
Primase	*dnaG*	Primosome; creates Okazaki fragment primers
Initiator protein	*dnaA*	Binds at origin of replication
DNA ligase	*lig*	Closes Okazaki fragments
Ssb protein	*ssb*	Binds single-stranded DNA
DNA topoisomerase I	*topA*	Relaxes supercoiled DNA
DNA topoisomerase type II (DNA Gyrase)		
α subunit	*gyrA*	Relaxes supercoiled DNA; ATPase
β subunit	*gyrB*	Relaxes supercoiled DNA
Topoisomerase IV	*parE*	Unconcatenates DNA circles
Termination protein	*tus*	Binds at termination sites

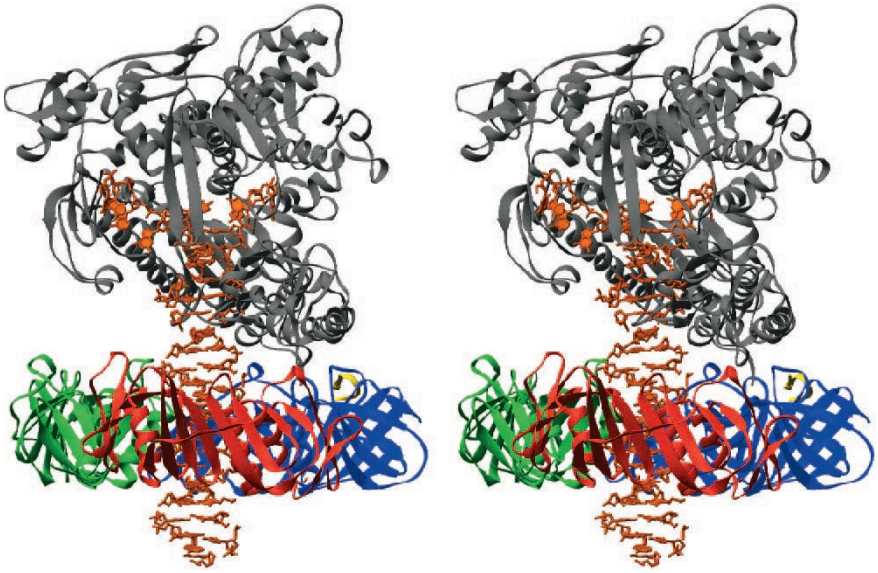


Figure 9.31 Stereo view of sliding clamp, DNA polymerase, and DNA from bacteriophage RB69. The clamp (*red, blue, green*) surrounds the DNA (*brown*) like a doughnut. The clamp is attached to the proximal segment of the DNA polymerase (*gray*). (From Yousif Shamoo and Thomas A. Steitz, "Building a replisome from interacting pieces" in *Cell,* 99:155–166, October 15. Reprinted by permission of *Cell.*)

E. coli. Several of the polymerases most likely both replicate and repair DNA.

Eukaryotes also have a clamp-loader complex, called replication factor C, and a six-unit clamp called the proliferating cell nuclear antigen. The RNA primers are removed during Okazaki fragment completion (maturation) by mechanisms similar to those in prokaryotes. In eukaryotes, RNAase enzymes remove the RNA primers in Okazaki fragments; a repair polymerase fills gaps; and a DNA ligase forms the final seal. Helicases, topoisomerases, and single-strand binding proteins play roles similar to those they play in prokaryotes. The completion of the replication of linear eukaryotic chromosomes involves the formation of specialized structures at the tips of the chromosomes, which we discuss in chapter 15. Thus, all of the enzymatic processes are generally the same in prokaryotes and eukaryotes. DNA replication developed in prokaryotes and was refined as prokaryotes evolved into eukaryotes.

T. Steitz and his colleagues have done much X-ray crystallography work that has given us an excellent look at the structure of a polymerase. (Most work has actually been done on a fragment of DNA polymerase I called the **Klenow fragment.**) The enzyme is shaped like a cupped right hand with enzymatic activity taking place in two places, separated by a distance of about two to three nucleotides (fig. 9.33). It is proposed that when the polymerization site senses a mismatch, the DNA is moved so that the 3′ end enters the exonuclease site, where the incorrect nucleotide residue is then cleaved. Polymerization then continues. There may be a general mode of polymerase action among diverse polymerases.

The replication of the *E. coli* chromosome may be controlled by the methylation state of several sequences within *oriC.* As we discuss in chapter 13, certain enzymes add methyl groups to specific DNA bases, and the presence or absence of these methyl groups can serve as signals to other enzymes.

Events at the Y-Junction

We now have the image of DNA replication proceeding as a primosome moves along the lagging-strand template, opening up the DNA (helicase activity), and creating RNA primers (primase activity) for Okazaki fragments. One DNA polymerase III moves along the leading-strand template, generating the leading strand by continuous DNA replication, whereas a second DNA polymerase III moves backward, away from the Y-junction, creating Okazaki fragments. **Single-strand binding proteins** (ssb proteins) keep single-stranded DNA stabilized (open) during

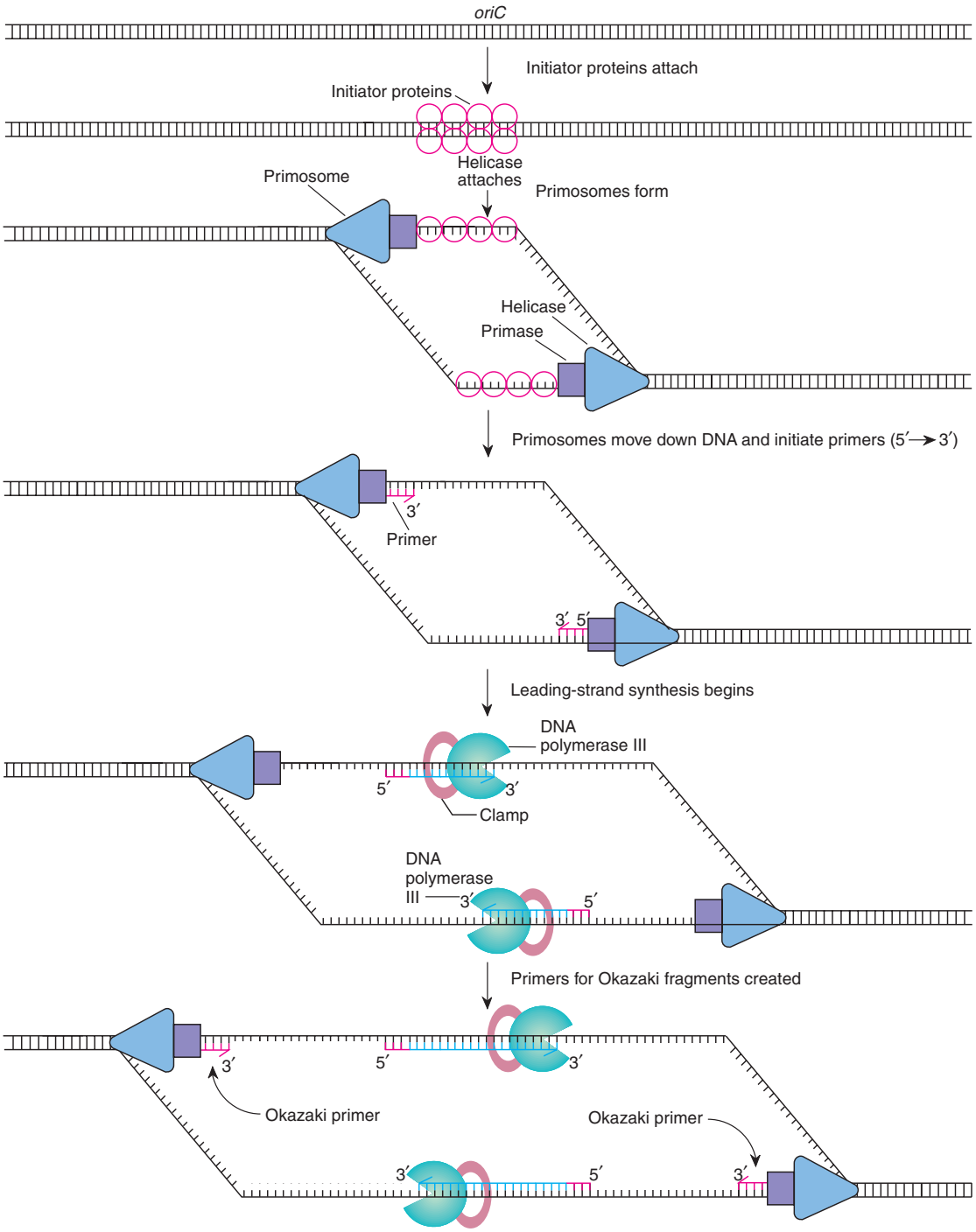


Figure 9.32 Events at the origin of DNA replication in *E. coli.* The DNA opens up at *oriC* to create two moving Y-junctions. Initiator proteins attach and then bind helicase. The helicase then binds primase, forming a primosome. After the primer forms and two copies of DNA polymerase III are bound, the polymerization process begins.

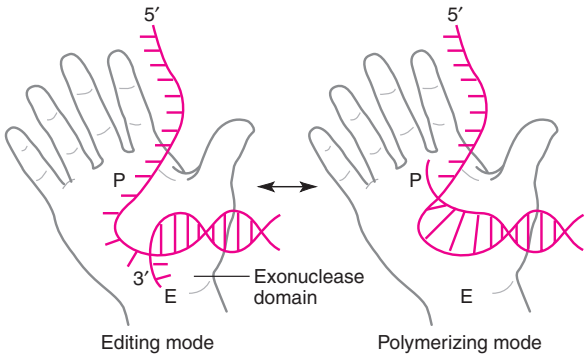


Figure 9.33 DNA polymerase (P) and exonuclease (E) activities of the Klenow fragment of DNA polymerase I in *E. coli.* On the *right,* 5′ → 3′ polymerization is occurring. On the *left,* the 3′ end of the nascent strand has been backed up into the exonuclease site, presumably when a mismatch was detected. (With permission from the *Annual Review of Biochemistry,* Volume 63. ©1994 by Annual Reviews. www.AnnualReviews.org.)

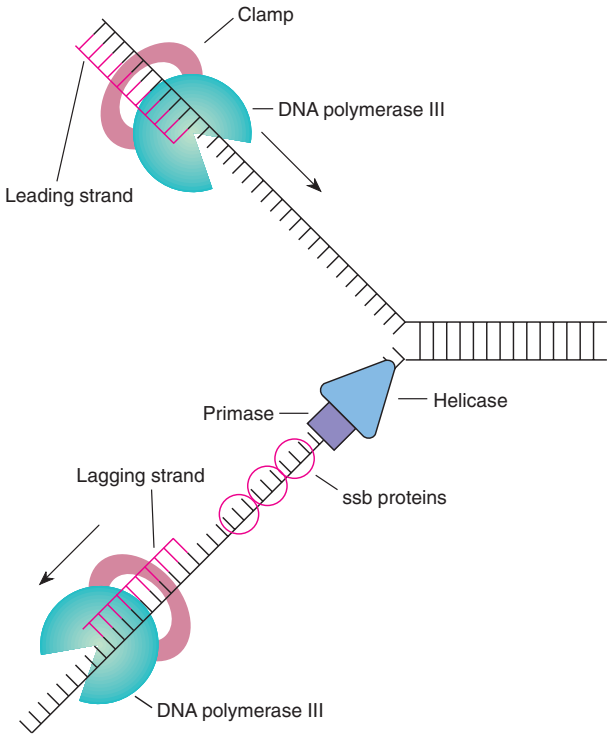


Figure 9.34 Schematic drawing of DNA replication at a Y-junction. Two copies of DNA polymerase III, ssb proteins, and a primosome (helicase + primase) are present.

this process, and DNA polymerase I and ligase connect Okazaki fragments (fig. 9.34).

This simple picture is slightly complicated by the fact that the lagging- and leading-strand synthesis is coordinated. B. Alberts suggested an explanation: the **replisome** model, in which both copies of DNA polymerase III are attached to each other and work in concert with the primosome at the Y-junction (fig. 9.35). According to this model, a single replisome, consisting of two copies of DNA polymerase III, a helicase, and a primase, moves along the DNA. The leading-strand template is immediately fed to a polymerase, whereas the lagging-strand template is not acted on by the polymerase until an RNA primer has been placed on the strand, meaning that a long (fifteen-hundred base) single strand has been opened up (fig. 9.35*a*).

As the replisome moves along, another single-stranded length of the lagging-strand template forms. At about the time that the Okazaki fragment is completed, a new RNA primer has been created (fig. 9.35*b*). The Okazaki fragment is released (fig. 9.35*c*), and a new Okazaki fragment is begun (polymerase cycling), starting with the latest primer (fig. 9.35*d*). This takes the replisome back to the same configuration as in figure 9.35*a,* but one Okazaki fragment farther along.

Figure 9.36 gives us a closer look at the details of the Y-junction at the moment of polymerase cycling. Primase, which is not highly processive, must be in touch with an ssb protein to stay attached to the DNA when forming a primer. At the appropriate moment, after the primer is formed, the clamp loader contacts the ssb, dislodging the primase. The clamp loader also loads a sliding clamp, which then recruits (attaches to) the polymerase that is creating the lagging strand. The polymerase then continues, creating the Okazaki fragment. The primase can later attach at a new point on the lagging-strand template to create the next primer.

Supercoiling

The simplicity and elegance of the DNA molecule masks an inevitable problem: coiling. Since the DNA molecule is made from two strands that wrap about each other, certain operations, such as DNA replication and its termination, face topological difficulties. Up to this point, we have seen the circular *E. coli* chromosome in its "relaxed" state (e.g., figs. 9.21 and 9.22). However, certain enzymes in the cell cause DNA to become overcoiled (positively **supercoiled**) or undercoiled (negatively supercoiled). Positive supercoiling comes about in two ways: either the DNA takes too many turns in a given length, or the molecule wraps around itself (fig. 9.37).

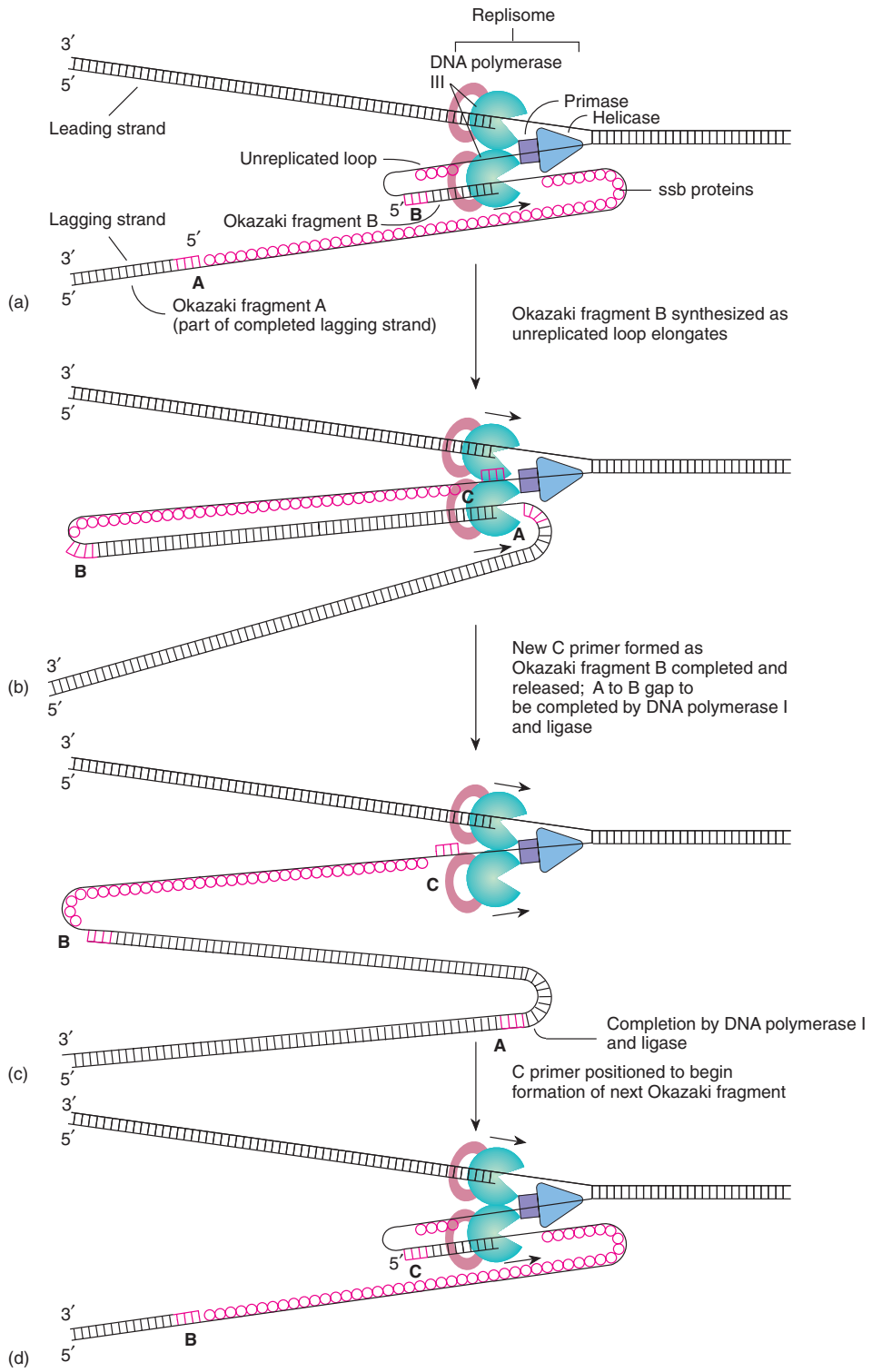


Figure 9.35 The replisome, which consists of two DNA polymerase III holoenzymes and a primosome (helicase + primase), coordinates replication at the Y-junction. Parts *b–d* show "polymerase cycling," in which the polymerase on the lagging-strand template releases a completed Okazaki fragment and then begins the next one.

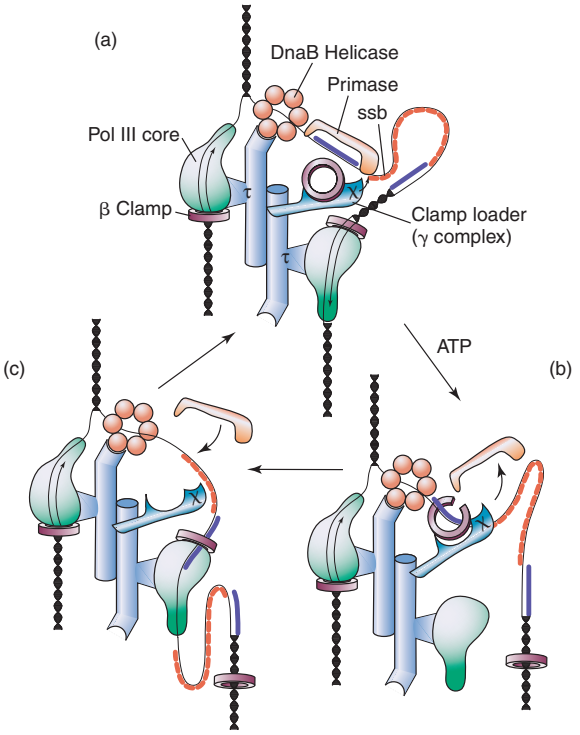


Figure 9.36 A close-up view of the Y-junction during polymerase cycling. The two polymerases (pol III core) are held together by τ subunits. Also pictured are the sliding clamp (β Clamp), clamp loader (γ complex), primase, helicase, and ssb proteins. In (*a*), the primase has just finished creating a primer. The χ subunit of the clamp loader contacts the ssb protein that is touching the primase; the primase is then dislodged (*b*). The clamp is loaded at the new primer and the polymerase on the lagging strand is cycled to the clamp to begin the next Okazaki segment (*c*). (Reprinted from *Cell,* Vol. 96, Yuzhakov et al., "Trading Places on DNA-a Three Point Switch Underlies Primer Handoff from Primase to the Replicative DNA Polymers," pp. 153–163, Copyright © 1999, with permission from Elsevier Science.)

Positive supercoiling occurs when the circular duplex winds about itself in the same direction as the helix twists (right-handed), whereas negative supercoiling comes about when the duplex winds about itself in the opposite direction as the helix twists (left-handed). The former increases the number of turns of one helix around the other (the **linkage number,** L), whereas the latter decreases it. The three forms of DNA in figure 9.37 all have the same sequence, yet they differ in linkage number. Accordingly, they are referred to as topological isomers **(topoisomers).** The enzymes that create or alleviate these states are called **topoisomerases.**

Topoisomerases affect supercoiling by either of two methods. Type I topoisomerases break one strand of a double helix and, while binding the broken ends, pass the other strand through the break. The break is then sealed (fig. 9.38). Type II topoisomerases (e.g., **DNA gyrase** in *E. coli*) do the same sort of thing, only instead of breaking one strand of a double helix, they break both and pass another double helix through the temporary gap. Four topoisomerases are active in *E. coli,* with somewhat confusing nomenclature: topoisomerases I and III are type I; topoisomerases II and IV are type II.

As DNA replication proceeds, positive supercoiling builds up ahead of the Y-junction. This is eliminated by topoisomerases that either create negative supercoiling ahead of the Y-junction in preparation for replication or alleviate positive supercoiling after it has been created.

Termination of Replication

The termination of the replication of a circular chromosome presents no major topological problems. At the end of the theta-structure replication (see fig. 9.22), both Y-junctions have proceeded around the molecule. The region of termination on the *E. coli* chromosome, the terminus region, is 180 degrees from *oriC* on the circular chromosome, between minutes 28 and 36. There are six terminator sites (*Ter*); three arrest the Y-junction from the left, and three arrest the one from the right when bound by a termination protein, the protein product of the *tus* gene. (*Tus* stands for terminus utilization substance; each *Ter* site is about twenty base pairs.) One interesting aspect of the termination of *E. coli* DNA replication is that the cells are viable even if the whole terminator region is deleted. There are fewer viable cells and some growth problems, but in general, *E. coli* can successfully terminate DNA replication even without formal termination sites. A topoisomerase, topoisomerase IV, then releases the two circles, and DNA polymerase I and ligase close them up (fig. 9.39).

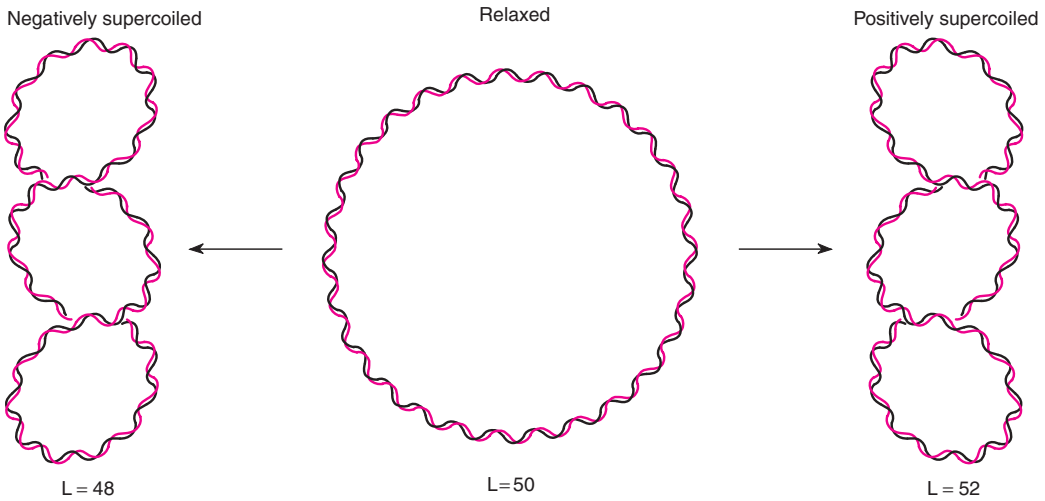


Figure 9.37 Positive and negative supercoils. Enzymes called topoisomerases can take relaxed DNA (*center*) and add negative (*left*) or positive (*right*) supercoils. *L* is the linkage number.

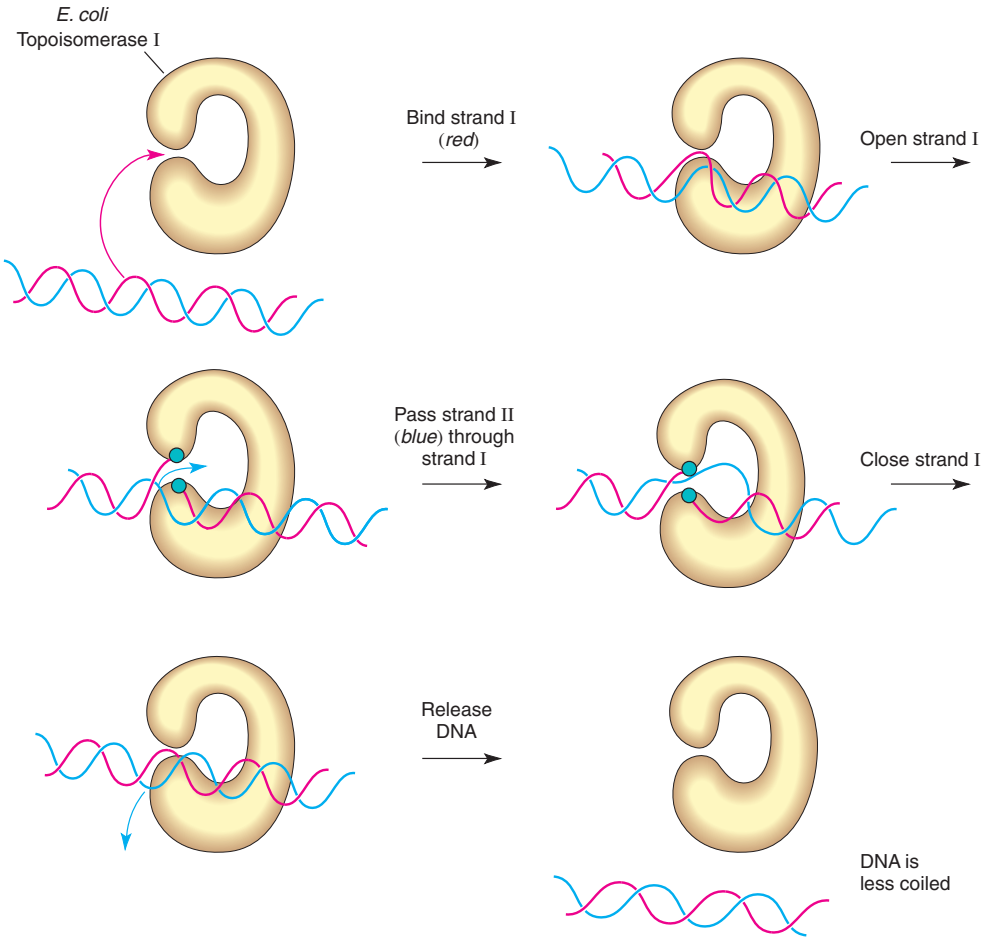


Figure 9.38 Topoisomerase I can reduce DNA coiling by breaking one strand of the double helix and passing the other strand through it.

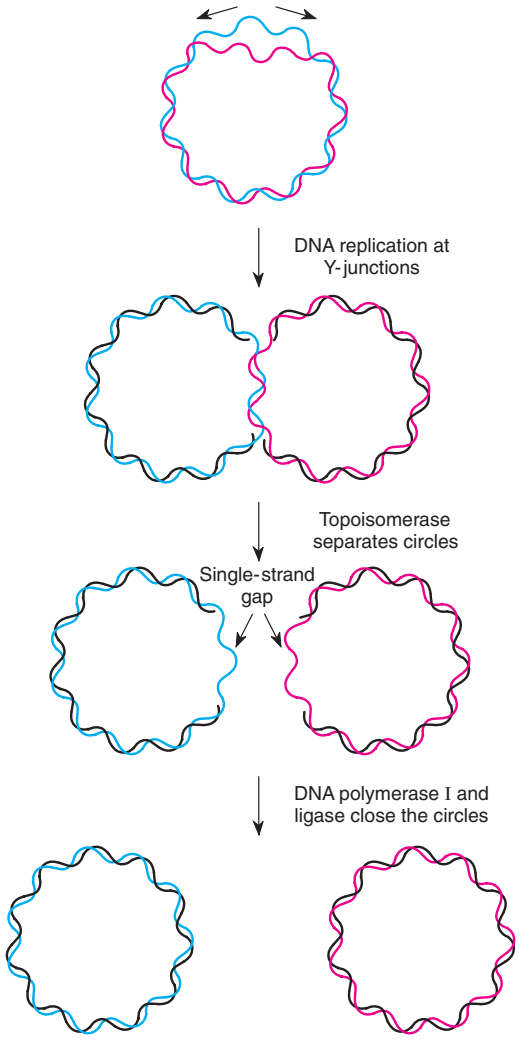


Figure 9.39 The replication of circular DNA terminates when topoisomerase separates the circles and DNA polymerase I and ligase close the gaps in each circle.

DNA Partitioning in E. coli

In chapter 3, we discussed processes that partition eukaryotic chromosomes between daughter cells during mitosis and meiosis. Until very recently, geneticists believed that the partitioning of the *E. coli* chromosome was a passive process, unlike that in eukaryotes. Now, however, we know that more complexity is involved in *E. coli* DNA partitioning. When DNA replication begins, the newly replicated origins of replication are segregated to opposite ends of the bacterial cell, acting as centromeres do. A ring of proteins, the products of the *FtsZ* gene, form a ring at the middle of the cell and begin to create the septum that will divide the cell into two. The full complexity involved in *E. coli* chromosomal partitioning should be uncovered in the near future.

REPLICATION STRUCTURES

The *E. coli* model of DNA replication that we have presented here is by way of the intermediate theta-structure (see fig. 9.22). Two other modes of replication occur in circular chromosomes: rolling-circle and D-loop.

Rolling-Circle Model

In the **rolling-circle** mode of replication, a nick (a break in one of the phosphodiester bonds) is made in one of the strands of the circular DNA, resulting in replication of a circle and a tail (fig. 9.40). This form of replication occurs in the F plasmid or *E. coli* Hfr chromosome during conjugation (see chapter 7). The F^+ or Hfr cell retains the circular daughter while passing the linear tail into the F^- cell, where replication of the tail takes place. Several phages also use this method, filling their heads (protein coats) with linear DNA replicated from a circular parent molecule.

D-Loop Model

Chloroplasts and mitochondria (in eukaryotic cells) have their own circular DNA molecules (see chapter 17) that appear to replicate by a slightly different mechanism. The origin of replication is at a different point on each of the two parental template strands. Replication begins on one strand, displacing the other while forming a displacement loop or **D-loop** structure (fig. 9.41). Replication continues until the process passes the origin of replication on the other strand. Replication then initiates on the second strand, in the opposite direction. Normal Y-junction replication, as described earlier, also occurs in mitochondrial DNA under some growth conditions.

EUKARYOTIC DNA REPLICATION

As we saw earlier, linear eukaryotic chromosomes usually have multiple origins of replication, resulting in figures referred to as "bubbles" or "eyes" (see fig. 9.24). Multiple origins allow eukaryotes to replicate their larger quantities of DNA in a relatively short time, even though eukaryotic DNA replication is considerably slowed by the presence of histone proteins associated

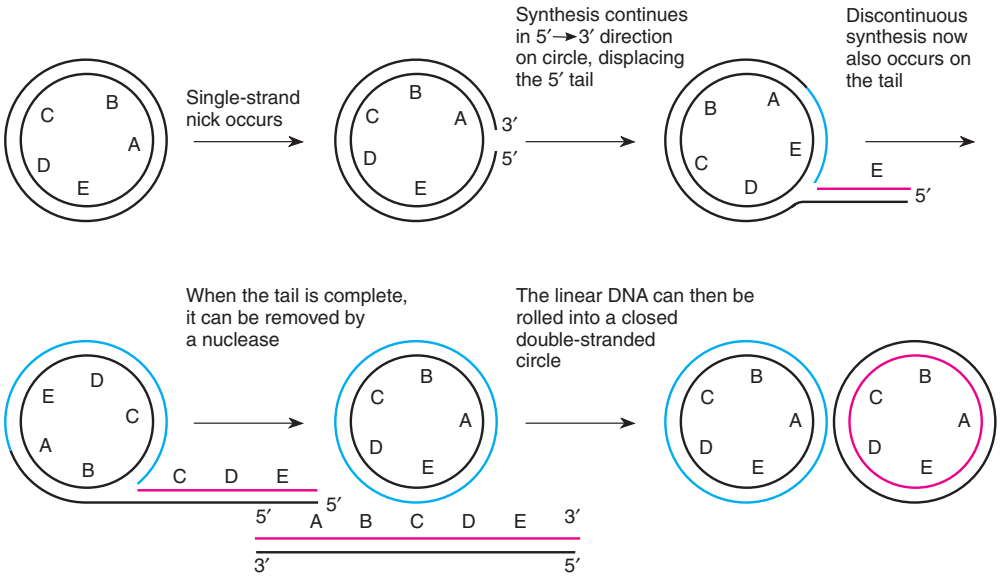


Figure 9.40 Rolling-circle model of DNA replication. The letters *A–E* provide landmarks on the chromosomes.

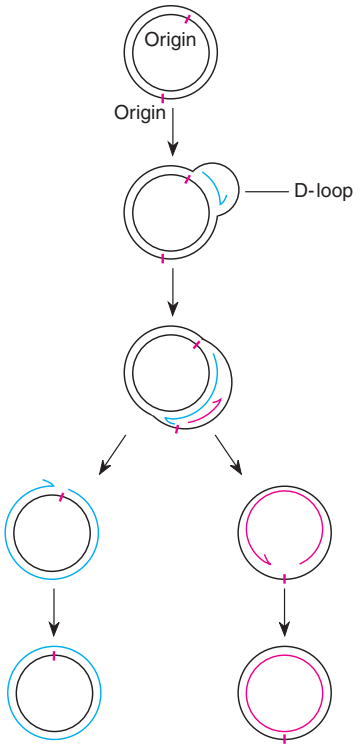


Figure 9.41 D-loops form during mitochondrial and chloroplast DNA replication because the origins of replication are at different places on the two strands of the double helix.

with the DNA to form chromatin (see chapter 15). For example, the *E. coli* replication fork moves through about twenty-five thousand base pairs per minute, whereas the eukaryotic Y-junction moves through only about two thousand base pairs per minute. The number of replicons in eukaryotes varies from about five hundred in yeast to as many as sixty thousand in a diploid mammalian cell.

In budding yeast, a lower eukaryote that is often used as a model organism, DNA replication initiates at sites called **autonomously replicating sequences (ARS).** Each consists of a specific 11-base-pair sequence plus two or three additional short DNA sequences encompassing 100–200 base pairs. Six proteins form a complex that binds to this sequence, referred to as the **origin recognition complex (ORC).** These proteins seem to be bound all the time, and thus additional proteins are needed to initiate DNA replication. Some of these additional proteins are cyclin-dependent kinases, proteins involved in the control of the cell cycle (chapter 3). This makes sense because in eukaryotes, DNA replication can take place only once during the cell cycle, during the S phase. Thus, the initiation of DNA replication must be tightly controlled to avoid multiple replication of some or all replicons.

SUMMARY

STUDY OBJECTIVE 1: To understand the properties that a genetic material must have 205–211.

A genetic material must be able to control the phenotype of a cell or organism (i.e., to direct protein synthesis), it must be able to replicate, and it must be located in the chromosomes. Avery and his colleagues demonstrated that DNA was the genetic material when they showed that the transforming agent was DNA. Griffith had originally demonstrated transformation of *Streptococcus* bacteria in mice. Hershey and Chase demonstrated that the DNA of bacteriophage T2 entered the bacterial cell. Fraenkel-Conrat demonstrated that in viruses without DNA (RNA viruses), such as tobacco mosaic virus, the RNA acted as the genetic material. Thus, by 1953, the evidence strongly suggested nucleic acids (DNA or, in its absence, RNA) as the genetic material.

STUDY OBJECTIVE 2: To examine the structure of DNA, the genetic material 211–224.

Chargaff showed a 1:1 relationship of adenine (A) to thymine (T) and cytosine (C) to guanine (G) in DNA. Wilkins, Franklin, and their colleagues showed, by X-ray crystallography, that DNA was a helix of specific dimensions. Following these lines of evidence, Watson and Crick in 1953 suggested the double-helical model of the structure of DNA. In their model, DNA is made up of two strands, running in opposite directions, with sugar-phosphate backbones and bases facing inward. Bases from the two strands form hydrogen bonds with each other with the restriction that only A and T or G and C can pair. This explains the quantitative relationships that Chargaff found among the bases. Melting temperatures of DNA also support this structural hypothesis because DNAs with higher G-C contents have higher melting, or denaturation, temperatures; G-C base pairs have three hydrogen bonds versus only two in an A-T base pair. The Watson-Crick DNA model represents the B form. DNA can exist in other forms, including the Z form, a left-handed double helix that may be important in controlling eukaryotic gene expression.

STUDY OBJECTIVE 3: To investigate the way in which DNA replicates 220–239.

DNA replicates by unwinding of the double helix, with each strand subsequently acting as a template for a new strand. This works because of complementarity—only A-T, T-A, G-C, or C-G base pairs form stable hydrogen bonds within the structural constraints of the model. This model of replication is *semiconservative.* Meselson and Stahl confirmed it in an experiment with heavy nitrogen. Autoradiographs of replicating DNA showed that replication proceeds bidirectionally from a point of origin. Prokaryotic chromosomes are circular, with a single initiation point of replication. Eukaryotic DNA is linear, with multiple initiation points of replication.

DNA polymerase enzymes add nucleotides only in the $5' \rightarrow 3'$ direction. Replication proceeds in small segments, working backward from the Y-junction on the $5' \rightarrow 3'$ template strand. Presumably, the $5' \rightarrow 3'$ restriction has to do with the proofreading DNA polymerases do to correct errors in complementarity. Polymerase III is the active replicating enzyme, and polymerase I is involved in DNA repair. Many other enzymes help create the Okazaki fragments, unwind DNA, and release the DNA from supercoiling. Prokaryotic and eukaryotic systems follow similar steps.

SOLVED PROBLEMS

PROBLEM 1: What evidence led to the idea that DNA was the genetic material?

Answer: Avery and his colleagues (MacLeod and McCarty) performed experiments showing that DNA was the transforming agent, and they are thus generally given credit for formalizing the notion that DNA, not protein, is the genetic material. Chargaff, Hershey and Chase, Fraenkel-Conrat, and several others also helped shape the general view. At the time that Watson and Crick published their model, the scientific community knew that DNA was the genetic material but didn't know its structure.

PROBLEM 2: How does DNA fulfill the requirements of a genetic material?

Answer: DNA is located in chromosomes, has a structure that is easily and accurately replicated, and has the sequence complexity to code for the fifty thousand or more genes that a eukaryotic organism has.

PROBLEM 3: What enzymes are involved in DNA replication in *E. coli?*

Answer: A replisome, consisting of a primosome (a primase and a helicase) and two polymerase III holoenzymes, forms at a Y-junction on DNA. One polymerase acts processively, synthesizing the leading strand, while the other forms Okazaki fragments initiated by primers created by the primase. DNA polymerase I completes the Okazaki fragments, eliminating the RNA primer of the previous Okazaki fragment and replacing it with DNA. Finally, DNA ligase connects the fragments. Also involved in the process are single-strand binding proteins and topoisomerases that relieve the DNA's supercoiling. Initiation involves initiation proteins at *oriC,* and termination requires termination proteins bound to the termination sites and a topoisomerase.

PROBLEM 4: What can be concluded about the nucleic acids in the following table?

Nucleic Acid Molecule	%A	%T	%G	%C	%U
a.	28	28	22	22	0
b.	31	0	31	17	21
c.	15	15	35	35	0

Answer: We must first look to see if U or T is present, for this will indicate whether the molecule is RNA or DNA, respectively. Molecule b is RNA; a and c are DNA. Now we look at base composition. In double-stranded molecules, A pairs evenly with T (or U) and G pairs with C. This relationship holds for molecules a and c, so they are double-stranded; molecule b is single-stranded. Finally, the melting temperature increases with the amount of G-C, so the melting temperature of c is greater than that of a.

EXERCISES AND PROBLEMS*

CHEMISTRY OF NUCLEIC ACIDS

1. If the tetranucleotide hypothesis were correct regarding the simplicity of DNA structure, under what circumstances could DNA be the genetic material?
2. Nucleic acids, proteins, carbohydrates, and fatty acids could have been mentioned as potential genetic material. What other molecular moieties (units) in the cell could possibly have functioned as the genetic material?
3. In what component parts do DNA and RNA differ?
4. Draw the structure of a short segment of DNA (three base pairs) at the molecular level. Indicate the polarity of the strands.
5. Roughly sketch the shape of B and Z DNA, remembering that B DNA is a right-handed helix and Z DNA is a left-handed helix.
6. Deduce whether each of the nucleic acid molecules in the following table is DNA or RNA and single-stranded or double-stranded.

Nucleic Acid Molecule	%A	%G	%T	%C	%U
a.	33	17	33	17	0
b.	33	33	17	17	0
c.	26	24	0	24	26
d.	21	40	21	18	0
e.	15	40	0	30	15
f.	30	20	15	20	15

7. A double-stranded DNA molecule is 28% guanosine (G).
   a. What is the complete base composition of this molecule?
   b. Answer the same question, but assume the molecule is double-stranded RNA.
8. The following are melting temperatures for five DNA molecules: 73° C, 69° C, 84° C, 78° C, 82° C. Arrange these DNAs in increasing order of percentage of G-C pairs.
9. We normally think that single-stranded nucleic acids should not melt, but many, in fact, do have a T_m. How can you explain this apparent mystery?
10. In a single-stranded DNA molecule, the amount of G is twice the amount of A, the amount of T is three times the amount of C, and the ratio of pyrimidines to purines is 1.5:1. What is the base composition of the DNA?
11. A double-stranded DNA measures 6.5 m in length. Approximately how many base pairs does it contain?

DNA REPLICATION—THE PROCESS

12. Diagram the results that Meselson and Stahl would have obtained (a) if DNA replication were conservative and (b) if it were dispersive.
13. What type of photo would J. Cairns have obtained if DNA replication were conservative? Dispersive?

*Answers to selected exercises and problems are on page A-10.

DNA REPLICATION—THE ENZYMOLOGY

14. Following is a section of a single strand of DNA. Supply a strand, by the rules of complementarity, that would turn this into a double helix. What RNA bases would primase use if this segment initiated an Okazaki fragment? In which direction would replication proceed?

5′-ATTCTTGGCATTCGC-3′

15. What is a primosome in *E. coli*? a replisome? What enzymes make up each? What is the relationship between these structures?

16. What are the differences between continuous and discontinuous DNA replication? Why do both exist?

17. Describe the synthesis of an Okazaki fragment.

18. Describe the enzymology of the origin, continuation, and termination of DNA replication in *E. coli.*

19. Can you think of any other mechanisms besides topoisomerase activity that could release supercoiling in replicating DNA?

20. Draw a diagram showing how topoisomerase II (gyrase) might work.

21. Retroviruses are single-stranded RNA viruses that insert their genomes into the host DNA during their life cycle. But only double-stranded DNA can be inserted into double-stranded DNA.

a. Propose a mechanism that retroviruses could use to insert their genomes.

b. What novel enzymes might such viruses require?

22. Propose a mechanism by which a single strand of DNA can make multiple copies of itself.

23. Progeria is a human disorder that causes affected individuals to age prematurely; a nine-year-old often resembles a sixty- to seventy-year-old individual in appearance and physiology. Suppose you extract DNA from a progeric patient and find mostly small DNA fragments rather than the expected long DNA molecules. What enzyme(s) might be defective in patients with progeria?

REPLICATION STRUCTURES

24. Under what circumstances would you expect to see a DNA theta structure? D-loop? rolling-circle? bubbles? What function does each structure serve?

EUKARYOTIC DNA REPLICATION

25. In developing sea urchins, just after fertilization, the cells divide every thirty to forty minutes. In the adult, the cells divide once every ten to fifteen hours. The amount of DNA per cell is the same in each case, but the DNA obviously replicates much faster in developing cells. Propose an explanation to account for the difference in replication time.

CRITICAL THINKING QUESTIONS

1. Mutants are used to study various aspects of the phenotype and genotype. How can we study genes that are critically important in the functioning of an organism? For example, how do we study mutations in the gene for DNA polymerase III in *E. coli,* when changes in this gene are usually lethal? Remember, to study the genes in bacteria, we need the bacteria to grow and form colonies in order to be scored for their phenotypes.

2. DNA and RNA differ in two major ways: DNA has deoxyribose sugar, whereas RNA has ribose, and DNA has thymine, whereas RNA has uracil. Why might those differences exist other than accidents of evolution?

Suggested Readings for chapter 9 are on page B-5.

10

GENE EXPRESSION

Transcription

STUDY OBJECTIVES

1. To examine the types of RNA and their roles in gene expression 245, 256
2. To look at the process of transcription, including start and stop signals, in both prokaryotes and eukaryotes 246
3. To investigate posttranscriptional changes in eukaryotic messenger RNAs, including an analysis of intron removal 260

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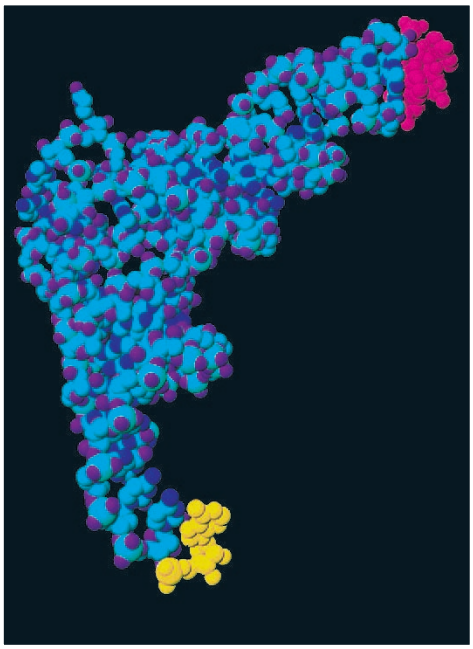


A computer model of the serine transfer RNA. The amino acid binding site is *yellow;* the anticodon is *red.* (© Ken Eward/SPL/Photo Researchers.)

In this chapter, we continue our study of genetics at the molecular level. After discussing the structure of DNA and the way in which it replicates in the last chapter, we turn our attention here and in the next chapter to the way in which the genetic material—primarily DNA—expresses itself. In this chapter, we concentrate on the conversion of DNA information into RNA information, the first step in gene expression. In the next chapter, we look at the conversion of RNA information into proteins. Later chapters discuss the control of these processes. We begin with prokaryotes and later in the chapter discuss the conceptually similar but functionally more complex process in eukaryotes.

All living things synthesize proteins. In fact, the types of proteins that a cell synthesizes determine the kind of cell it is. Hence, the genetic material must determine the types and quantities of proteins a cell synthesizes. Proteins (polypeptides) are made up of strings of amino acids (three hundred to five hundred, on average) joined together by peptide bonds. (We cover protein structure and synthesis in chapter 11.) Each protein contains a unique combination of only twenty amino acids. The amino acid sequence is specified by the sequence of nucleotides in DNA or RNA. In all prokaryotes, eukaryotes, and DNA viruses, the gene is a sequence of nucleotides in DNA that codes for the sequence of RNA. That RNA then determines which amino acids are included in a polypeptide. RNA usually serves as an intermediary between DNA and proteins. (In RNA viruses, the RNA may serve as a template for the eventual synthesis of DNA, or the RNA may serve as genetic material without DNA ever being formed. We will consider these cases at the end of the chapter.)

In 1958, Francis Crick originally described the flow of genetic information as the **central dogma:** DNA transfers information to RNA, which then directly controls protein synthesis (fig. 10.1). DNA also controls its own replication. **Transcription** is the process of synthesizing RNA from a DNA template using the rules of complementarity—the DNA information is rewritten, but in the same nucleotide language. RNA controls the synthesis of proteins in a process called **translation** because the information in the language of nucleotides is *translated* into information in the language of amino acids.

In the previous chapter, we introduced the idea of proteins that recognize specific DNA sequences and bind to those sequences. Specifically, we introduced the initiator proteins that bind to *oriC* and the proteins that bind to the terminator sequences. DNA polymerases and some of the other proteins involved in DNA replication bind to DNA, but they do not necessarily bind to any specific sequences. Proteins that recognize specific DNA sequences are critically important to the transcriptional process. In the next chapter, we spend more time on proteins, discussing their structures and how they are synthesized. It is sufficient to say here that specific proteins recognize specific DNA sequences. They do so by interdigitating the amino acid side chains of the proteins into the grooves of the DNA, thereby recognizing specific sequences by hydrogen bonding and other electrostatic interactions between the side chains of the amino acids of the proteins and the bases of the DNA (fig. 10.2). Proteins can have parts that recognize DNA sequences and parts that recognize other proteins or that perform other enzymatic activities such as hydrolyzing ATP.

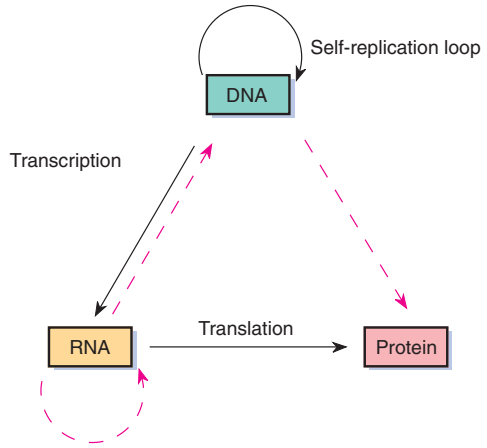
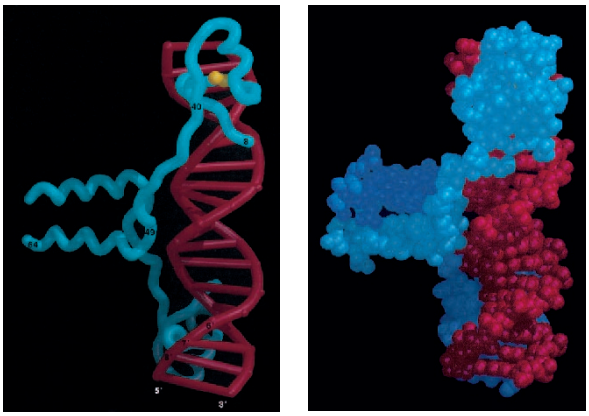


Figure 10.1 Crick's original central dogma depicted the flow of genetic information. Dashed *red* lines indicate the possible information transfers unconfirmed in 1958, when Crick proposed the central dogma.



(a) (b)

Figure 10.2 Computer model of the interaction of a yeast transcriptional factor, GAL4 (*blue*), and a seventeen-base-pair region of DNA (*red*). Zinc ions are in *yellow.* The protein is a dimer; only the DNA recognition region and associated part are shown. Part (*b*) is a space-filling model of part (*a*). (Reprinted with permission from *Nature,* 2 April 1992, Vol. 356, p. 411, fig. 3b,c. Copyright 1992 Macmillan Magazines Limited.)

TYPES OF RNA

In the protein synthesis process, three different kinds of RNA serve in three different roles. The first type is **messenger RNA (mRNA),** which carries the DNA sequence information to particles in the cytoplasm known as **ribosomes,** where the messenger RNA is translated. The second type is **transfer RNA (tRNA),** which brings the amino acids to the ribosomes, where protein synthesis takes place. The third type of RNA is a structural and functional part of the ribosome called **ribosomal RNA (rRNA).** The general relationship of the roles of these three types of RNA is diagrammed in figure 10.3. In addition, small RNAs play other roles in cellular metabolism, some of which are described later in the chapter.

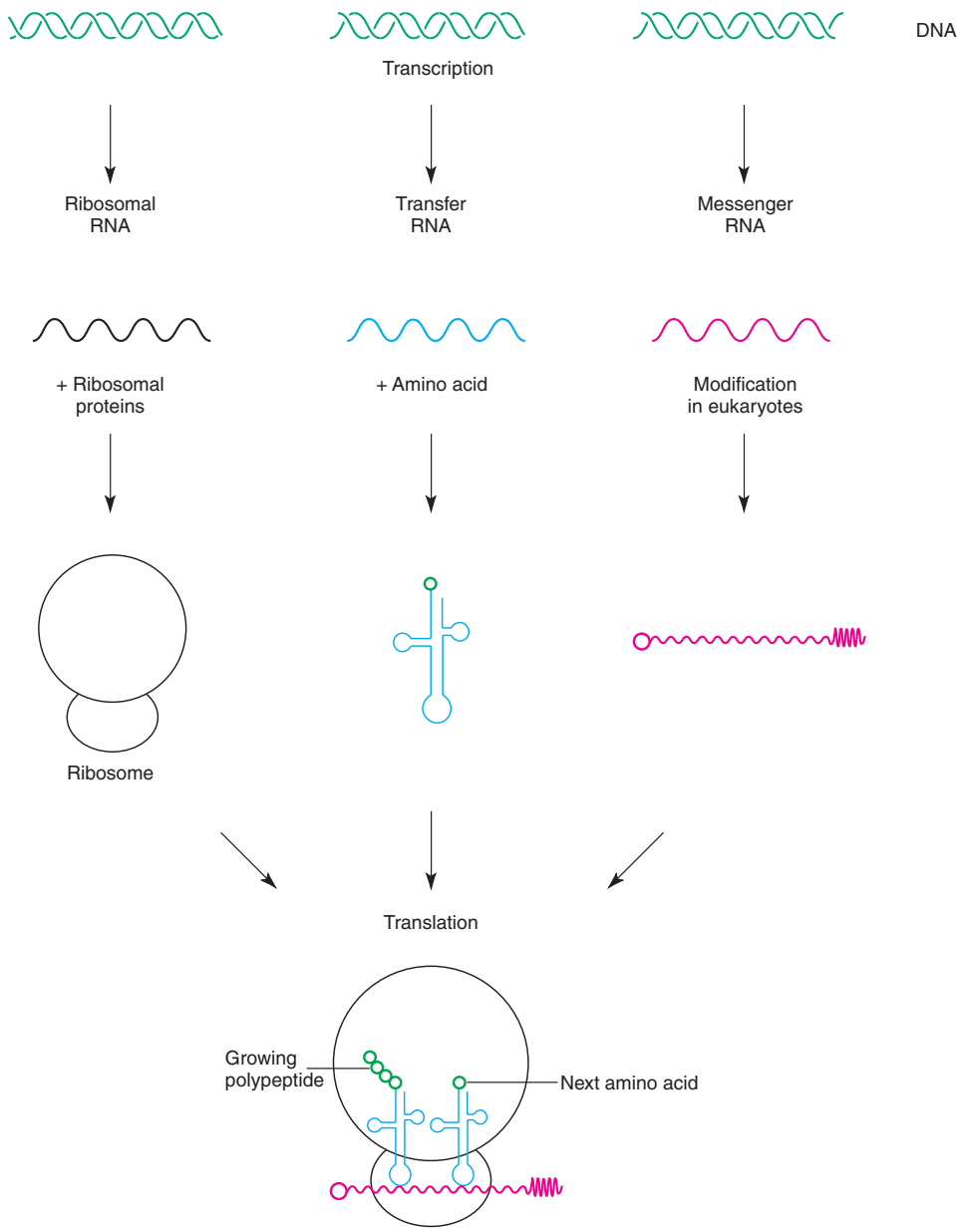


Figure 10.3 Relationship among the three types of RNA—ribosomal, transfer, and messenger—during protein synthesis. All three types are found together at the ribosome during protein synthesis.

We know that DNA does not take part directly in protein synthesis because, in eukaryotes, translation occurs in the cytoplasm, whereas DNA remains in the nucleus. We suspected for a long time that the genetic intermediate in prokaryotes and eukaryotes was RNA because the cytoplasmic RNA concentration increases with increasing protein synthesis, and the cytoplasmic RNAs carry nucleotide sequences complementary to the cell's DNA. Proof of an RNA intermediate came when it was shown that messenger RNA directs protein synthesis.

PROKARYOTIC DNA TRANSCRIPTION

DNA-RNA Complementarity

What proof do we have that a messenger RNA exists? That is, what proof convinced geneticists that gene-sized RNAs (not transfer RNAs or ribosomal RNAs) were found in the cytoplasm that were complementary to the DNA in the nucleus? At least two lines of evidence exist. First, it was shown that the RNAs produced by various organisms have base ratios very similar to the base ratios in the same organisms' DNA (table 10.1). The second line of evidence comes from experiments by B. Hall, S. Spiegelman, and others using **DNA-RNA hybridization.** This technique denatures DNA by heating, which causes the two strands of the double helix to separate. When the solution cools, a certain proportion of the DNA strands rejoin and rewind—that is, complementary strands "find" each other and re-form double helices. When RNA is added to the denatured DNA solution and the solution is cooled slowly, some of the RNA forms double helices with the DNA if the RNA fragments are complementary to a section of the DNA (fig. 10.4). The existence of extensive complementarity between DNA and RNA is a persuasive indication that DNA acts as a template for complementary RNA.

In another experiment, DNA-RNA hybridization showed that bacteriophage infection led to the production of phage-specific messenger RNA. Gene-sized pieces of RNA extracted from *Escherichia coli* before and after bacteriophage T2 infection were tested to see if they hybridized with the DNA of the T2 phage or with the DNA of the *E. coli* cell. The RNA in the *E. coli* cell was found to hybridize with the *E. coli* DNA before infection but with the T2 DNA after infection. Thus it is apparent that when the phage attacks the *E. coli* cell, it starts to manufacture RNA complementary to its own DNA and stops the *E. coli* DNA from serving as a template.

Having reached the conclusion that RNA is transcribed (synthesized) from a DNA template and then directs protein synthesis, we look at two questions. First, is this RNA single- or double-stranded? Second, is it synthesized (transcribed) from one or both strands of the parental DNA?

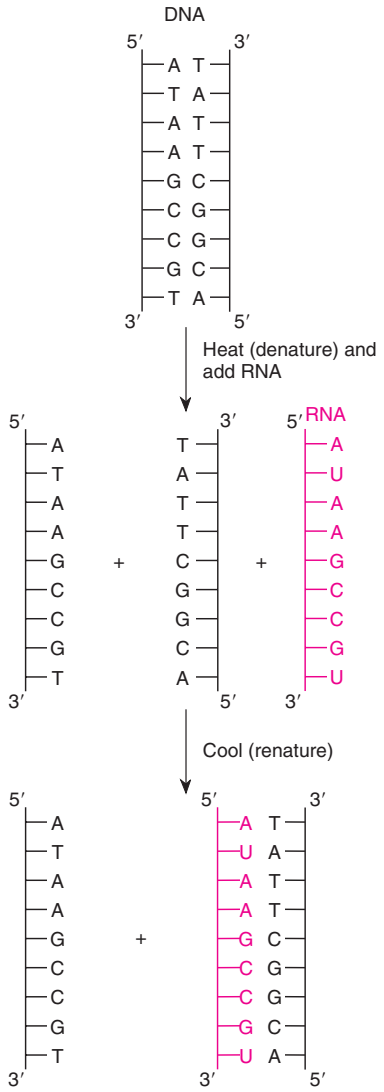


Figure 10.4 DNA-RNA hybridization occurs between DNA and complementary RNA.

Table 10.1 **Correspondence of Base Ratios Between DNA and RNA of the Same Species**

	RNA % G + C	DNA % G + C
E. coli	52	51
T2 phage	35	35
Calf thymus gland	40	43

For the most part, cellular RNA does not exist as a double helix. It can form double helical sections when complementary parts come into apposition (e.g., see fig. 10.16), but its general form is not a double helix. The simplest, and most convincing, evidence for this is that complementary RNA bases do not occur in corresponding proportions (Chargaff's ratios). That is, in RNA, uracil does not usually occur in the same quantity as adenine, nor does cytosine occur in the same quantity as guanine (table 10.2).

The answer to the second question is that RNA is not usually copied from both strands of any given segment of the DNA double helix, although rare exceptions do occur. Consider a sequence of nucleotides on one strand of a DNA duplex that specifies a sequence of amino acids for a protein, with the complementary nucleotide sequence also specifying the amino acid sequence for another functional protein. Since most enzymes are three hundred to five hundred amino acids long, the virtual impossibility of this task is obvious. It was, therefore, assumed *a priori* that, for any particular gene—that is, in any particular segment of DNA—the sequence on only one strand is transcribed and its complementary sequence is not. There is now considerable evidence to support this assumption.

The most impressive evidence that only one DNA strand transcribes RNA comes from work done with bacteriophage SP8, which attacks *Bacillus subtilis.* This phage has an interesting property—a great disparity in the purine-pyrimidine ratio of the two strands of its DNA. The disparity is significant enough that the two strands can be separated by density using density-gradient centrifugation. After denaturation and separation of the two strands, DNA-RNA hybridization can be carried out separately on each of the two strands with the RNA produced after the virus infects the bacterium. J. Marmur and his colleagues found that hybridization occurred only between the RNA and the heavier of the two DNA strands. Thus, only the heavy strand acted as a template for the production of RNA during the infection process.

The idea that only one strand of DNA serves as a transcription template for RNA has also been verified for several other small phages. However, when we get to larger viruses and cells, we find that either of the strands may be transcribed, but only one strand is used as a template in any one region. This was clearly shown in phage T4 of *E. coli,* where certain RNAs hybridize with one DNA strand, and other RNAs hybridize with the other. Let us now look at the transcription process in prokaryotes, then proceed to examine the three types of RNA in detail, and finally look at transcription in eukaryotes.

Table 10.2 Base Composition in RNA (percentage)

	Adenine	Uracil	Guanine	Cytosine
E. coli	24	22	32	22
Euglena	26	19	31	24
Poliovirus	30	25	25	20

Prokaryotic RNA Polymerase

In prokaryotes, transcription of RNA is controlled by **RNA polymerase.** Using DNA as a template, this enzyme polymerizes ribonucleoside triphosphates (RNA nucleotides). The complete RNA polymerase enzyme of *E. coli*—the holoenzyme—is composed of a core enzyme and a **sigma factor.** The core enzyme is composed of four subunits: α (two copies), β, and β'; this core is the component of the holoenzyme that actually carries out polymerization. The sigma factor is involved in recognizing transcription start signals on the DNA. Following the initiation of transcription, the sigma factor disassociates from the core enzyme.

Logically, transcription should not be a continuous process like DNA replication. If there were no control of protein synthesis, all the cells of a higher organism would be identical, and a bacterial cell would be producing all of its proteins all of the time. Since some enzymes depend on substrates not present all of the time, and since some reactions in a cell occur less frequently than others, the cell—be it a bacterium or a human liver cell—needs to regulate its protein synthesis. One of the most efficient ways for a cell to exert the necessary control over protein synthesis is to perform transcription selectively. Transcription of nongenic regions or of genes coding for unneeded enzymes is wasteful. Therefore, RNA polymerase should be selective. It should use as transcription templates only those DNA segments (genes or small groups of genes) whose products the cell needs at that particular time.

The mechanisms of transcriptional control need to be examined in two ways. First, we need to understand how the beginnings and ends of transcribable sections (a single gene or a series of adjacent genes) are demarcated. Second, we need to understand how the cell can selectively repress or enhance transcription of certain of these transcribable sections. The latter issues—the keys to bacterial efficiency and eukaryotic growth and development—are covered in chapters 14 and 16, respectively.

RNA polymerase must be able to recognize both the beginnings and the ends of genes (or gene groups) on the DNA double helix in order to initiate and terminate transcription. It must also be able to recognize the correct DNA strand to avoid transcribing the DNA strand that is not informational. RNA polymerase accomplishes those tasks by recognizing certain start and stop signals in DNA, called initiation and termination sequences, respectively.

Prokaryotic Initiation and Termination Signals for Transcription

The DNA region that RNA polymerase associates with immediately before beginning transcription is known as the **promoter.** The promoter is an important part of gene expression in both prokaryotes and eukaryotes. Promoters contain the information for transcription initiation and are the major sites in which gene expression is controlled.

Without the sigma factor, the core enzyme of RNA polymerase binds randomly along the DNA. Formation of the holoenzyme brings about high affinity of RNA polymerase for DNA sequences in the promoter region. Termination of transcription comes about when the polymerase enzyme recognizes a DNA region known as a **terminator sequence.** Let us elaborate on the various stages of transcription (in this section and in boxes 10.1 and 10.2).

Promoters

The RNA polymerase molecule covers a region of about sixty base pairs of DNA. This was determined by causing the polymerase to bind to DNA and then digesting the mixture with nucleases, in a technique known as **footprinting** (fig. 10.5). The polymerase "protects" or prevents degradation of the region it covers. The undigested DNA is then isolated and its size determined. Geneticists have gained much new information about the nature of recognition regions within promoters through recombinant DNA technology and nucleotide sequencing techniques (see chapter 13). Sequencing of numerous promoters has shown that they contain common sequences. If the promoter nucleotide sequences align with each other, and each has exactly the same series of nucleotides in a given segment, we say that the sequence of that segment comprises an invariant or **conserved sequence.** If, however, there is some variation in the sequence, but certain nucleotides occur at a high frequency (significantly greater than by chance), we refer to those nucleotides as making up a **consensus sequence.** Surrounding a point in prokaryotic promoters about ten nucleotides before the first transcribed base is just such a consensus sequence—TATAAT. This sequence is known as a **Pribnow box** after one of its discoverers (fig. 10.6).

The nucleotides in the Pribnow box are mostly adenines and thymines, so the region is primarily held together by only two hydrogen bonds per base pair. Since local DNA denaturation occurs during transcription by RNA polymerase (the DNA is opened to allow transcription), fewer hydrogen bonds make this process easier energetically. When the polymerase is bound at the promoter region (fig. 10.6), it is in position to begin polymerization six to eight nucleotides down from the Pribnow box.

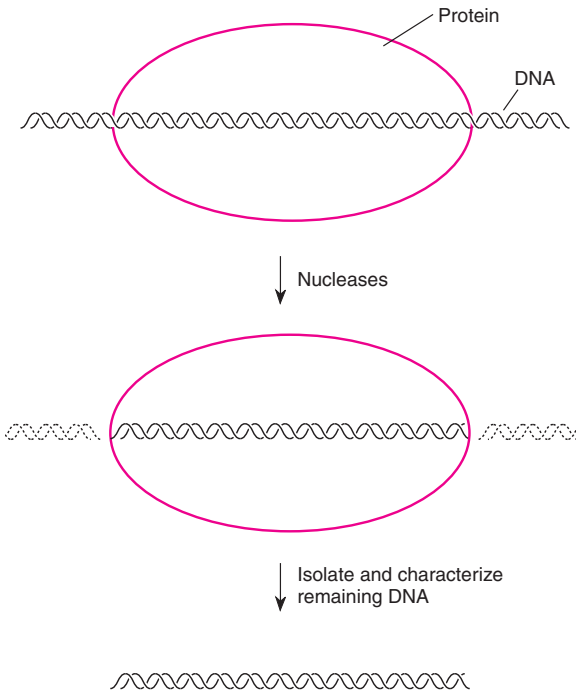


Figure 10.5 Footprinting technique. DNA in contact with a protein (e.g., RNA polymerase) is protected from nuclease degradation. The protected DNA is then isolated and characterized.

Figure 10.6 Nucleotide sequences of the promoter region and the first base transcribed from several different genes. Lambda (λ), T7, and ϕ×174 are bacteriophages. *Lac* is an *E. coli* gene, and SV40 is an animal virus. Only the SV40 promoter has the actual consensus sequence of TATAAT. Even when other sequenced promoters not shown here are Included, no base is found 100% of the time (conserved).

The sequences shown in figure 10.6 are those of the **coding strand** of DNA. It is a general convention to show the coding strand because both that strand and messenger RNA have the same sequences, substituting U for T in RNA; they are both complementary to the same **template strand** (also referred to as the **anticoding strand** or **noncoding strand;** fig. 10.7). Another convention is to indicate the first base transcribed by number +1 and to use positive numbers to count farther down the DNA in the **downstream** direction of transcription. If transcription is proceeding to the right, the direction to the left is called **upstream,** with bases indicated by negative numbers (fig. 10.8). Under this convention, the Pribnow box is often referred to as the −10 sequence.

Figure 10.8 also indicates another region with similar sequences among many promoters centered near −35 and referred to as the −35 sequence. The consensus sequence at −35 is TTGTCA. Mutation studies have attempted to determine the relative roles of the −10 and −35 sequences in transcription. In other words, mutations of bases in the −10 and −35 regions were examined to determine how they affected transcription initiation. The conclusions from these studies are that both regions contribute to the efficiency of polymerase binding. In other words, the more each sequence differs from the consensus sequence, the less frequently that promoter initiates transcription. The sigma factor recognizes both the −35 and the −10 sequences. The sigma factor is also sensitive to the spacing between these sequences, preferring (being most efficient at) seventeen base pairs.

Farther upstream from the −35 sequence is a recognition element in bacterial promoters that are very strongly expressed, such as the ribosomal RNA genes (fig. 10.8). This **upstream element,** or **UP element,** is about twenty base pairs long, is centered at −50, and is rich in A and T. By mutational studies, it has been shown that adding this element to promoters that don't normally have it greatly increases the rate of transcription. There are other recognition sites in prokaryotes, both upstream and downstream, at which various proteins attach that can enhance or inhibit transcription by direct contact with the polymerase (the α and σ subunits). We discuss these in chapter 14 under control of transcription. They are not part of what we think of as the core promoter, the DNA sequence needed for efficient binding of RNA polymerase.

Since the holoenzyme recognizes consensus sequences in a promoter, it is not surprising that some promoters are bound more efficiently than others or that different sigma factors exist within a cell. In *E. coli,* the major sigma factor is a protein of 70,000 daltons, referred to as σ^{70}. (One dalton is an atomic mass of 1.0000, approximately equal to the mass of a hydrogen atom.) The existence of about five less common sigma factors provides the cell with a mechanism for transcribing different genes under different circumstances. For example, in an *E. coli* cell subjected to elevated temperatures, a group of new proteins, referred to as **heat shock proteins,** appear, acting to protect the cell to some extent against the elevated temperatures. These proteins all appear at once because they have promoters that a different sigma factor recognizes, one with a molecular weight of 32,000 daltons (σ^{32}); this new sigma factor is produced by the cell after

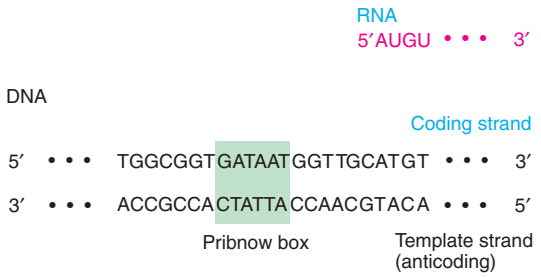


Figure 10.7 The template (anticoding) strand of DNA is complementary to both the coding strand and the transcribed RNA. The sequences are from the promoter of the λP_R region (see fig. 10.6).

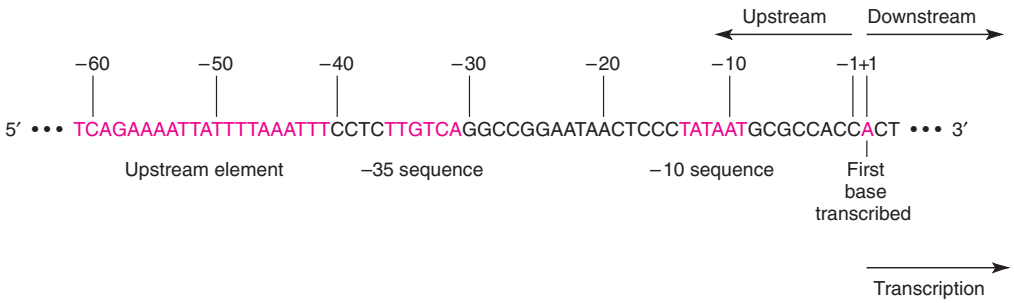


Figure 10.8 Promoter of the *Escherichia coli* ribosomal RNA gene, *rrnB.* Note the −10 and −35 sequences and the upstream element. The first base transcribed (the transcriptional start site) is noted (+1), as well as the upstream, downstream, and transcription directions. (Data from W. Ross, et al., 1993. *Science* 262:1407.)

BOX 10.1

Experimental Methods

Observing Transcription in Real Time

The overwhelming evidence that molecular events, such as transcription, take place comes from genetic and biochemical analyses and occasionally an electron micrograph of one type or another (fig. 1). Thus, it is refreshing and illuminating to be able to observe some of the processes we know are taking place in real time; that is, to sit at a microscope and actually see these events happen. Such a study on transcription was published in 1991 in *Nature* by four scientists at Washington University in St. Louis.

Although new methods of microscopy are being developed, normally we cannot see these molecular events taking place; the components are too small. Making them visible in electron microscopes usually requires fixation that destroys the ability of the components to actually continue their tasks. The Washington University group overcame this by attaching a gold particle to DNA, thus rendering the motion of that DNA visible under the light microscope (fig. 2). The scientists immobilized the RNA polymerase to a glass coverslip; thus, as transcription took place, the DNA moved and the length of the tether of the gold particle increased. At first they stopped the process by limiting the concentration of nucleoside triphosphates (NTPs). They

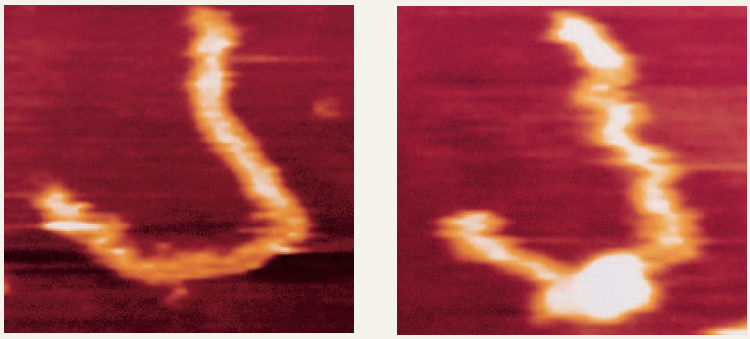


Figure 1 Visualizing transcription. Image of DNA before (*a*) and after (*b*) *E. coli* RNA polymerase (bright oblong object in *b*) binds to a promoter. Pictures are by scanning force microscopy, a new laser technique that images molecules in water. Image sizes are 300 by 300 nm. *Dark brown* represents substrate level; the highest point is *white* at about 10 nm high. Intermediate colors represent intermediate heights. (Courtesy of Martin Guthold and Carlos Bustamante, Institute of Molecular Biology and HHMI, University of Oregon.)

heat shock. We discuss heat shock proteins and other systems of transcriptional control in chapters 14 and 16.

From mutational studies of promoters and the proteins in the RNA polymerase holoenzyme, we now have a picture of a holoenzyme that sets down on a DNA promoter because the sigma factor recognizes the −10 and −35 elements, the α proteins recognize the UP element, and the α and σ subunits recognize proteins bound to various other upstream elements, when present (fig. 10.9*a*). This initiation complex is initially referred to as a closed complex because the DNA has not melted, which is the next step in transcription initiation (fig. 10.9*b*). After the transcription of 5–10 bases, the sigma factor is released (fig. 10.9*c* and *d*).

About seventeen base pairs of DNA are opened, and as transcription proceeds, about twelve bases of RNA form a DNA-RNA duplex at the point of transcription. Some of this information comes from studies with potassium permanganate ($KMnO_4$), which modifies DNA bases that are single-stranded but not double-stranded. Thus, the lengths of melted DNA can be determined experimentally. Also used is the technique of **photocrosslinking,** in which two moieties such as DNA and one or two proteins are caused to be permanently crosslinked, verifying their close contact. This is done by attaching a chemical crosslinking element to one of the moieties and then causing crosslinking to occur by shining light, usually ultraviolet, on the mixture.

Transcription, like DNA replication, always proceeds in the 5′ → 3′ direction. That is, a single base is added *de novo* and then new RNA nucleotides are added to the 3′-OH free end, as in DNA replication. However, unlike

could then observe the motion of the gold ball when no transcription was taking place. The scientists predicted that an immobilized gold ball would not move, and a tethered gold ball would show a limited amount of Brownian motion. That is, it would show a limited amount of blur in light microscope video images averaged over time. However, as soon as NTPs were added, any tethered gold ball would show an increased blur as it moved out of the field of vision and eventually would be released when transcription was completed. That is exactly what they saw (fig. 3). Thus, they succeeded in watching transcription take place in real time.

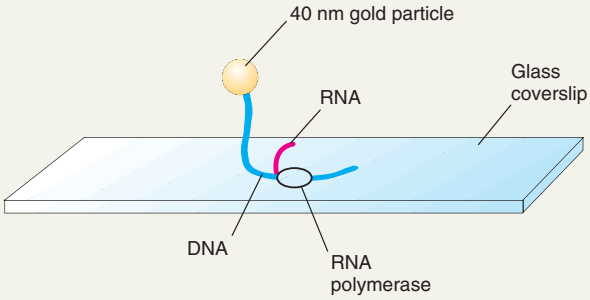


Figure 2 An experimental design in visualizing transcription in real time under the light microscope. Here, an RNA polymerase is immobilized on a coverslip, waiting for nucleoside triphosphates (NTPs) to be added. The gold particle is tethered by the DNA, allowing us to keep track visually of the end of the DNA.

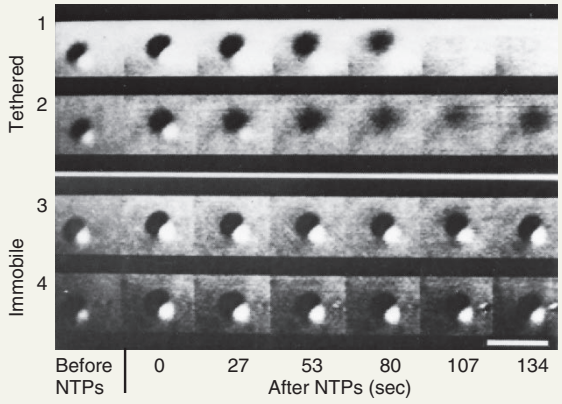


Figure 3 Enhanced light microscope images of the gold particles. In *3* and *4,* presumably immobilized particles show no change in focus over time. In *1* and *2,* Brownian motion—and hence blur—increases through time consistent with the lengthening of the tether (transcription). The particle in *1* was released 87 seconds (and in *2,* 135 seconds) after NTPs were added. Scale bar is 1 μm. (Transcription by single molecules of RNA polymerase observed by light microscopy. Robert Landick, Department of Biology, Washington University, St. Louis, MO.)

DNA polymerase, prokaryotic RNA polymerase does not seem to proofread as it proceeds. That is, RNA polymerase evidently does not verify the complementarity of the new bases added to the growing RNA strand. This deficiency is not serious; since many messenger RNAs are short-lived and many copies are made from actively transcribed genes, an occasional mistake will probably not produce permanent or overwhelming damage. If a particular RNA is not functional, a new one will be made soon. Evolutionarily speaking, it seems that it is more important to make RNA quickly than to proofread each RNA made.

Terminators

Transcription continues as RNA polymerase adds nucleotides to the growing RNA strand according to the rules of complementarity (C, G, A, and U of RNA pairing with G, C, T, and A of DNA, respectively). The polymerase moves down the DNA until the RNA polymerase reaches a stop signal, or terminator sequence. Two types of terminators, rho-dependent and rho-independent, differ in their dependency on the **rho protein** (Greek letter ρ). The functional form of rho is a hexamer, six identical copies of the protein. **Rho-independent terminators** cause termination of transcription even if rho is not present. **Rho-dependent terminators** require the rho protein; without it, RNA polymerase continues to transcribe past the terminator in a process known as **read-through.** Both types of terminators sequenced so far have one thing in common: They include a sequence and its inverted form separated by another short sequence, all together forming an **inverted-repeat sequence.** The

BOX 10.2

Experimental Methods

Polymerase Collisions: What Can a Cell Do?

Both RNA polymerase and DNA polymerase move along the DNA of a cell during a cell cycle. The DNA polymerase moves along at about ten times the speed of the transcribing enzyme. Since many genes are usually active in a cell, the interaction (collision) of the two enzymes is inevitable. What happens when this collision takes place? What does the cell do? Although we cannot directly observe these interactions, various bits of data suggest that a head-on collision could be fatal to the cell, and certain patterns of gene placement minimize the chance of a head-on collision.

B. Brewer first analyzed the problem of the coexistence of these two enzymes in a paper published in 1988 in the journal *Cell.* In evolutionary terms, the cell could obviate the problems of a head-on collision by either avoiding them or resolving them. Resolution would entail some sort of right-of-way settlement when the two enzymes met; for example, the RNA polymerase could drop off the DNA when a confrontation takes place. The cell might avoid confrontations if the genes are oriented so that transcription occurs for the most part in the same direction as DNA replication. That is, DNA replication begins at *oriC,* with Y-junctions proceeding to the left and the right until they meet 180 degrees later. Thus, to avoid head-on collisions, genes on the left and right arcs of the bacterial chromosome could be transcribed away from the origin of replication (fig. 1).

Brewer analyzed the orientation of genes on the *E. coli* chromosome; more recently, D. Zeigler and D. Dean did the same for the chromosome of *Bacillus subtilis.* In *B. subtilis,* 95% (91 of 96) of the genes analyzed were in the proper orientation to avoid a head-on collision of polymerases. Among the exceptions were sporulation genes, genes that would not be transcribed during DNA synthesis and whose orientation is thus not relevant to DNA polymerase activity. In *E. coli,* Brewer found that, overall, 74% (375 of 501) of the genes she looked at were oriented to avoid head-on collisions. Brewer's data were more impressive when she broke them down according to transcription function and activity.

For genes that transcribe very actively most of the time, the orientation is about 90% in the "safe" direction. For regulatory genes that are transcribed only very rarely, the orientation is random (50% safe). For other genes, the orientation was 72% in the safe direction. Thus, an organization clearly exists within the bacterial chromosome that helps to avoid head-on collisions of the two polymerases.

Brewer also provided evidence that a head-on collision between polymerases could be fatal to the cell. Studies selected inversions of the *E. coli* chromosome to see the effects of collision. (Inversions are regions that have been cut out and put back in the opposite orientation.) It was impossible to isolate inversion mutations that changed the orientation of genes in respect to *oriC.* Thus, it appears that a cell may not be able to resolve a head-on collision of polymerases and that evolution has solved the problem by having gene transcription generally oriented in the same direction as DNA replication.

More amazingly, Alberts and his colleagues recently studied what happens when a replication fork catches up to a stalled RNA polymerase. Not only does the replication fork pass the transcription apparatus, but the RNA polymerase can resume transcription after the replication fork passes without loss of the transcript. Although there are contrary observations in other systems, it appears that gene orientation and the behavior of polymerases allow cells to survive with both replication and transcription occurring on the same DNA.

terminator in figure 10.10 has the sequence AAAGGCTCC, 5′ → 3′, from both the left on the coding strand and from the right on the template strand. A four-base-pair sequence separates the inverted repeats. Inverted repeats can form a **stem-loop structure** by pairing complementary bases within the transcribed messenger RNA.

Both rho-dependent and rho-independent terminators have the stem-loop structure in RNA just before the last base transcribed. Rho-independent terminators, as figure 10.10 shows, also have a sequence of thymine-containing nucleotides after the inverted repeat, whereas rho-dependent terminators do not. Although the exact sequence of events at the terminator is not fully known, it appears that the RNA stem-loop structure forms and causes the RNA polymerase to pause just after completing it. This pause may then allow termination under two different circumstances.

In rho-independent terminators, the pause may occur just after the sequence of uracils is transcribed (fig. 10.11). Uracil-adenine base pairs have two hydrogen bonds and are thus less stable thermodynamically than guanine-cytosine base pairs. Perhaps during the pause, the uracil-adenine base pairs spontaneously denature, releasing the transcribed RNA and the RNA polymerase,

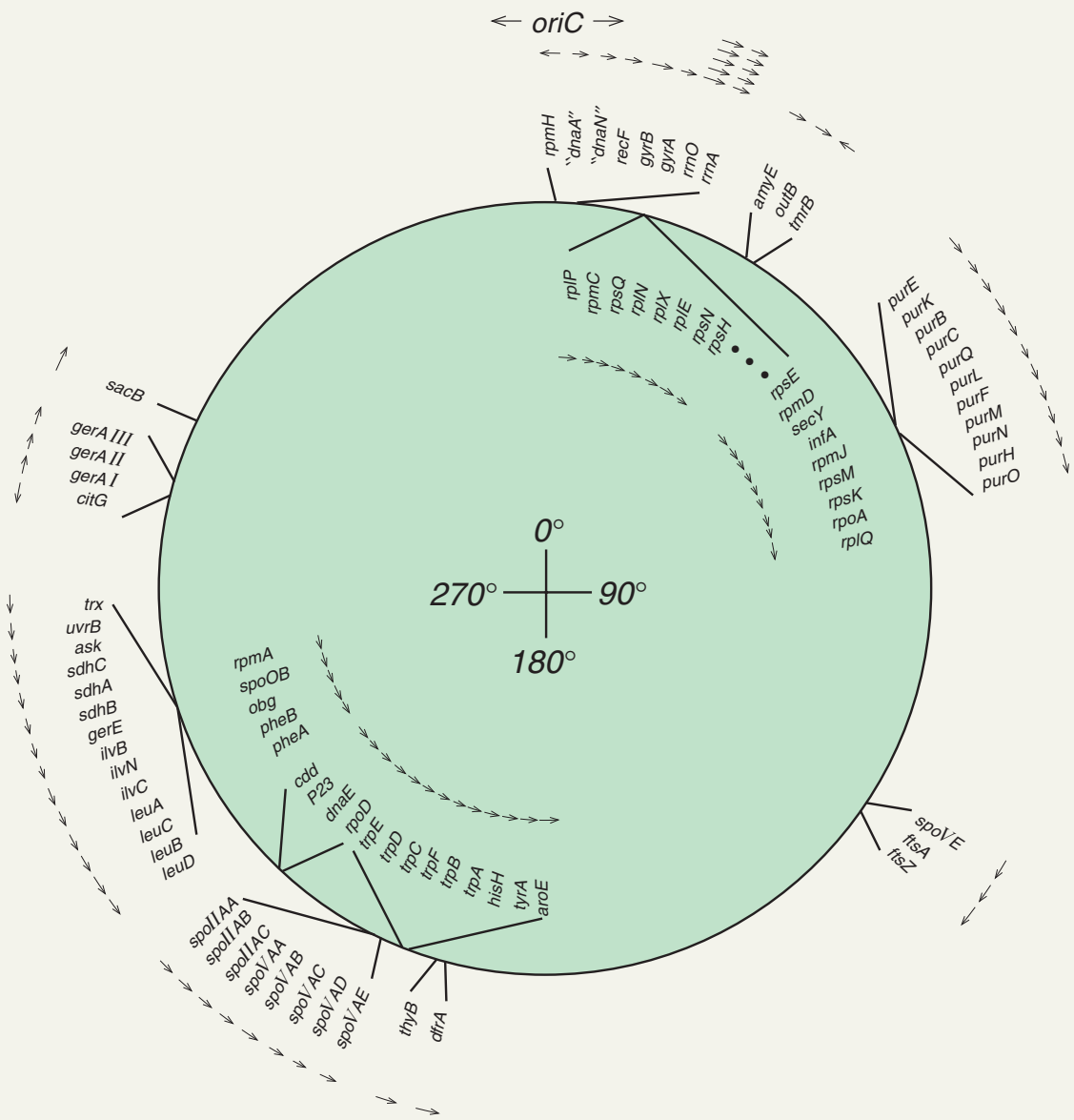


Figure 1 Location and orientation of gene transcription on the chromosome of *Bacillus subtilis* (*arrows*). DNA replication begins at *oriC* and terminates approximately 180 degrees from the origin of replication. Note that the overwhelming number of arrows point away from the origin of replication toward the termination point. (From D. R. Zeigler and D. H. Dean, "Orientation of genes in the *Bacillus subtilis* chromosome," *Genetics,* 125:703–8. Copyright © 1990 Genetics Society of America.)

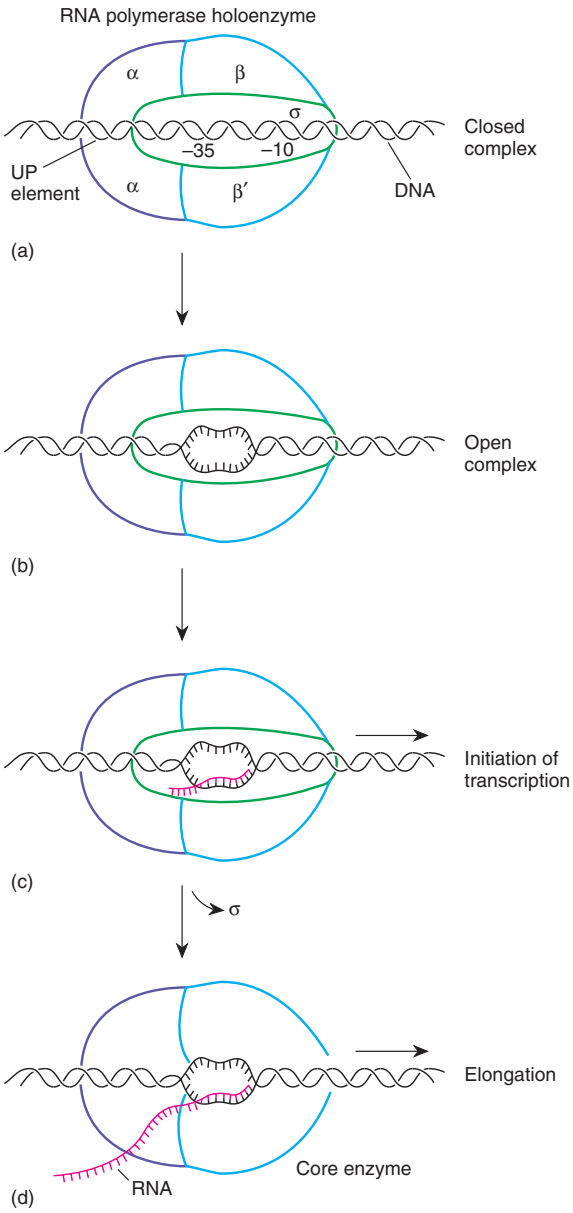


Figure 10.9 Transcription begins after RNA polymerase attaches to the promoter, with specificity imparted by the sigma factor. The DNA opens to form the open complex, transcription begins, the sigma factor leaves, and elongation commences.

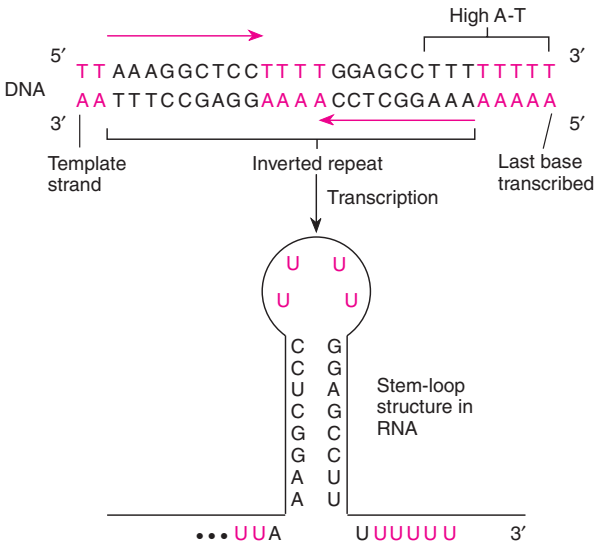


Figure 10.10 An inverted-repeat base sequence characterizes terminator regions of DNA. Stem-loop structures can occur as the RNA forms because of complementary sequences. The 3′ poly-U tail indicates a rho-independent terminator.

terminating the process, and making the polymerase available for further transcription of other promoters.

Rho-dependent terminators do not have the uracil sequence after the stem-loop structure. Here, termination depends on the action of rho, which appears to bind to the newly forming RNA. In an ATP-dependent process, rho travels along the RNA at a speed comparable to the transcription process itself (fig. 10.11). Possibly, when RNA polymerase pauses at the stem-loop structure, rho catches up to the polymerase and unwinds the DNA-RNA hybrid, leting the DNA, RNA, and polymerase fall free. Rho can do this because it has DNA-RNA helicase (unwinding) properties.

The process of transcription termination is probably more complex than described. Significant interactions may take place with other proteins, and particular sequences surrounding the termination sequence may also be significant in the termination process. This is an area of active research.

Figure 10.12 shows an overview of transcription. The information of a gene, coded in the sequence of nucleotides in the DNA, has been transcribed into a complementary sequence of nucleotides in the RNA. This RNA transcript contains a complement of the template strand of the gene's DNA and thus acts as a messenger from the gene to the cell's protein-synthesizing complex. The transcript contains nucleotide sequences that will be translated into amino acids—coding segments—as well as noncoding segments before and after. The translatable segment, or gene, almost always begins with a three-base sequence, AUG, which is known as an initiator codon, and ends with one of the three-base sequences, UAA, UAG, or UGA, known as nonsense codons. (We discuss these signals in chapter 11.)

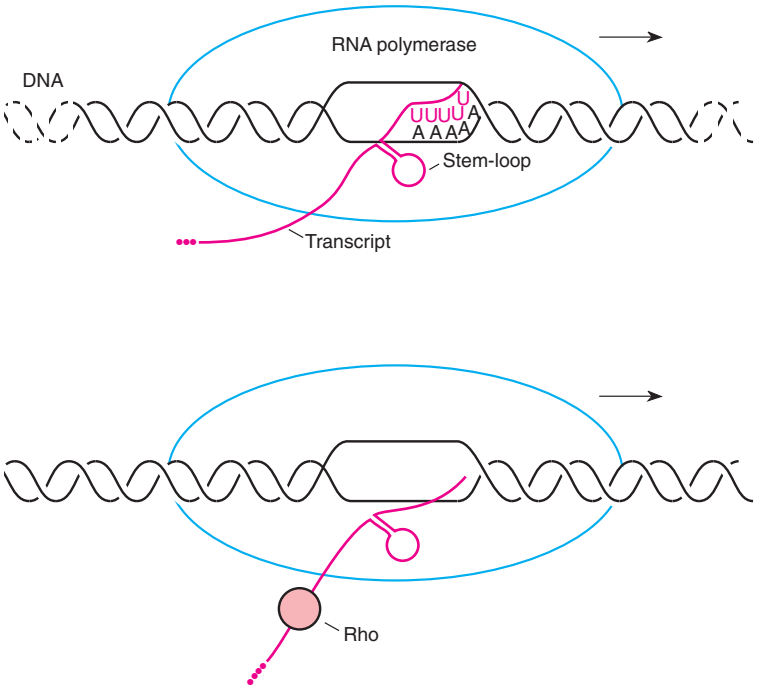


Figure 10.11 Rho-independent (*top*) and rho-dependent (*bottom*) termination of transcription are preceded by a pause of the RNA polymerase at a terminator sequence. Presumably, the stem-loop structure in the nascent RNA causes the pause in both cases.

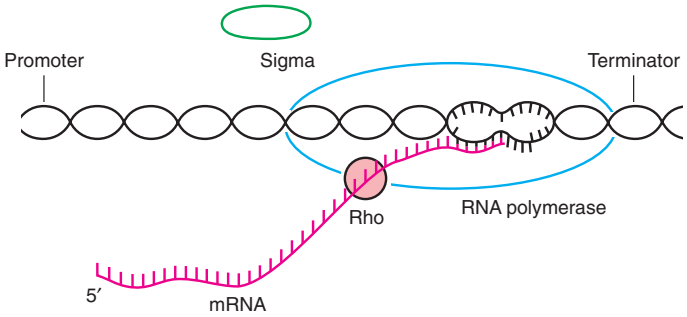


Figure 10.12 Transcription overview and RNA polymerase molecules. RNA polymerase is transcribing near the terminator. The rho factor—actually made up of six subunits—is shown on the newly formed RNA. The sigma factor is shown nearby, detached from the core polymerase.

The portion of the RNA transcript that begins at the start of transcription and goes to the translation initiator codon (AUG) is referred to as a **leader,** or 5′ untranslated sequence. The length of RNA from the nonsense codon (UAA, UAG, or UGA) to the last nucleotide transcribed is the **trailer,** or 3′ untranslated sequence. These sequences play a role in recognizing messenger RNA and ensuring its structural stability at the ribosome during the process of translation; the leader region can also have regulatory functions (see chapter 14). Figure 10.13 diagrams a complete prokaryotic RNA transcript. In this simplified drawing, the transcript has only one gene (AUG → UAA). However, the average prokaryotic transcript contains the information for several genes. We will say more about the parts of a transcript later in this chapter and the next. Now we turn our attention to the types of transcripts: ribosomal, transfer, and messenger RNA.

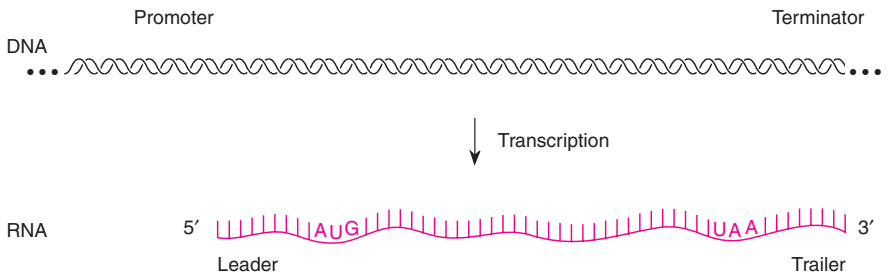


Figure 10.13 Transcribed piece of prokaryotic RNA and its DNA template region. Note the promoter and terminator regions on the DNA and the leader and trailer regions on the RNA. The initiation (AUG) and nonsense (UAA) codons for protein synthesis are shown. These signals are read at the ribosome at the time of translation.

RIBOSOMES AND RIBOSOMAL RNA

Ribosomes are organelles in the cell, composed of proteins and RNA (ribosomal RNA, or rRNA), where protein synthesis occurs. In a rapidly growing *E. coli* cell, ribosomes can make up as much as 25% of the mass of the cell. Ribosomes, as well as other small particles and molecules, are measured in units that describe their rate of sedimentation during density-gradient centrifugation in sucrose. This technique gives information on size and shape (due to the speed of sedimentation) while simultaneously isolating the molecules. Isolation by centrifugation in sucrose is a relatively gentle isolation technique; the molecules still retain their biological properties and can be used for further experimentation. In the 1920s, physical chemist T. Svedberg developed ultracentrifugation, giving his name to the unit of sedimentation: the **Svedberg unit,** S.

In sucrose density-gradient centrifugation, the gradient is formed by layering on decreasingly concentrated sucrose solutions. In a related technique, cesium chloride density-gradient centrifugation, mentioned in chapter 9, the gradient develops during centrifugation. The sucrose centrifugation is stopped after a fixed time, whereas in the cesium chloride technique, the system spins until it reaches equilibrium. The sucrose method tends to be more rapid. Samples can be isolated from a sucrose gradient by punching a hole in the bottom of the tube and collecting the drops in sequentially numbered containers. The first (lowest-numbered) containers will contain the heaviest molecules (with the highest S values).

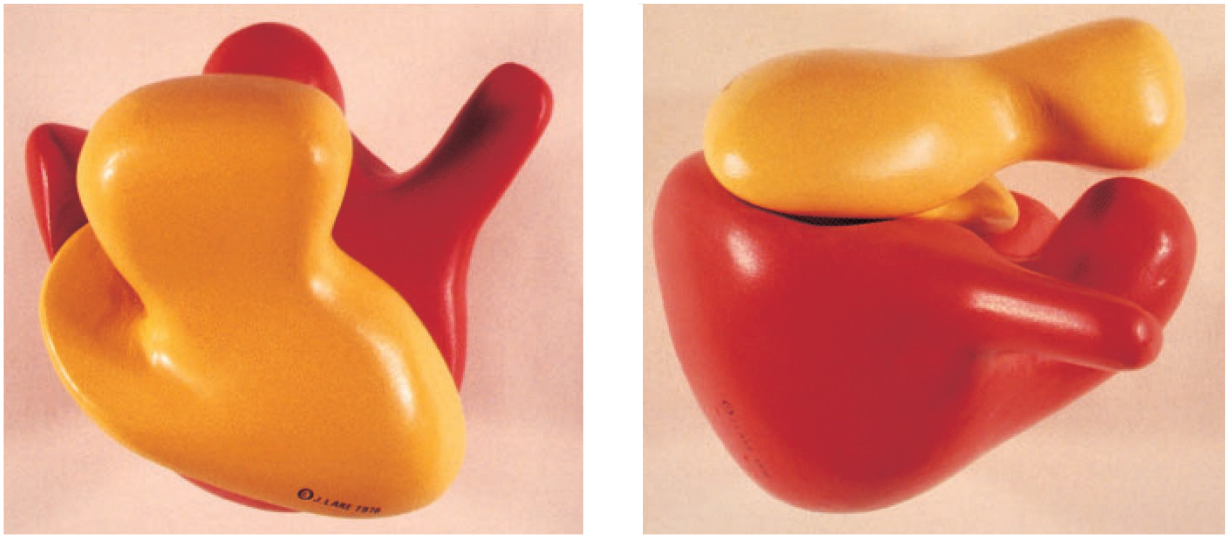
Ribosomes in all organisms are made of two subunits of unequal size. The sedimentation value is 50S (Svedberg units) for the large one in *E. coli* and 30S for the smaller one. Together they sediment at about 70S. Eukaryotic ribosomes vary from 55S to 66S in animals and 70S to 80S in fungi and higher plants. Most of our discussion will be confined to the well-studied ribosomes of *E. coli.*

Each ribosomal subunit comprises one or two pieces of ribosomal RNA and a fixed number of proteins. The 30S subunit of *E. coli* has twenty-one proteins and a 16S molecule of ribosomal RNA, and the 50S subunit has thirty-four proteins and two pieces of ribosomal RNA—one 23S and one 5S section (fig. 10.14). Advances in understanding ribosomal structure have come about after protein chemists isolated and purified all the proteins of the ribosome. This allowed researchers to experiment on the proper sequence needed to assemble the subunits and also allowed them to develop immunological techniques to show the positions of many proteins in the completed ribosomal subunits.

In *E. coli,* all three ribosomal RNA segments are transcribed as a single long piece of RNA that is then cleaved and modified to form the final three pieces of RNA (16S, 23S, and 5S). The region of DNA that contains the three ribosomal RNA molecules also contains genes for four transfer RNAs (fig. 10.15). There appear to be about five to ten copies of this region in each chromosome of *E. coli.* The occurrence of the three ribosomal RNA segments on the same piece of RNA ensures a final ratio of 1:1:1, the ratio needed for ribosomal construction.

TRANSFER RNA

During protein synthesis (see fig. 10.3), a messenger RNA, carrying the information transcribed from the gene (DNA), is bound to the ribosome. Amino acids are brought to the ribosome attached to transfer RNAs. The code is read in sequences of three nucleotides, called **codons.** The nucleotides of the codon on messenger RNA are complementary to and pair with a sequence of three bases—the **anticodon**—on a transfer RNA. Each different transfer RNA carries a specific amino acid. Thus, the transfer RNA recognizes the specificity of the genetic code (fig. 10.16).



(a) **30S**
Twenty-one proteins
One 16S rRNA

(b) **50S**
Thirty-four proteins
One 23S rRNA
One 5S rRNA

Figure 10.14 The *E. coli* ribosome. (*a*) and (*b*) show models of the 70S ribosome of *E. coli,* revealing the relationship of the small (*yellow*) and large (*red*) subunits at the time of translation. The 30S ribosomal subunit is composed of twenty-one proteins and one 16S piece of ribosomal RNA. The 50S subunit is composed of thirty-four proteins and two pieces of ribosomal RNA, 23S and 5S. ([*a* and *b*] James A. Lake, *Journal of Molecular Biology* 105 (1976):131–59. Reproduced by permission of Academic Press.)

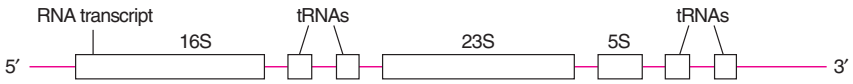


Figure 10.15 The *E. coli* transcript that contains the three ribosomal RNA segments also contains four tRNAs and some spacer RNA (*red*) that separates the tRNA and rRNA genes.

The correct amino acid is attached to its transfer RNA by one of a group of enzymes called **aminoacyl-tRNA synthetases.** One specific aminoacyl synthetase exists for every amino acid, but the synthetase may recognize more than one transfer RNA because there are more transfer RNAs (and codons) than there are amino acids. (In chapter 11 we discuss the genetic code in more detail.) R. W. Holley, a Nobel laureate, and his colleagues were the first to discover the nucleotide sequence of a transfer RNA; in 1964, they published the structure of the alanine transfer RNA in yeast (fig. 10.17). The average transfer RNA is about eighty nucleotides long.

Similarities of All Transfer RNAs

Transfer RNAs have several unusual properties. For one, all the different transfer RNAs of a cell have the same general shape; when purified, the heterogeneous mixture of all of a cell's transfer RNAs can form very regular crystals. The regularity of the shape of transfer RNAs makes sense. During the process of protein synthesis, two transfer RNAs attach next to each other on a ribosome, and a peptide bond forms between their amino acids. Thus, any two transfer RNAs must have the same general dimensions as well as similar structures so that they can be recognized and positioned correctly at the ribosome.

An obvious feature of the transfer RNA in figure 10.17 is that it has **unusual bases.** When this transfer RNA is originally transcribed from DNA, it is about 50% longer than the final eighty nucleotides. In fact, some transcripts contain two copies of the same transfer RNA, or sometimes several different transfer RNA genes are part of the same transcript (see fig. 10.15). The original transcription of transfer RNAs is completely regular: It does not involve unusual bases. The transcript is then processed down to the final size of a transfer RNA by various nucleases that remove trailing and leading pieces of RNA. In eukaryotes, a CCA sequence of nucleotides is added at the 3′ end by a nucleotidyl

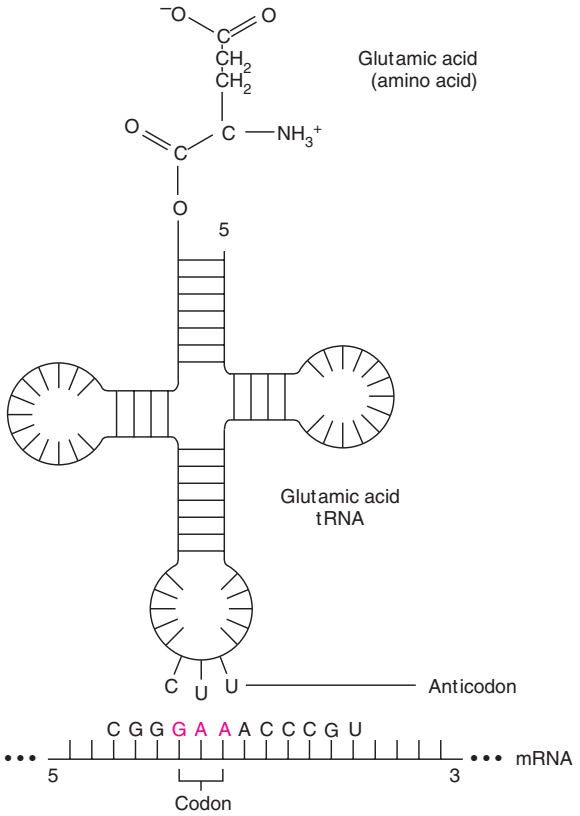


Figure 10.16 Specificity of the genetic code manifests itself in the transfer RNA, in which a particular anticodon is associated with a particular amino acid. In this case, glutamic acid is attached to its proper transfer RNA, which has the anticodon CUU.

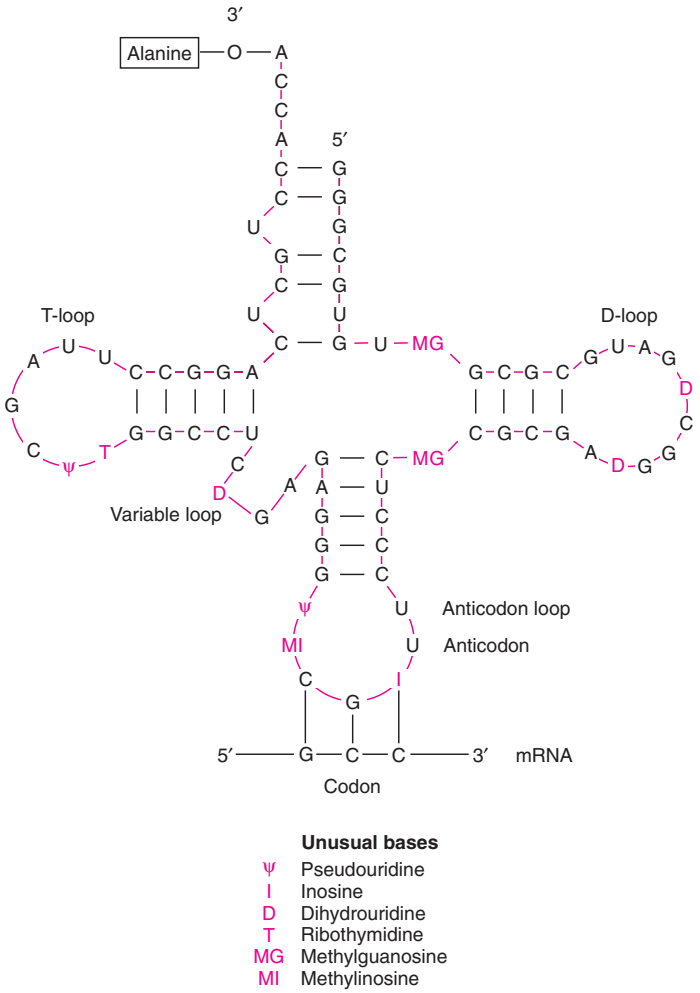


Figure 10.17 Structure and sequences of alanine transfer RNA in yeast. Note the modified bases in the loops. The anticodon of the transfer RNA is shown paired with its complementary codon in the DNA. (Source: Data from R. W. Holley, et al., "Structure of a ribonucleic acid," *Science,* 147:1462–65, 1965.)

transferase enzyme. Then the transfer RNA is further modified, frequently by the addition of methyl groups to the bases already in the RNA (fig. 10.18). Presumably, these unusual bases disrupt normal base pairing and are in part responsible for the loops the unpaired bases form (see fig. 10.17).

Transfer RNA Loops

It is believed that the first loop on the 3′ side (the T- or T-ψ-C-loop) is involved in making the transfer RNA recognizable to the ribosome. The ribosome must hold each transfer RNA in the proper orientation to check the complementarity of the anticodon of the transfer RNA and the codon of the messenger RNA. The center loop of transfer RNA is the anticodon loop. The aminoacyl-tRNA synthetases seem to recognize many points all over the transfer RNA molecule (see chapter 11).

The amino acid is attached to the ACC sequence on the 3′ end of the transfer RNA. The ribosome-binding loop on all transfer RNAs has the T-ψ-C-G sequence. The anticodon on all is bounded by uracil on the 5′ side and a purine on the 3′ side. Thus, there is a good deal of general similarity among all the transfer RNAs, consistent with the fact that they all enter protein synthesis in the same way. The actual shape of the functional transfer RNA in the cell is not an open cloverleaf, as shown in figure 10.17; rather, the whole molecule exhibits helical twisting due to pairing of complementary regions (fig. 10.19).

Earlier we considered a rough definition of a gene as a length of DNA that codes for one protein. But we have just encountered an inconsistency—genes code for both transfer RNAs and ribosomal RNAs, yet neither is eventu-

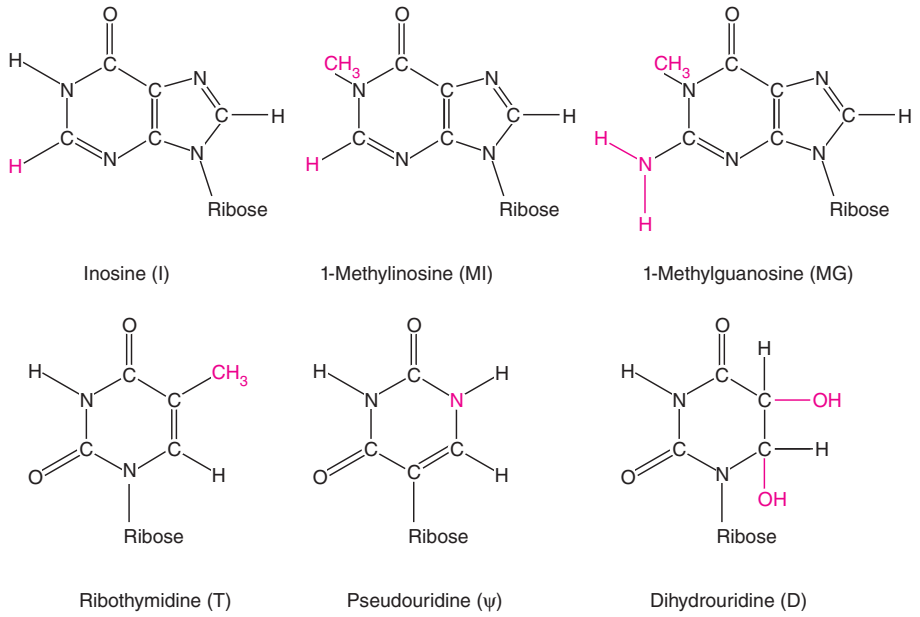


Figure 10.18 Structures of the modified bases found in alanine transfer RNA of yeast. The various modifications of normal bases are shown in *red.*

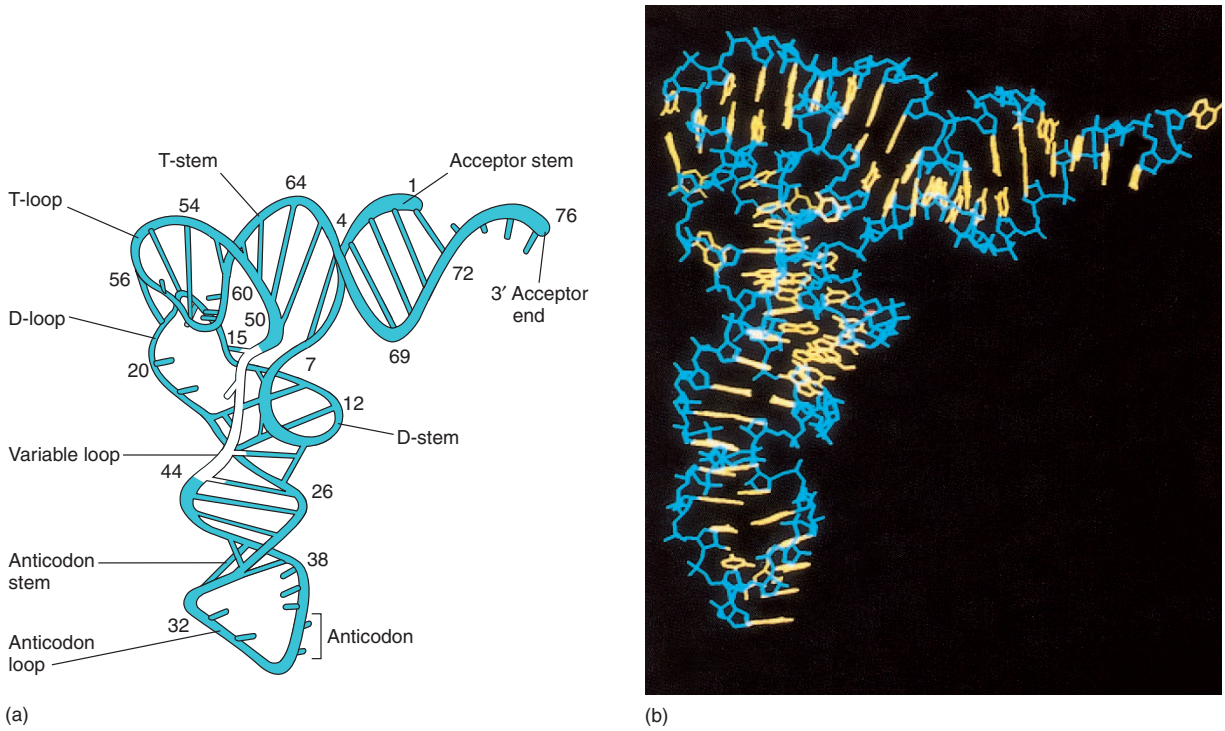


Figure 10.19 Structure of yeast phenylalanine transfer RNA. (*a*) A diagram showing coiling of the sugar-phosphate backbone. (*b*) A molecular model with bases in *yellow* and backbone in *blue.* The two parts of the figure (*a* and *b*) are in the same orientation.
([*b*] Courtesy of Alexander Rich.)

ally translated into a protein. Their transcripts function as final products without ever being translated. Thus, transfer RNA and ribosomal RNA are the major exceptions to the general rule that a gene codes for a protein.

EUKARYOTIC DNA TRANSCRIPTION

The Nucleolus in Eukaryotes

Eukaryotes have four segments of ribosomal RNA in the ribosome, compared with three in prokaryotes. The smaller ribosomal subunit has an 18S piece of RNA, and the larger subunit has 5S, 5.8S, and 28S segments. All but the 5S ribosomal RNA section are transcribed as part of the same piece of RNA. However, eukaryotic cells have many copies of these ribosomal RNA genes, depending on the species. For example, the fruit fly, *Drosophila melanogaster,* has about 130 copies of the DNA region that the larger segments of ribosomal RNA are transcribed from. These regions occur in tandem on the sex (X and Y) chromosomes and are known collectively as the nucleolar organizer (see chapter 3). The smallest ribosomal RNA subunit is also produced from a duplicated gene, but at a different point in the genome. For example, in *D. melanogaster,* the 5S subunit is produced on chromosome 2.

Eukaryotes—unlike prokaryotes, which have only one RNA polymerase—have three RNA polymerases. Eukaryotic RNA polymerase I (or polymerase A) transcribes only the nucleolar organizer DNA. RNA polymerase II (or polymerase B) transcribes most genes. RNA polymerase III (or polymerase C) transcribes small genes, primarily the 5S ribosomal RNA gene and transfer RNA genes (table 10.3). In addition, mitochondria, chloroplasts, and some phages have other RNA polymerases.

Table 10.3 **Prokaryotic and Eukaryotic RNA Polymerases**

Enzyme	Function
Prokaryotic	
RNA polymerase	Transcribes DNA template
Primase	Primer synthesis during DNA replication
Eukaryotic	
RNA polymerase I	Transcribes nucleolar organizer
RNA polymerase II	Transcribes most genes
RNA polymerase III	Transcribes 5S rRNA and tRNA genes
Primase	Primer synthesis during DNA replication

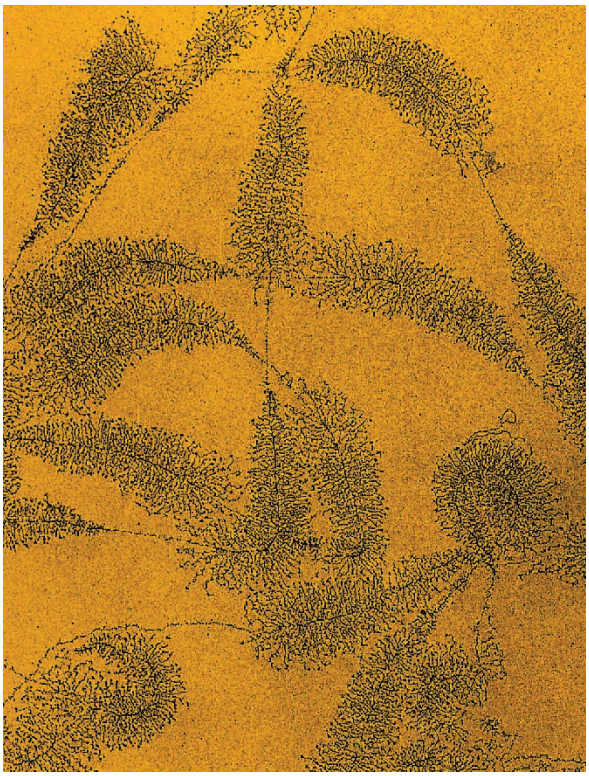


Figure 10.20 Transcription in the nucleolus of the newt, *Triturus.* Tandem repeats of the large ribosomal RNA genes are being transcribed. The polarity of the process (progressing from small to large transcripts), as well as the spacer DNA (*thin lines* between transcribing areas), is clearly visible. Magnification 18,000×. (© O. L. Miller, B. R. Beatty, D. W. Fawcett/Visuals Unlimited.)

At the nucleolar organizer, the nucleolus forms the familiar dark blob found in eukaryotic nuclei. The nucleolus is the place where ribosomes are assembled. The various ribosomal proteins that have been manufactured in the cytoplasm migrate to the nucleus and eventually to the nucleolus, where, with the final forms of the ribosomal RNAs, they are assembled into ribosomes.

In the nucleolar organizer, an untranscribed region of spacer DNA separates each repeat of the large ribosomal RNA gene. This is shown in figure 10.20 and diagrammed in figure 10.21. In the electron micrograph in figure 10.20, the polarity of transcription is evident from the short RNA at one end of the transcribing segment and the long RNA at the other end, with a uniform gradation between. Notice that many RNA polymerases are transcribing each region at the same time. The regions between the transcribed DNA segments are the spacer DNA regions.

Like transfer RNAs, ribosomal RNAs are also modified: some uridines are converted to pseudouridines, and some

ribose sugars are methylated. These conversions take place in the nucleolus, orchestrated by particles composed of small RNA segments and protein. The RNA segments are referred to as **small nucleolar RNAs (snoRNAs)** and, when combined with protein, are referred to as **small nucleolar ribonucleoprotein particles (snoRNPs).** Each different snoRNP has a snoRNA that is complementary to the regions surrounding the nucleotide to be modified. Thus, sites for modification are chosen based on complementarity to a snoRNA, which then somehow directs the modification to take place.

Differences Between Eukaryotic and Prokaryotic Transcription

Although all aspects of transcription differ to some extent between prokaryotes and eukaryotes, we will look at two major differences here: the coupling of transcription and translation that is possible in prokaryotes, and the extensive **posttranscriptional modifications** that occur in eukaryotic messenger RNA. In *E. coli,* translation of the newly transcribed messenger RNA into a protein can take place before transcription is complete (fig. 10.22). The messenger RNA is synthesized in the 5′ → 3′ direction, and it is

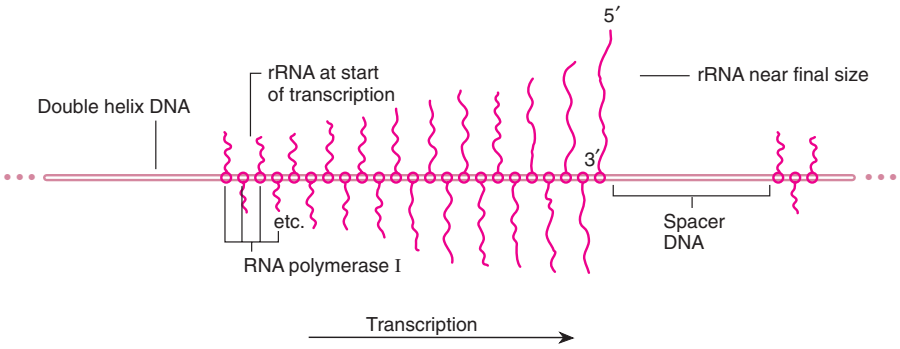
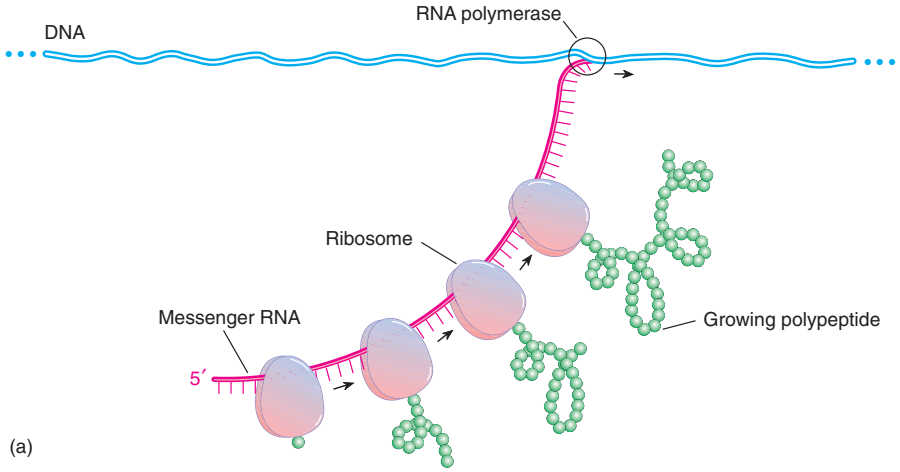


Figure 10.21 Details of the transcription of the large ribosomal RNA genes shown in figure 10.20. Note the polarity of the process and the spacer DNA, as seen in figure 10.20.



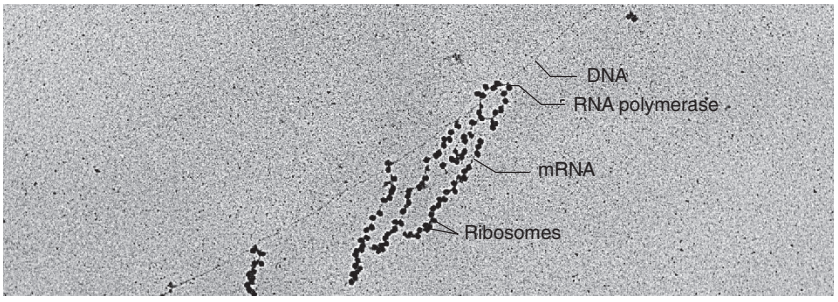


Figure 10.22 (*a*) In prokaryotes, translation of messenger RNA by ribosomes begins before transcription is complete. Ribosomes attach to the growing mRNA strand when the 5′ end becomes accessible. They then move along the RNA as it elongates. When the first ribosome moves from the 5′ end, a second ribosome can attach, and so on. (*b*) Electron micrograph of events diagrammed in (*a*). The growing polypeptides cannot be seen in this preparation. Magnification 44,000×.
([*b*] Courtesy of O. L. Miller, Jr.)

near the 5′ end that translation begins. As soon as the 5′ end of the RNA is available, a ribosome can attach to the messenger RNA and move along it in the 5′ → 3′ direction, lengthening the growing polypeptide as it moves. When the first ribosome moves away from the 5′ end of the transcript, a second ribosome can attach and begin translation. These processes are repetitive, as electron micrographs (fig. 10.22*b*) clearly show. In eukaryotes, however, messenger RNA is synthesized in the nucleus, but protein synthesis takes place in the cytoplasm. (This regional division of labor is not present in *E. coli* because, among other reasons, the bacterium has no nucleus.) Before a eukaryotic messenger RNA leaves the nucleus, it is highly modified by processes that generally do not occur in prokaryotes.

Promoters

Eukaryotic promoters are somewhat similar to prokaryotic promoters; both are regions of DNA at the beginnings of genes with signals that allow RNA polymerase to attach and begin transcription. In eukaryotes, however, more proteins are involved in promoter recognition, and more proteins are involved in the control of transcription, many recognizing signals thousands of base pairs away. We discuss these control processes in eukaryotes in chapter 16.

All three eukaryotic RNA polymerases (I, II, and III) recognize a seven-base sequence, TATAAAA, located at about −25 on the promoter DNA. It is similar to the −10 sequence in prokaryotes and is called the **TATA box** (or **Hogness box** after its discoverer, D. Hogness). Since RNA polymerase II transcribes most of the genes in eukaryotes, we turn our attention specifically to it.

Among the large number of promoters that have been sequenced, a few lack the TATA box, yet are still transcribed. Transcription initiation in these promoters appears to be controlled by a CT-rich area, called the **initiator element (Inr),** at +1 of the transcript (close to the transcription start site), coupled with a **downstream promoter element (DPE)** at about +28 to +34 of the transcript. In TATA-less promoters, a protein called TFIID requires both these elements to bind. The initiator element has a consensus sequence of TCA(G or T)T(T or C), and the downstream promoter element has the consensus sequence of (A or G)G(A or T)CGTG. We will concentrate on RNA polymerase II genes with TATA boxes.

Yeast RNA polymerase II is a protein of twelve subunits. This enzyme cannot locate promoters or attach to DNA in a stable fashion. To attach at the beginnings of genes, RNA polymerase II must interact with several proteins called **general transcription factors.** In eukaryotes, general transcription factors are named after the polymerase they work with. Thus, the transcription factor that recognizes the TATA box for polymerase II genes is called TFIID (D being the fourth letter of the alphabet for the fourth transcription factor so named). TFIID is composed of one

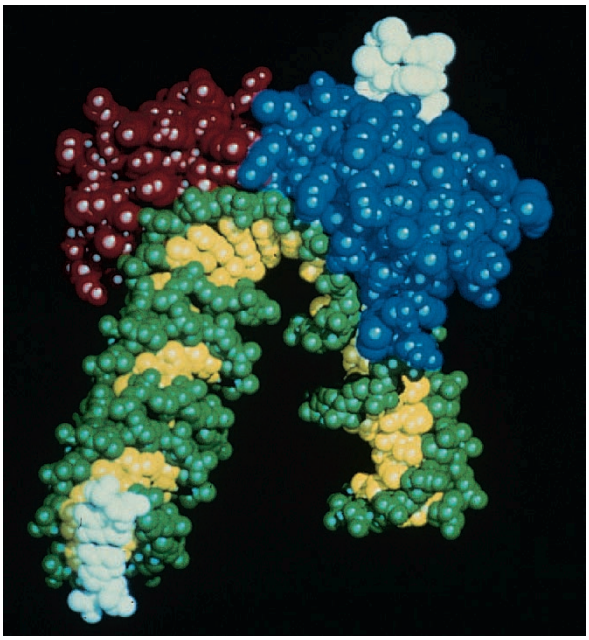


Figure 10.23 Molecular space-filling model of a yeast TATA-binding protein attached to a TATA box on the DNA. The DNA sugar-phosphate backbone is *green* and the bases are *yellow.* The protein has twofold symmetry (*red* and *blue*). Note the bending of the DNA through 80 degrees, which also opens up the minor groove of the DNA. The *upper white* atoms are the N-terminus of the TATA-binding protein; the *lower white* atoms are the first base pair at which transcription begins. (Courtesy of J. L. Kim and S. K. Burley. From J. L. Kim, J. H. Geiger, S. Hahn, and P. B. Sigler, "Crystal Structure of a Yeast TBP TATA-box Complex." *Nature* 365 (6446): 520–27, Oct. 7, 1993. © Macmillan Magazines, Ltd. Figure adapted from the work of S. K. Burley.)

subunit that recognizes the TATA sequence, called **TATA-binding protein (TBP),** and up to a dozen other proteins called **TBP-associated factors (TAFs),** which recognize the initiator element, when present, and aid in regulating transcription. TFIID is, in essence, similar to the sigma factors of prokaryotic RNA polymerase. One interesting aspect of the binding of TBP is that it causes a significant bending and opening of the DNA (fig. 10.23). This bending may be an important signal for other binding proteins.

Once TFIID binds to the TATA box, a cascade of recruitment (binding) of other transcription factors takes place. Transcription factors IIA, IIB, and IIF bind, as does RNA polymerase II in an unphosphorylated state. Then transcription factors IIE and IIH bind, forming a **preinitiation complex (PIC),** equivalent to the *E. coli* holoenzyme (fig. 10.24*a*). The RNA polymerase II is then phosphorylated, presumably by TFIIH, which is a kinase; at this point, most of the transcription factors drop off, leaving the **elongation complex,** which carries out a

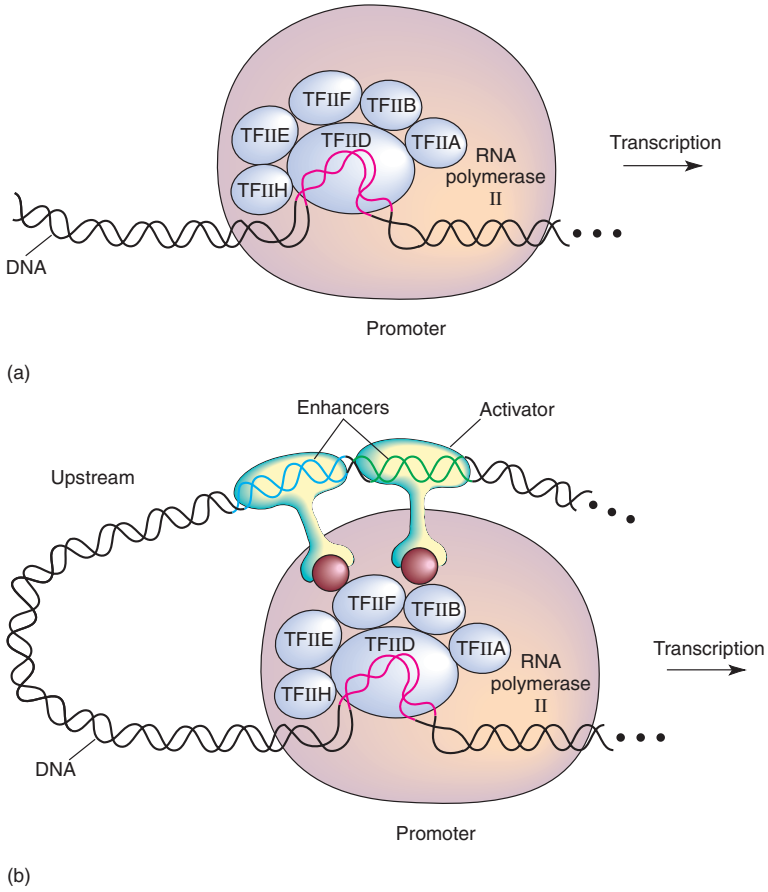


Figure 10.24 (*a*) An RNA polymerase II pre-initiation complex at a promoter. TFIID binds to the TATA box (*red*). The other transcription factors are then recruited with the polymerase. (*b*) Two activators (*yellow*) are shown bound at one end (their DNA domains) to enhancers (*blue* and *green*) upstream on the DNA. The activators are bound at their other ends (their transcriptional activation domains) to other proteins associated with the polymerase machinery. Phosphorylation of the polymerase initiates activated transcription.

Table 10.4 Putative Roles of the General Transcription Factors of RNA Polymerase II

General Transcription Factor	Function
TFIID, TBP	Recognizes TATA box
TFIID, TAFs	Recognizes initiator element and regulatory proteins
TFIIA	Stabilizes TFIID
TFIIB	Aids in start-site selection by RNA polymerase II
TFIIE	Controls TFIIH functions; enhances promoter melting
TFIIF	Destabilizes nonspecific interactions of RNA polymerase II and DNA
TFIIH	Melts promoter with helicase activity; activates RNA polymerase II with kinase activity

Source: Data from R. G. Roeder, "The Role of General Initiation Factors in Transcription by RNA Polymerase II" in *Trends in Biochemical Sciences,* 21:327–35, 1996.

basal rate of transcription (fig. 10.25). TFIIH also has a role here, since it is also a helicase. Table 10.4 summarizes the postulated roles of the general transcription factors.

For activated transcription, a high level of transcription, to take place, other factors are needed that are involved in controlling which promoters are actively transcribed. These other factors are **activators** or **specific transcription factors** that bind to DNA sequences called **enhancers.** Enhancers are often hundreds or thousands of base pairs upstream from the promoter (fig. 10.24*b*).

Note that much of this information has been gathered by footprinting, mutational studies, cloning and isolating the genes and proteins involved, and then reconstituting various purified combinations in the test tube. These studies are combined with kinetic research to determine which arrangements are stable, immunological research

to isolate various components with antibodies, and photocrosslinking studies to determine which moities are in contact with each other.

These specific transcriptional activators have domains (regions) that recognize their specific enhancer sequences, regions that recognize proteins associated with the polymerase (general transcription factors), and regions that allow the joint attachment of other transcription factors (fig. 10.24*b*). Similar to activators and enhancers, repressors can bind to silencer regions of DNA, often far upstream of the promoters, to repress transcription. Thus, many genes are associated with numerous and complex arrangements of transcription factors, providing elaborate control of transcription (see chapter 16).

For specific transcription factors to attach to both enhancers and the polymerase machinery, possibly thousands of base pairs apart, the DNA must bend to allow them to come into the range of the polymerase. Electron micrographs clearly show this DNA bending and looping (fig. 10.26).

Although RNA polymerases I and III seem to have termination signals similar to rho-independent promoters in prokaryotes, termination of transcription of RNA polymerase II genes is more complex, coupled with further processing of the mRNA.

Before we move on, several other points merit discussion. First, unlike prokaryotic RNA polymerases, eukaryotic RNA polymerases do proofread (showing $3' \rightarrow 5'$ exonuclease activity). Second, as we will discuss in chapter 15, eukaryotic DNA is complexed with histone proteins that can interfere with transcription. In turn, part of the RNA polymerase II complex is made up of proteins that can disrupt the histones bound to the DNA.

In addition, the RNA polymerase II complex contains proteins that act as mediators between activators and the polymerase holoenzyme. This complex coordination of the initiation of transcription in eukaryotes has been termed **combinatorial control;** the huge initiation complex may contain 85 or more different polypeptides.

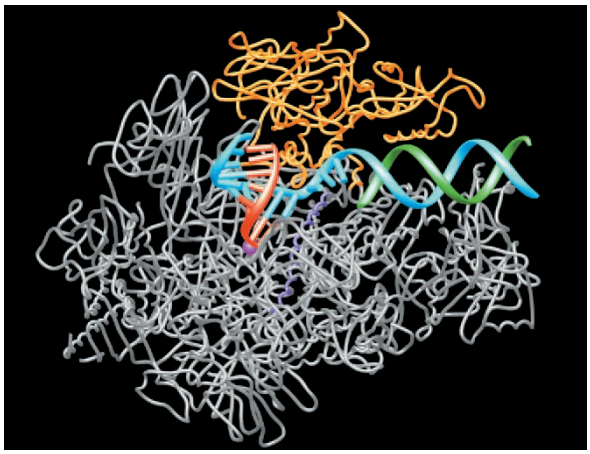
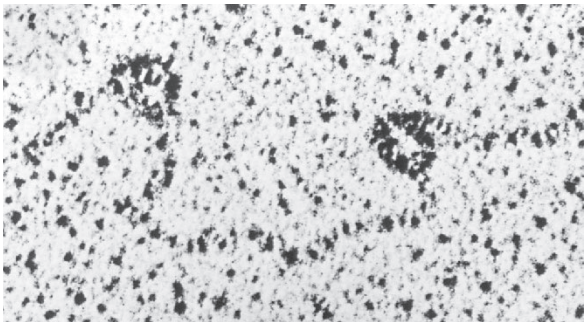
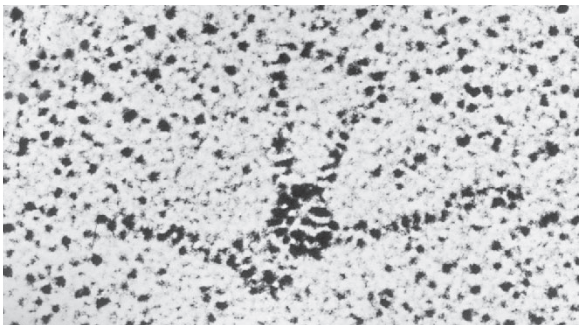


Figure 10.25 The RNA polymerase II elongation complex with part of the protein structure removed to show the DNA and RNA within the cleft of the protein. The DNA is *blue* (template strand) and *green* (nontemplate strand) with the RNA *red*. The majority of protein is shown as *gray*; the part in *yellow* is a domain that appears to open for DNA loading and is in a closed state during elongation, thus acting as a clamp on the DNA and RNA. Closure of the clamp allows for the high stability of transcribing complexes and thus for processivity of the polymerase. The *purple* part is a helix that crosses the major cleft of the enzyme. The DNA template strand is led over this helix towards the active site. The *pink* sphere is a magnesium ion in the active site, where RNA synthesis occurs. (P. Cramer, D. A. Bushnell and R. D. Kornberg. RNA polymerase II at 2.8A resolution and A. L. Gnatt, P. Cramer, J. Fu, D. A. Bushnell and R. D. Kornberg, Structure of an RNA polymerase II transcribing complex. Reprinted by permission of the authors.)



(a)



(b)

Figure 10.26 The interaction between an activator and RNA polymerase (in this case, in prokaryotes). (*a*) In this system, the RNA polymerase of *E. coli* (the more heavily stained sphere) is controlled by an activator called NtrC (the more lightly stained sphere). The activator is bound to an enhancer, and the polymerase is bound to the promoter. (*b*) The activator has bound to the polymerase, causing a looping of the DNA. Compare with figure 10.24. (Courtesy of Sydney Kustu.)

Finally, transcription in the archaea, although under much simpler control than in the eukaryotes, resembles transcription in eukaryotes rather than prokaryotes.

The study of the details of the transcription process—its initiation, control, and termination—is one of the most active and exciting areas in modern genetics.

Caps and Tails

Eukaryotic transcription results in a **primary transcript.** In contrast to most prokaryotic transcripts that contain information from several genes, virtually all transcripts from higher eukaryotes contain the information from just one gene. (Transcripts from several genes are found in some lower eukaryotes, such as nematode worms.) Three major changes occur in primary transcripts of RNA polymerase II before transport into the cytoplasm: modifications to the 5′ and 3′ ends and removal of intervening sequences. We refer to these changes as posttranscriptional modifications.

At the 5′ end of polymerase II transcripts, 7-methyl guanosine is added in the "wrong" direction, 5′ → 5′ (fig. 10.27). This **cap** allows the ribosome to recognize the beginning of a messenger RNA. At the other end, the 3′ end of polymerase II transcripts, a sequence of twenty to two hundred adenine-containing nucleotides, known as a **poly-A tail,** is added by the enzyme poly-A polymerase. Polyadenylation takes place after the 3′ end of the transcript is removed by a nuclease that cuts about twenty nucleotides downstream from the signal 5′-AAUAAA-3′. The tail adds stability to the molecule and aids in its transportation from the nucleus.

When messenger RNAs were first studied in eukaryotes, the messenger RNAs in the nucleus were found to be much larger than those in the cytoplasm and were called **heterogeneous nuclear mRNAs,** or **hnRNAs.** It now turns out that these were primary transcripts, RNAs that had not had any of the major posttranscriptional modifications. In essence, they were premessenger RNAs.

Introns

Eukaryotes have segments of DNA within genes that are transcribed into RNA but never translated into protein sequences. These **intervening sequences,** or **introns,** are removed from the RNA in the nucleus before its transport into the cytoplasm (fig. 10.28). P. Sharp and his colleagues at MIT and R. Roberts, T. Broker, L. Chow, and their colleagues at the Cold Spring Harbor Laboratory first discovered introns in 1977. (Sharp and Roberts were awarded 1993 Nobel prizes for their work.) An example of a gene with introns appears in figure 10.29. The segments of the gene between introns, which are transcribed and translated—and hence exported to the cytoplasm and expressed—are termed **exons.** The results of intron removal are clear when a messenger RNA with its introns removed is hybridized with the original gene (fig. 10.30). The DNA forms double-stranded structures with the exons in RNA. The introns in DNA have nothing to pair with in the RNA, so they form single-stranded loops. Introns also occur in eukaryotic transfer RNA and ribosomal RNA genes.

For introns to be removed, the ends of the exons must be brought together and connected in a process called *splicing.* At least two types of splicing occur, although they are related: self-splicing and protein-mediated splicing.

Self-Splicing

In 1982, Thomas Cech and his colleagues, building on the work of others, including Sidney Altman, who showed that RNA can have catalytic properties, discovered self-splicing by RNA. (Cech and Altman were awarded 1989

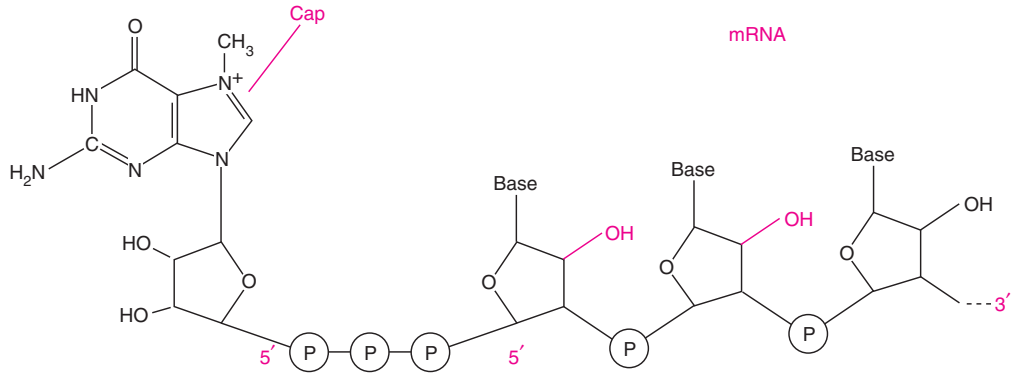


Figure 10.27 A cap of 7-methyl guanosine is added in the "wrong" direction (5′ → 5′), to the 5′ end of eukaryotic mRNAs. In some cases, the 2′ -OH groups on the second or second and third riboses (*red*) are methylated.

Richard J. Roberts (1943–).
(Courtesy of Richard J. Roberts.)

Philip A. Sharp (1944–).
(Courtesy of Dr. Philip A. Sharp.)

Thomas Broker (1944–).
(Courtesy of Dr. Thomas Broker.)

Louise T. Chow (1943–).
(Courtesy of Dr. Louise Chow.)

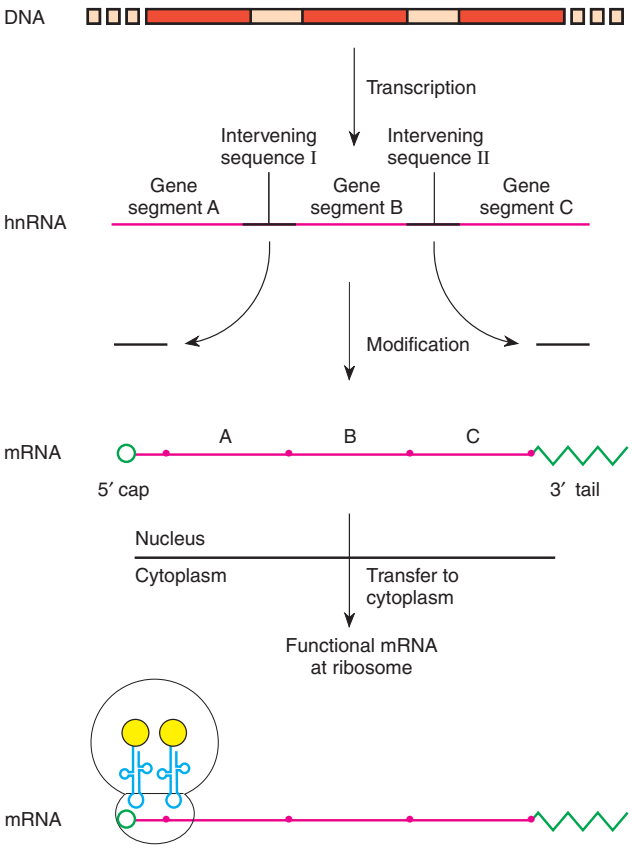


Figure 10.28 In eukaryotic DNA, intervening sequences, or introns, are removed from the RNA in the nucleus before the mRNA is transported into the cytoplasm and translated. Other modifications consist of splicing, 5′ capping, and 3′ polyadenylation.

Nobel prizes in chemistry.) Working with an intron in the 35S ribosomal RNA precursor in the ciliated protozoan, *Tetrahymena,* Cech and his colleagues found that they could induce intron removal in vitro with no proteins present. A guanine-containing nucleotide (GMP, GDP, or GTP) had to be present. Figure 10.31 diagrams how self-splicing occurs. The intron is acting as an enzyme; we call an RNA with enzymatic properties a **ribozyme.**

During self-splicing, the U-A bond at the left (5′) side of the intron is transferred to the GTP. The U that is now unbonded displaces the G at the right (3′) side of the intron, reconnecting the RNA with a U-U connection and releasing the intron (fig. 10.31). Since all bonds are reversible transfers (transesterifications) rather than new bonds, no external energy source is required. Self-splicing introns of this type are called **group I introns.** An extensive secondary structure (RNA stem-loops) that forms is also important in intron removal (box 10.3).

Although the first enzymatic activity of the ribozyme is its own removal, its secondary structure after removal gives it the ability to further catalyze reactions (fig. 10.32). The reactions that ribozymes catalyze are transesterifications and the hydrolysis reaction of splitting an RNA molecule into two parts. Ribozymes can also perform other functions, including peptide bond formation, covered in chapter 11. Currently, at least seven different classes of ribozymes are known, based on their enzymatic properties. A ribozyme that can split other RNAs and that occurs in small plant pathogens is called a **hammerhead ribozyme** (fig. 10.33) because of its shape. Because these RNA molecules are small, they have the potential to be modified in the laboratory for specific purposes related to clinical treatment and further study of RNA processing.

Self-splicing has also been found in genes in the mitochondria of yeast. These introns are referred to as **group II introns** because they use a different mechanism of splicing that does not require an external nucleotide. Instead, the first bond is transferred within the intron to an adenosine, forming a lariat structure (fig. 10.34). In order for the lariat to form, the ribose of the adenosine must make three phosphodiester bonds (fig. 10.35).

```
                10        20        30        40        50        60        70        cAp       90
  0  GGCCAATCTGCTCACACAGGATAGAGAGGGCAGGAGCCAGGCAGAGCATATAAGGTGAGGTAGGATCAGTTGCTCCTCACATTTGCTTCTGACATAGTTG

100  TGTTGACTCACAACCCCAGAAACAGACATCATGGTGCACCTGACTGATGCTGAGAAGGCTGCTGTCTCTTGCCTGTGGGGAAAGGTGAACTCCATGAAG
                                                 MetValHisLeuThrAspAlaGluLysAlaAlaValSerCysLeuTrpGlyLysValAsnSerAspGluV
200  TTGGTGGTGAGGCCCTGGGCAGGTTGGTATCCAGGTTACAAGGCAGCTCAAGAAGAAGTTGGGTGCTTGGAGACAGAGGTCTGCTTTCCAGCAGACAC
     alGlyGlyGluAlaLeuGlyArg  30
300  TAACTTTCAGTGTCCCCTGTCTATGTTTCCCTTTTTAGGCTGCTGGTTGTCTACCCTTGGACCCAGCGGTACTTTGATAGCTTTGGAGACCTATCCTCTG
                                          31  LeuLeuValValTyrProTrpThrGlnArgTyrPheAspSerLeuLysGlyTh
400  CCTCTGCTATCATGGGTAATGCCAAAGTGAAGGCCCATGGCAAGAAGGTGATAACTGCCTTTAACGATGGCCTGAATCACTTGGACAGCCTCAAGGGCAC
     laSerAlaIleMetGlyAsnAlaLysValLysAlaHisGlyLysLysValIleThrAlaPheAsnAspGlyLeuAsnHisLeuAspSerLeuLysGlyTh
500  CTTTGCCAGCCTCAGTGAGCTCCACTGTGACAAGCTGCATGTGGATCCTGAGAACTTCAGGGTGAGTCTGATGGGCACCTCCTGGGTTTCCTTCCCCTGC
     rPheAlaSerLeuSerGluLeuHisCysAspLysLeuHisValAspProGluAsnPheArg  104
600  TATTCTGCTCAACCTTCCTATCAGAAAAAAAGGGGAAGCGATTCTAGGGAGCAGTCTCCATGACTGTGTGTGGAGTGTTGACAAGAGTTCGGATATTTTA

700  TTCTCTACTCAGAATTGCTGCTCCCCCTCACTCTGTTCTGTGTTGTCATTTCCTCTTTCTTTGGTAAGCTTTTTAATTTCCAGTTGCATTTTACTAAATT

800  AATTAAGCTGGTTATTTACTTCCCATCCTGATATCAGCTTCCCCTCCTCCTTTCCTCCCAGTCCTTCTCTCTCTCCTCTCTCTTTCTCTAATCCTTTCCT

900  TTCCCTCAGTTCATTCTCTCTTGATCTACGTTTGTTTGTCTTTTTAAATATTGCCTTGTAACTTGCTCAGAGGACAAGGAAGATATGTCCCTGTTTCTTC

1000 TCATAGCTCAAGAATAGTAGCATAATTGGCTTTTATGCAGGGTGACAGGGGAAGAATATATTTTACATATAAATTCTGTTTGACATAGGATTCTTGTGGT

1100 GGTTTGTCCAGTTTAAGGTTGCAAACAAATGTCTTTGTAAATAAGCCTGCAGGTATCTGGTATTTTTGCTCTACAGTTATGTTGATGGTTCTTCCATATT

1200 CCCACAGCTCCTGGGCAATATGATCGTGATTGTGCTGGGCCACCTTGGCAAGGATTTCACCCCCGCTGCACAGGCTGCCTTCCAGAAGGTGGTGGCT
        105  LeuLeuGlyAsnMetIleValIleValLeuGlyHisHisLeuGlyLysAspPheThrProAlaAlaPheGlnLysValValAla
1300 GGAGTGGCCACTGCCTTGGCTCACAAGTACCACTAAACCCCCTTTCCTGCTCTTGCCTGTGAACAATGGTTAATTGTTCCCAAGAGAGCATCTGTCAGTT
     GlyValAlaThrAlaLeuAlaHisLysTyrHisTer                                                  pA
1400 GTTGGCAAAATGATAGACATTTGAAAATCTGTCTTCTGACAAATAAAAAGCATTTATGTTCACTGCAATGATGTTTTAAATTATTTGTCTGTGTCATAGA

1500 AGGGTTTATGCTAAGTTTTCAAGATACAAAGAAGTGAGGGTTCAGGTCTCGACCTTGGGGAAATAAA
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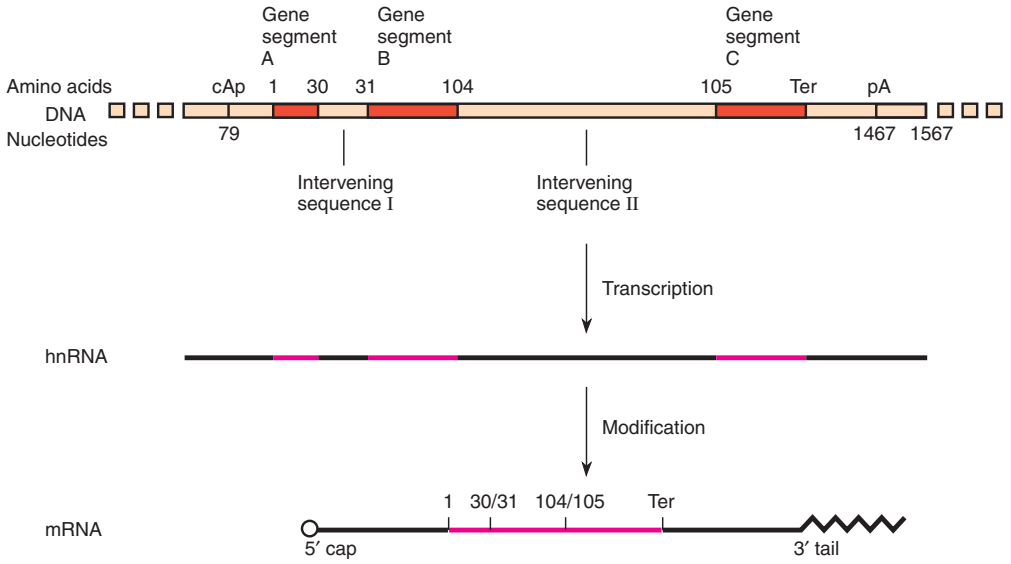
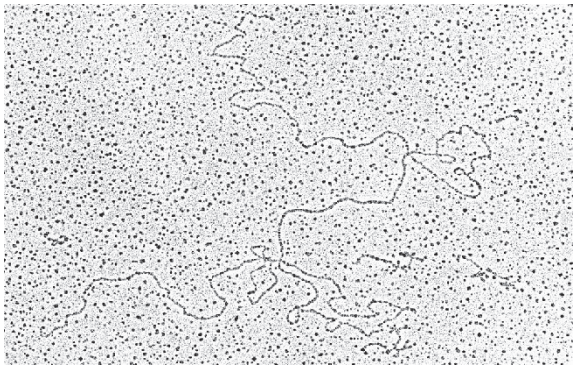
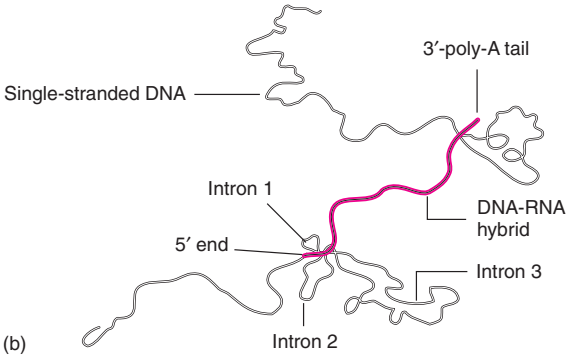


Figure 10.29 Nucleotide sequence of the mouse β-globin major gene. The coding DNA strand is shown; cAp (position 79) indicates the start of the capped mRNA; pA indicates the start of the poly-A tail (position 1467); numbers inside the sequence are adjacent amino acid positions; Ter is the termination codon (position 1334). The three-letter abbreviations (e.g., Met, Val, His) refer to amino acids (see chapter 11). The TATA box begins at position 49. (Source: National Institutes of Health Research by David A. Konkel, et al., "The sequence of the chromosomal mouse β-globin major gene: Homologies in capping, splicing and poly (A) Sites," *Cell,* 15:1125–32, 1978.)



(a)



(b)

Figure 10.30 The mRNA of adenovirus hybridized with its DNA. Three introns are visible as single-stranded DNA loops. They form single-stranded loops because they have nothing in the RNA molecule to hybridize with. Also visible is the poly-A tail of the mRNA. (*a*) Electron micrograph, (*b*) explanatory diagram. ([*a*] Courtesy of Louise T. Chow and Thomas Broker.)

Thomas Cech (1947–). (Courtesy of Dr. Thomas Cech. Photo by Ken Abbott.)

Sidney Altman (1939–). (Courtesy of Dr. Sidney Altman. Photo: Michael Marsland, Yale University Office of Public Affairs.)

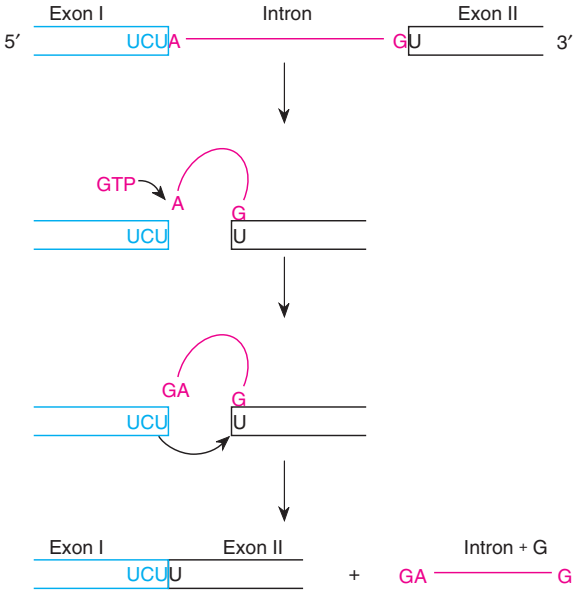


Figure 10.31 Self-splicing of a ribosomal RNA precursor in *Tetrahymena.* An external GTP is required. Two bond transfers produce a shortened RNA and a free intron.

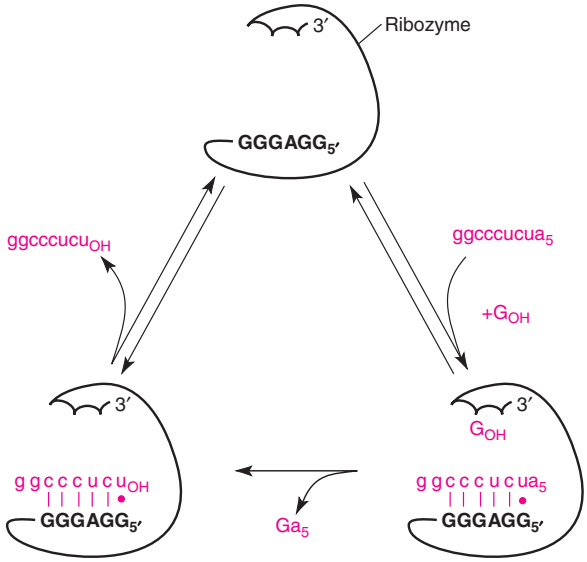
Protein-Mediated Splicing (the Spliceosome)

Eukaryotic nuclear messenger RNAs also have their introns removed by way of a lariat structure, just as in type II introns, but with the help of RNA-protein particles. Figure 10.36 shows consensus sequences in nuclear messenger RNA for the majority of introns. At the left (5′) side of the intron, the GU sequence is invariant, as is the AG at the right (3′) side. The right-most A of the UACUAAC sequence is the branch point of the lariat and is also invariant. (In DNA nucleotides, UACUAAC is TACTAAC; therefore, that region is sometimes referred to as the **TACTAAC box.**)

Unlike the mitochondrial group II introns, however, nuclear messenger RNAs have their introns removed with the help of a protein-RNA complex called a **spliceosome,** named by J. Abelson and E. Brody. The splicing apparatus in eukaryotic messenger RNAs consists of several components called **small nuclear ribonucleoproteins** (discovered and named by J. Steitz and colleagues), abbreviated as **snRNPs** and pronounced "snurps." Five of these particles take part in splicing, each composed of one or more proteins and a small RNA molecule; they are designated U1, U2, U4, U5, and U6. The RNA molecules range in size from 100 to 215 bases. The snRNPs and their associated proteins are located in twenty to forty small regions in the nucleus called *speckles* because of their appearance in the fluorescent microscope.

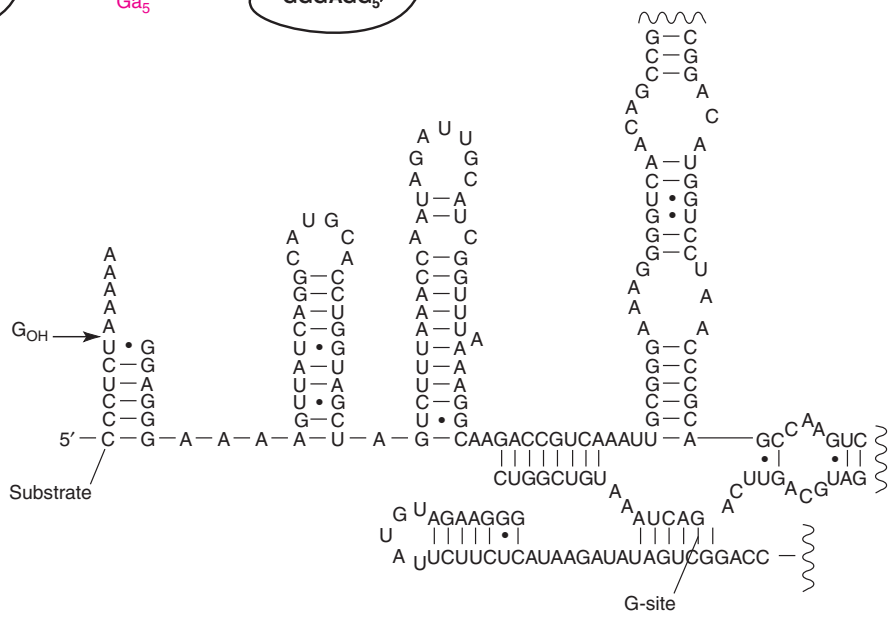
The RNAs of these particles have been sequenced, and sequencing shows they have regions of complementarity to either sites in the exons, sites in the introns, or sites in the other snRNP RNAs (table 10.5). These sequences, together with the experimental techniques of photocrosslinking and the creation of selective mutations (using techniques of site-directed mutagenesis described in chapter 13) have given us insight into the splicing mechanism. Photocrosslinking tells us which components are in contact. Mutations change pairings of components and may disrupt the structure. The change can be *rescued* (the pairing restored) by making a second change in the complementary RNA. When this happens successfully, the

Joan A. Steitz (1941–).
(Courtesy of Dr. Joan A. Steitz.)



(a)

Figure 10.32 The intron removed from the ribosomal RNA of *Tetrahymena* can catalyze the removal of the 3′ end of an RNA, diagrammed here as five AMP residues (a_5) from the sequence 5′-GGCCCUCUA$_5$-3′. The intron is called the *Tetrahymena* ribozyme. Any sequence can be removed from an RNA as long as there is a sequence complementary to the GGGAGG-5′ of the ribozyme to bring the RNA into position. In (*a*), the reaction needs an external guanine-containing nucleotide (G_{OH}); substrate nucleotides are in lowercase letters. This transesterification requires no external energy. In (*b*), the secondary structure of the ribozyme is shown. G_{OH} is the site of cleavage, and the position of the G-binding site is shown. Further structure must develop to bring the G-site to the substrate. *Wavy lines* represent additional structure not shown. (Reprinted with permission from Ann Marie Pyle, et al., "RNA substrate binding site in the catalytic core of the *Tetrahymena* ribozyme," *Nature,* Volume 358, 1992. Copyright © 1992 Macmillan Magazines, Ltd.)



(b)

Table 10.5 **The Five Small Nuclear Ribonucleoproteins (snRNPs) Involved in Nuclear Messenger RNA Intron Removal and Their RNAs**

snRNP RNA	Partial Sequence	Complementarity	Role
U1	3′-UCCAUUCAUA	5′ end of intron	Recognizes and binds 5′ site of intron
U2	3′-AUGAUGU	Branch point of intron	Binds branch point of intron
U4	3′-UUGGUCGU . . . AAGGGCACGUAUUCCUU	U6	Binds to (inactivates) U6
U5	3′-CAUUUUCCG	Exon 1 and exon 2	Binds to both exons
U6	3′-CGACUAGU . . . ACA	U2, 5′ site	Displaces U1 and binds 5′ site and U2 at branch point

Source: With permission from the *Annual Review of Genetics*, Volume 28 © 1994 by Annual Reviews www.AnnualReviews.org.

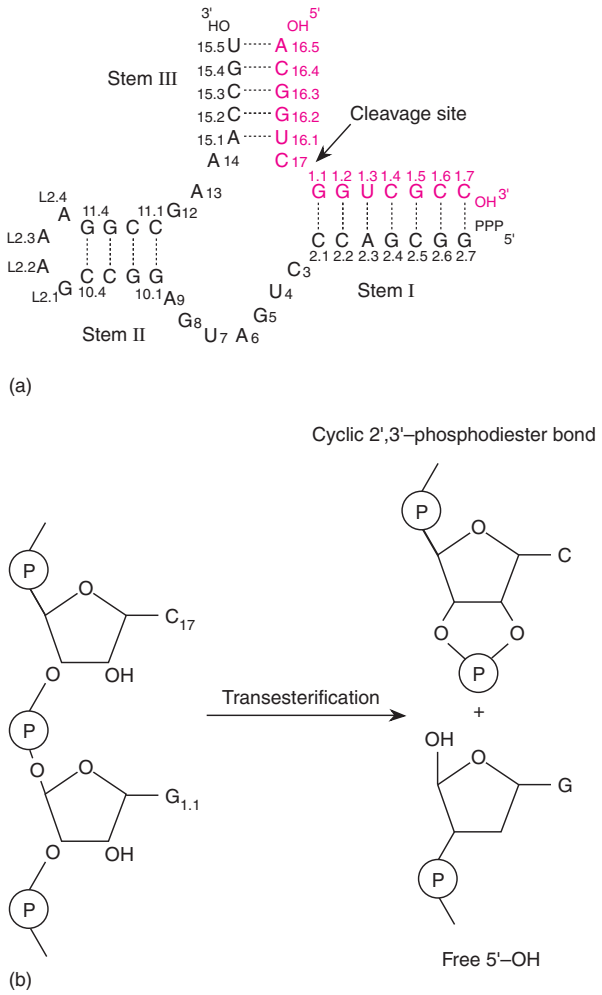


Figure 10.33 The hammerhead ribozyme, first seen in the RNAs of certain viruses (stems I, II, and III). (*a*) The cleavage point of the substrate (*red*) is shown using original sequence numbering, relating to the three stems of the hammerhead-shaped structure. (*b*) The cleavage, a transesterification, creates a cyclic 2′,3′-phosphodiester bond and a free 5′-OH.
(Reprinted with permission from *Nature,* Vol. 372, Heinz W. Pley et al., "Three Dimensional Structure of a Hammerhead Ribozyme." Copyright © 1994 Macmillan Magazines Limited.)

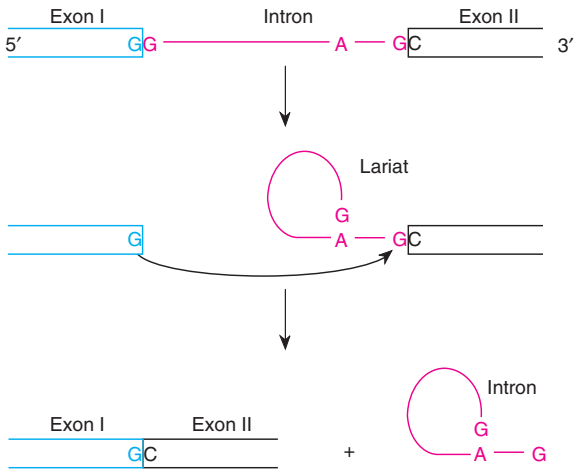


Figure 10.34 Self-splicing of a group II intron results in a lariat configuration of the released intron. No external GTP is required since the first bond transfer takes place with an internal nucleotide, forming the loop of the lariat. A second bond transfer releases the lariat-shaped intron.

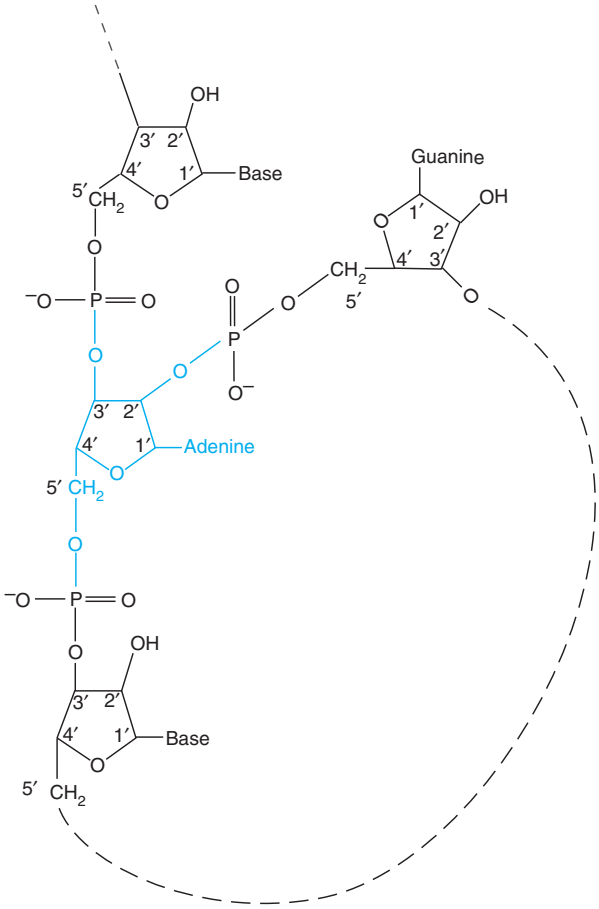


Figure 10.35 The lariat branch point (see fig. 10.34), formed during removal of a group II intron, occurs as three phosphodiester bonds form at the same ribose sugar. (The lariat loop is formed by the 2′-phosphodiester bond.)

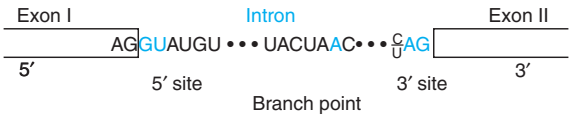


Figure 10.36 Consensus sequences of nuclear introns, showing the 5′ and 3′ sites and the branch point. Letters in *blue* (GU, A, AG) represent invariant bases. The last A (in *blue*) of the UACUAAC sequence is the lariat branch point.

presumed pairing is then confirmed. For example, if an A-U base pair occurs between two pieces of RNA, changing the A to a C disrupts the pairing. However, if the U is converted to a G, the pairing is restored (complementary A-U bases are converted to complementary C-G bases via a noncomplementary C-U intermediate). From these techniques, we believe that the following sequence of events takes place.

First, the U1 snRNP binds at the 5′ site of the intron and the U2 snRNP binds at the branch point (fig. 10.37). The U4, U5, and U6 snRNPs form a single particle. The U4 snRNP releases, freeing the U6 snRNP to bind to the 5′ site, displacing the U1 snRNP. (The U1 snRNP, with the help of other proteins, may bind at the 5′ site simply to mark it and initiate the process.) The U6 snRNP then also binds the U2 snRNP, allowing the lariat to form in the intron. The U5 snRNP binds the two exon ends together, allowing the splice to be completed as the lariat is removed.

The splicing machinery for the majority of introns also includes numerous other polypeptides called *auxiliary* and *splicing factors;* the entire splicing process requires about 50 polypeptides. A second, less common, intron, called the U12-dependent intron, with different consensus sequences, also exists. It is removed by a similar splicing process involving different snRNPs (U11, U12) as well as many components shared with the major spliceosome.

Currently, we believe the splicing out of the intron may be autocatalyzed, just as in the type II self-splicing introns. The spliceosome may have evolved to ensure control over the process, allowing different introns to splice with differing efficiencies and allowing **alternative splicing** to take place. In many eukaryotic genes, alternative paths of splicing can take place—different splice sites may be chosen or splices may be avoided entirely. Thus, a single gene can produce several different proteins, depending on splicing choice. For example, in yeast, the gene *RPL32* codes for a ribosomal protein. When this protein accumulates in excess, it somehow causes intron removal to fail. The result is a nonfunctional messenger RNA and no further RPL32

BOX 10.3

Experimental Methods

Are Viroids Escaped Introns?

Viroids are small (less than four hundred nucleotides), single-stranded RNA circles that act as plant pathogens. They do not have protein coats. In addition, they do not seem to code for any protein. The nature of their pathogenicity is not well understood. In 1986, Gail Dinter-Gottlieb, at the University of Colorado, pointed out numerous regions of homology between viroids and group I introns, supporting proposals by Francis Crick and Theodor Diener that viroids are escaped introns.

Many group I introns form circles after they are released. The self-splicing group I intron of *Tetrahymena thermophila* is 399 bases in circular form, whereas the potato spindle tuber viroid (PSTV) is 359 bases. These similarities of size and shape prompted the search for base homologies. In figure 1, we compare

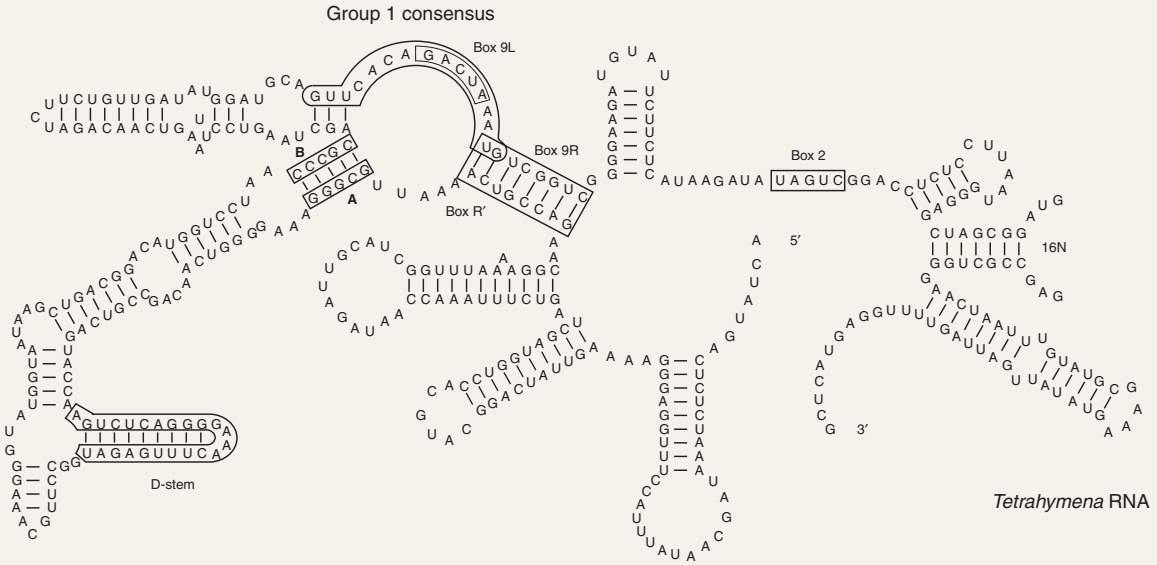


Figure 1 Self-splicing group I *Tetrahymena* intron (*left page*) and potato spindle tuber viroid (PSTV, *right page*); 16N in the *left figure* refers to sixteen nucleotides not shown. Note the similarities around the group 1 consensus area. (From G. Dinter-Gottlieb, *Proceedings of the National Academy of Sciences,* page 6251, 1986.)

protein produced. In human beings, the gene *RBP-MS* can produce at least twelve different transcripts, depending on alternative splicing.

One other mode of protein-mediated intron removal is known. Nuclear transfer RNAs have introns that are not self-splicing but are removed by an endonuclease; the exons are subsequently joined by a ligase. Archaean bacteria seem to have this type of intron.

Intron Function and Evolution

Since the discovery of introns, geneticists have been trying to figure out why they exist. Several views have arisen. Walter Gilbert suggested that introns separate exons (coding regions) into functional domains—that is, they separate different exons that presumably have specific tasks. In a given protein, one exon might code for a membrane-binding region, one might code for the active site of the enzyme, and one might code for ATPase activity. By recombinational mechanisms, or by excluding an exon during intron removal, **exon shuffling** would allow the rapid evolution of new proteins whose structures would be conglomerates of various functional domains. In a 1990 article in *Science,* Gilbert, with two colleagues, calculated that all proteins in eukaryotes can be accounted for by as few as one thousand to seven thousand

the *Tetrahymena* intron with PSTV. Note that both have an extensive secondary structure (stem-loops) and similarities of some sequences. Most notable is the box 9L similarity. This box lies within a 16-base consensus sequence of all group I introns and has similarities to the corresponding sequence in PSTV. Note the general shape around the group I consensus region: two stems to the left and one to the right with some homologies. Note also the D-stem similarities.

These similarities strongly indicate that viroids and group I introns are related. Whether viroids are escaped introns or both evolved from a common ancestor has, as yet, not been resolved.

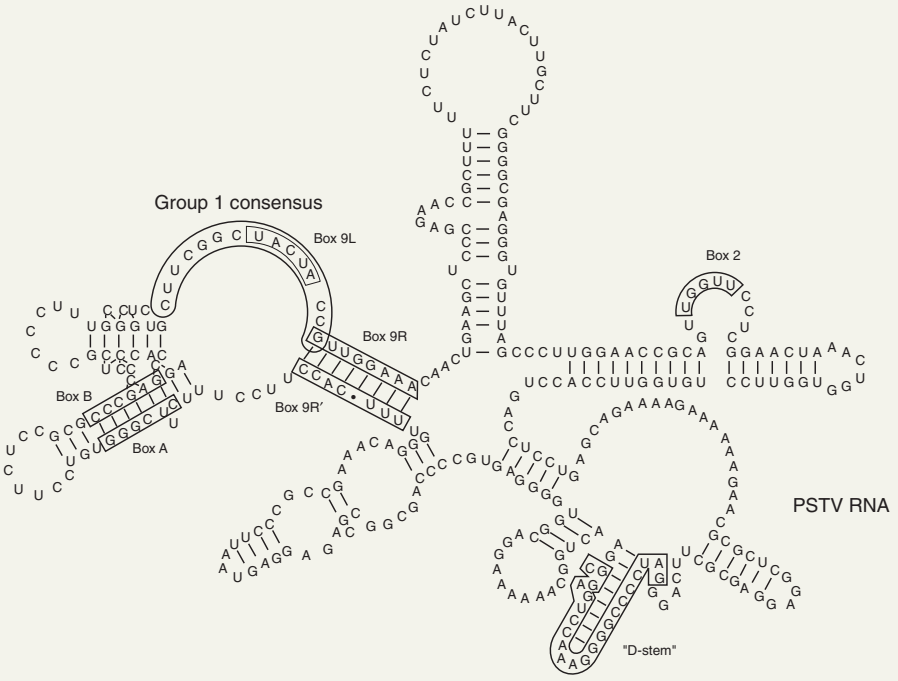


Figure 1 (continued)

exons; all proteins may be conglomerates of this primordial number. However, this view is controversial.

J. Darnell and W. F. Doolittle have expanded Gilbert's idea of exon shuffling into the *introns-early* view. They suggest that introns arose before the first cells evolved. After eukaryotes evolved from prokaryotes, the prokaryotes lost their introns. This is supported by the evidence that, generally, prokaryotes lack introns. This view is also consistent with the opinion that the original genetic material was RNA. In this "RNA world," introns arose as part of the genetic apparatus; they were the first enzymes (ribozymes).

An alternative view is that introns arose later in evolution, after the eukaryotes split from the prokaryotes. At first, the justification for this *introns-late* view was that introns evolved late to give the organism the ability to evolve quickly to new environments by an exon-shuffling type of mechanism. However, evolutionary biologists don't accept the rationale of evolution based on future needs. An alternative explanation is that introns are actually invading "selfish DNA," DNA that can move from place to place in the genome without necessarily providing any advantage to the host organism. We call these "jumping genes" transposons and discuss them at length in chapters 14 and 16. Thus, both time frames for the development of introns—late or early—have conceptual support.

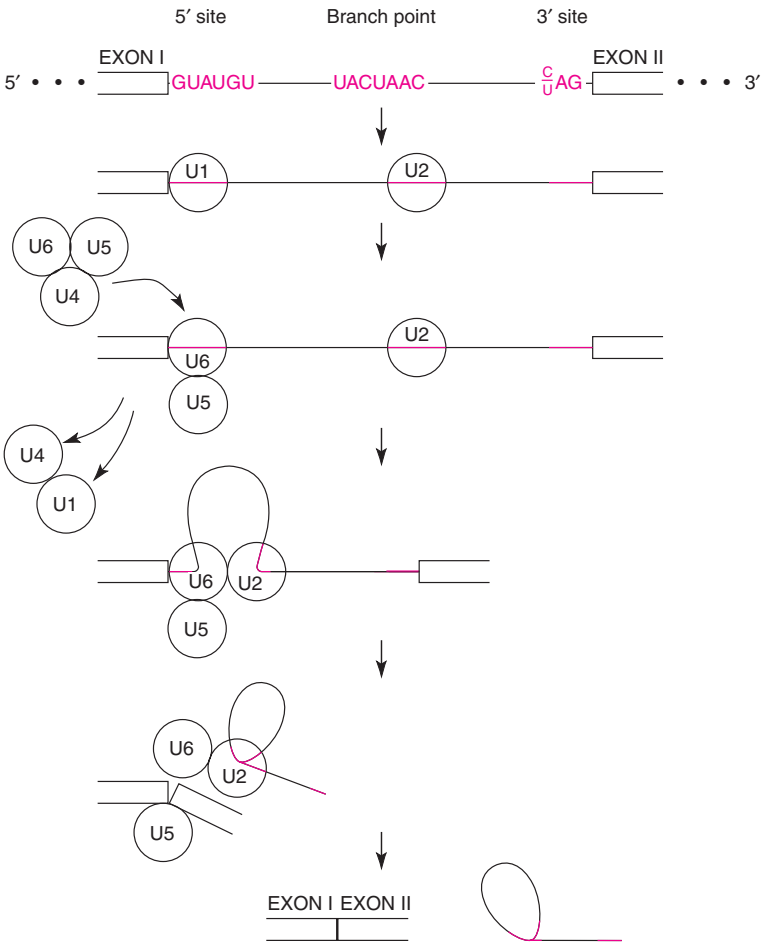


Figure 10.37 Sequence of steps, explained in the text, in which U1, U2, U4, U5, and U6 snRNPs take part in intron removal in a nuclear RNA.

Evidence exists to support both views. Gilbert's exon-shuffling view is supported by the analysis of some genes that do indeed fit the pattern of exons coding for functional domains of a protein. (Analysis consists of DNA sequencing, RNA sequencing, and protein structural analysis.) For example, the second of three exons of the globin gene binds heme. Similarly, the human low-density lipoprotein receptor is a mosaic of exon-encoded modules shared with several other proteins. Autocatalytic properties of introns lend credence to the view that RNA was the original genetic material and that introns can move within a genome.

Additional evidence for the introns-early hypothesis includes the discovery of several introns in phage genes and introns in transfer RNA and ribosomal RNA genes in ancient bacteria (archaebacteria). Until recently, however, no introns were known in the true bacteria (eubacteria). That changed with recent work from the labs of D. Shub and J. Palmer, who independently discovered an intron in a transfer RNA gene in seven species of cyanobacteria (blue-green algae of the eubacteria). This intron was suspected to exist because it occurred in the equivalent chloroplast gene; the chloroplast evolved from an invading cyanobacterium. However, this discovery has been viewed as supporting both the introns-early and introns-late view. The introns-early supporters say this evidence confirms that introns arose before the eukaryotes-prokaryotes split. Introns-late supporters say they expect to see some introns in prokaryotes because of the mobility these bits of genetic material have.

Both the introns-early and the introns-late views may be correct. It is possible that introns arose early, were lost by the prokaryotes, which prioritized small genomes and rapid, efficient DNA replication, and later evolved to produce exon shuffling in eukaryotes.

RNA Editing

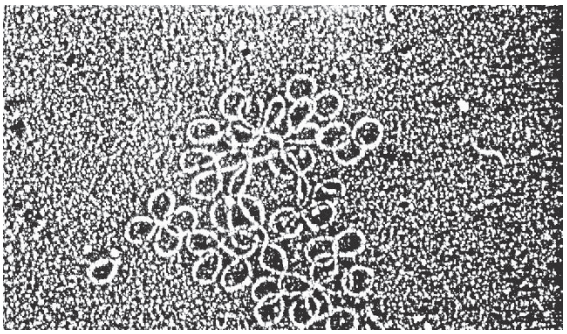
In the last few years, several examples have arisen in which DNA sequence does not predict protein sequence. In several cases, changes in the protein occur that could have only come about by inserting or deleting nucleotides in the messenger RNA before it is translated. This insertion or deletion is almost exclusively of uridines. The process is termed **RNA editing.**

RNA editing was particularly evident in the mitochondrial proteins of a group of parasites, the trypanosomes (some of which cause African sleeping sickness); in one case, more than 50% of the nucleotides in the messenger RNA were added uridines. Uridines were also deleted from the original sequence. These parasites had another mysterious trait—the existence of minicircles and maxicircles of DNA in specialized mitochondria called *kinetoplasts.* In the average kinetoplast, there are about fifty maxicircles and about five thousand minicircles, concatenated like chain links (fig. 10.38*a*). The maxicircles contain genes for mitochondrial function (see chapter 17); as L. Simpson and his colleagues showed in 1990, both maxicircles and minicircles are templates for **guide RNA (gRNA),** RNA that guides the process of messenger editing.

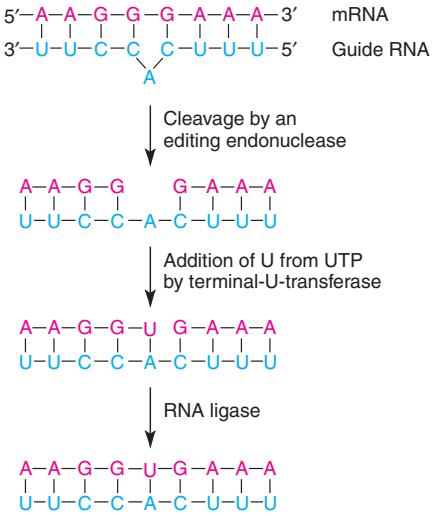
The guide RNA forms a complement with the messenger RNA to be edited; however, the guide RNA has the sequence complementary to that of the *final* messenger RNA, the one with bases added. Since the bases have not yet been added, a bulge occurs in the guide RNA where the complement to be added is (fig. 10.38*b*). The messenger RNA is then cleaved opposite the bulge by an editing endonuclease. A uridylate (U) is brought into the messenger RNA as a complement to the adenine (A) with the enzyme terminal-U-transferase. An RNA ligase then closes the nick in the messenger RNA, which now has a uridylate added.

An exciting outcome of this research, aside from learning about a novel mechanism of messenger RNA processing, is the possibility of clinical rewards. Anytime there is a specialized pathway in a parasite not found in its host, it is possible to use that pathway to attack the parasite. Thus, this research might lead to new ways of combating these trypanosome parasites.

RNA editing also occurs in other species and by different mechanisms. For example, in the apolipoprotein-B (*apoB*) gene in mammals, one gene produces two forms of the protein. In one case, nucleotide 6666, a cytosine, is modified by deamination to a uracil in the messenger RNA, resulting in the termination of translation and a protein about half the normal size. RNA editing also occurs in plant mitochondria and chloroplasts in which the usual change is also a cytosine to a uracil. RNA editing is thus routinely seen in specific examples of posttranscriptional RNA modification in both animals and plants.



(a)



(b)

Figure 10.38 RNA editing. (*a*) Eight hundred seventy base pair minicircles of DNA from *Leishmania tarentolae.* (*b*) Mechanism by which a guide RNA is involved in the editing of a messenger RNA. After the cycle shown, a uridine-containing nucleotide has been added to the messenger RNA. The guide RNA has the sequence complementary to the messenger RNA with the base already added. ([*a*] Courtesy of Larry Simpson.)

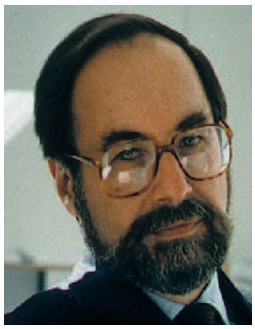
UPDATED INFORMATION ABOUT THE FLOW OF GENETIC INFORMATION

The original description of the central dogma included three information transfers that were presumed to occur even though they had not been observed (see fig. 10.1). Since then, researchers have documented these three transfers: reverse transcription, RNA self-replication, and the direct involvement of DNA in translation (fig. 10.39).

Reverse Transcription

First, the return arrow from RNA to DNA in figure 10.39 indicates that RNA can be a template for DNA synthesis. All RNA tumor viruses, such as Rous sarcoma virus, as well as the AIDS virus, can make an RNA-dependent DNA polymerase (often referred to as **reverse transcriptase**) that synthesizes a DNA strand complementary to the viral RNA. (H. Temin and D. Baltimore received Nobel prizes for their discovery of this polymerase enzyme.) This enzyme is involved in a tumor virus's infection of a normal cell and the transformation of that cell into a cancerous cell. When the viral RNA enters a cell, it brings reverse transcriptase with it. The enzyme synthesizes a DNA-RNA double helix, which then is enzymatically converted into a DNA-DNA double helix that can integrate into the host chromosome. After integration, the DNA is transcribed into copies of the viral RNA, which are both translated and packaged into new viral particles that are released from the cell to repeat the infection process. (We cover this material in more detail in chapters 13 and 16.)

Howard Temin (1934–1994). (Courtesy of Dr. Howard Temin. UW photo media.)



David Baltimore (1938–). (Courtesy of Kucerea and Company/Laxenburger Strasse 58.)

RNA Self-Replication

The second modification to the original central dogma is the verification that RNA can act as a template for its own replication. This process has been observed in a small class of phages. These **RNA phages,** such as R17, f2, MS2, and Qβ, are the simplest phages known. MS2 contains about thirty-five hundred nucleotides and codes for only three proteins: a coat protein, an attachment protein (responsible for attachment to and subsequent penetration of the host), and a subunit of the enzyme **RNA replicase.** The RNA replicase subunit combines with three of the cell's proteins to form RNA replicase, allowing the single-stranded RNA of the phage to replicate itself.

Since the new protein needed to construct the RNA replicase enzyme must be synthesized before the phage can replicate its own RNA, the phage RNA must first act as a messenger when it infects the cell. Thus, protein synthesis is taking place without a preceding transcription process. The viral genetic material, its RNA, is first used as a messenger in the process of translation and then used as a template for RNA replication.

DNA Involvement in Translation

In the mid-1960s, B. J. McCarthy and J. J. Holland showed that under certain experimental conditions, denatured (single-stranded) DNA could bind to ribosomes and be translated into proteins. The experimental conditions usually involved the addition of antibiotics that interacted with the DNA or the ribosome. Direct translation of DNA is not known to occur naturally.

Even in our updated central dogma (fig. 10.39), no arrows originate at the protein. In other words, protein cannot self-replicate, nor can it use amino acid sequence information to reconstruct RNA or DNA. Crick has called these arrows "forbidden transfers." We know of no cellular machinery that can produce these forbidden processes. In the next chapter, we continue this discussion of protein synthesis by describing the process of translation, in which the information in messenger RNA is used to form the sequences of amino acids in proteins.

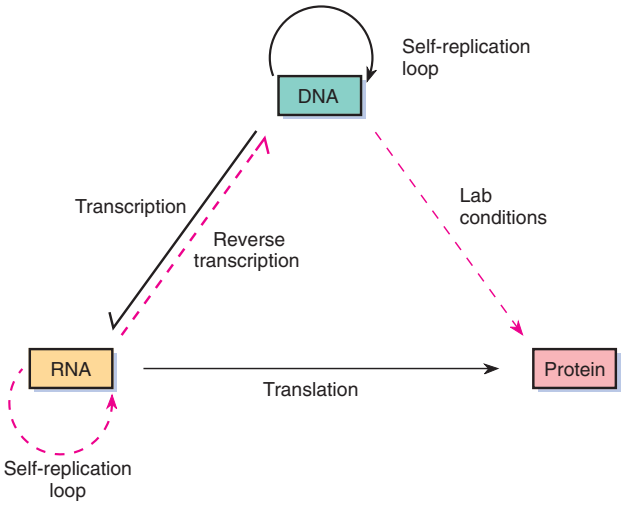


Figure 10.39 An updated version of Crick's central dogma, showing all known paths of genetic information transfer. Paths confirmed since Crick proposed the original central dogma appear as dashed *red* lines (reverse transcription, RNA self-replication, and direct DNA translation). Direct DNA translation is known only under laboratory conditions: the process apparently does not occur naturally. There is no known information flow beginning with protein.

SUMMARY

The central dogma is a description of how genetic information is transferred among DNA, RNA, and protein. In chapter 9, we described the DNA self-replication loop. In this chapter, we described the transcriptional process, in which DNA acts as a template for the production of RNA.

STUDY OBJECTIVE 1: To examine the types of RNA and their roles in gene expression 245–246, 256–260

Messenger RNA (mRNA) is a complementary copy of the DNA of a gene that carries the information of the gene to the ribosomes, where protein synthesis actually takes place. Transfer RNAs (tRNAs) transport the amino acid building blocks of proteins to the ribosome. Complementarity between the messenger RNA codon and the transfer RNA anticodon establishes the amino acid sequence in the synthesized protein ultimately specified by the gene. Ribosomal RNA (rRNA) is also involved in this process of gene-directed protein synthesis.

STUDY OBJECTIVE 2: To look at the process of transcription, including start and stop signals, in both prokaryotes and eukaryotes 246–256

Intracellular RNA is single-stranded, although extensive intramolecular stem-loop structures may form. At any one gene, RNA is transcribed from only one strand of the DNA double helix. The transcribing enzyme is RNA polymerase. In *E. coli,* the core enzyme, when associated with a sigma factor, becomes the holoenzyme that recognizes the transcription start signals in the promoter. Several consensus sequences define a promoter. In prokaryotes, termination of transcription requires a sequence on the DNA, called the terminator, that causes a stem-loop structure to form in the RNA. Sometimes the rho protein is required for termination (in rho-dependent, as compared with rho-independent, termination). In eukaryotes, there are three RNA polymerases. Eukaryotic genes have promoters with sequences analogous to those in prokaryotic promoters as well as enhancers that work at a distance.

The ribosome is made of two subunits, each with protein and RNA components. Transfer RNAs are charged with their particular amino acids by enzymes called aminoacyl-tRNA synthetases. Each transfer RNA has about eighty nucleotides, including several unusual bases. All transfer RNAs have similar structures and dimensions. Transfer RNAs and ribosomal RNAs are modified from their primary transcripts.

STUDY OBJECTIVE 3: To investigate posttranscriptional changes in eukaryotic messenger RNAs, including an analysis of intron removal 260–276

Prokaryotic messenger RNAs are transcribed with a leader before, and a trailer after, the translatable part of the gene. In prokaryotes, translation begins before transcription is completed. In eukaryotes, these processes are completely uncoupled—transcription is nuclear and translation is cytoplasmic. Eukaryotic messenger RNA is modified after transcription: a cap and tail are added, and intervening sequences (introns) are removed, before transport into the cytoplasm. Introns can be removed by self-splicing or with the aid of the spliceosome, composed of small nuclear ribonucleoproteins (snRNPs). It is not known whether introns arose early or late in evolution or what their functions are. In some organisms, such as trypanosomes, RNAs can be edited further by the addition or deletion of nucleotides under the direction of guide RNA.

The study of several RNA viruses has shown that RNA can act as a template to replicate itself and to synthesize DNA; under laboratory conditions, DNA can be translated directly into protein. These discoveries add new directions of information transfer to the central dogma.

SOLVED PROBLEMS

PROBLEM 1: What would be the sequence of segments on a prokaryotic messenger RNA with more than one gene present?

Answer: The transcript would have unmodified 5′ (leader) and 3′ (trailer) ends. Reading the sequence of nucleotides on the RNA, you would come across an initiation codon (AUG) and then, after perhaps nine hundred more nucleotides, a termination codon (UAA, UAG, or UGA). The nine hundred nucleotides would be those translated into the protein. Then there would be a spacer region of nucleotides, followed by another initiation codon, intervening nucleotides that are translated into amino acids, and a termination codon. This sequence of initiation codon, codons to be translated, a termination codon, and spacer RNA would be repeated for as many genes as are present in the messenger RNA.

PROBLEM 2: Can one nucleotide be a conserved sequence?

Answer: Conserved sequences are invariant sequences of DNA or RNA recognizable to either a protein or a

complementary sequence of DNA or RNA. However, in group II introns, an adenine is needed near the 3′ end of the intron for lariat formation. Thus, this single nucleotide, given its relative position in the intron and possible surrounding bases, is a conserved sequence of one.

PROBLEM 3: Why might *E. coli* not have a nucleolus?

Answer: The nucleolus is the site of ribosomal construction in eukaryotes. It is centered at the nucleolus organizer, the tandemly repeated gene coding for the three larger pieces of ribosomal RNA. In *E. coli,* there are only five to ten copies of the ribosomal RNA gene, whereas there is usually an order of magnitude or more copies in eukaryotes. Thus, the simplest reason that a nucleolus is not visible in *E. coli* is because there are too few copies of the gene around which a nucleolus forms.

PROBLEM 4: If this sequence of bases represents the start of a gene on double-stranded DNA, what is the sequence of the transcribed RNA, what is its polarity, and what is the polarity of the DNA?

G C T A C G G A T T G C T G
C G A T G C C T A A C G A C

Answer: Begin by writing the complementary strand to each DNA strand: C G A U G C C U A A C G A C for the top, and G C U A C G G A U U G C U G for the bottom. Now look for the start codon, AUG. It is present only in the RNA made from the top strand, so the top strand must have been transcribed. The polarity of the start codon is 5′-A U G-3′. Since transcription occurs 5′ → 3′, and since nucleic acids are antiparallel, the left end of the top DNA strand is the 3′ end.

EXERCISES AND PROBLEMS*

TYPES OF RNA

1. Diagram the relationships of the three types of RNA at a ribosome. Which relationships make use of complementarity?

PROKARYOTIC DNA TRANSCRIPTION

2. How could DNA-DNA or DNA-RNA hybridization be used as a tool to construct a phylogenetic (evolutionary) tree of organisms?
3. Assume that prokaryotic RNA polymerase does not proofread. Do you expect high or low levels of error in transcription as compared with DNA replication? Why is it more important for DNA polymerase than RNA polymerase to proofread?
4. What are the transcription start and stop signals in eukaryotes and prokaryotes? How are they recognized? Can a transcriptional unit include more than one translational unit (gene)? (*See also* EUKARYOTIC DNA TRANSCRIPTION)
5. What is a consensus sequence? a conserved sequence?
6. What would the effect be on transcription if a prokaryotic cell had no sigma factors? no rho protein?
7. Draw a double helical section of prokaryotic DNA containing transcription start and stop information. Give the base sequence of the messenger RNA transcript.
8. In what ways does the transcriptional process differ in eukaryotes and prokaryotes? (*See also* EUKARYOTIC DNA TRANSCRIPTION)
9. What is a stem-loop structure? an inverted repeat? a tandem repeat? Draw a section of a DNA double helix with an inverted repeat of seven base pairs.
10. What is the function of each of the following sequences: TATAAT, TTGACA, TATA, TACTAAC? What is a Pribnow box? a Hogness box? (*See also* EUKARYOTIC DNA TRANSCRIPTION)
11. What is footprinting? How did it help define promoter sequences?
12. What are the differences between rho-dependent and rho-independent termination of transcription?
13. What are the differences between a σ^{70} and a σ^{32}?
14. Draw a typical mature messenger RNA molecule of a prokaryote and a eukaryote. Label all regions. (*See also* EUKARYOTIC DNA TRANSCRIPTION)
15. Determine the sequence of both strands of the DNA from which this RNA was transcribed. Indicate the 5′ and 3′ ends of the DNA and, with an arrow, which strand was transcribed.

5′-C C A U C A U G A C A G A C C C U U G C U A A C G C-3′

16. The following DNA fragment was isolated from the beginning of a gene. Determine which strand is transcribed, indicate the polarity of the two DNA strands, and then give the sequence of bases in the resultant messenger RNA and its polarity.

C C C T A C G C C T T T C A G G T T
G G G A T G C G G A A A G T C C A A

*Answers to selected exercises and problems are on page A-11.

17. The following DNA fragment represents the beginning of a gene. Determine which strand is transcribed, and indicate polarity of both strands in the DNA.

A T G A T T T A C A T C T A C A T T T A C A T T
T A C T A A A T G T A G A T G T A A A T G T A A

18. The following sequence of bases in a DNA molecule is transcribed into RNA:

C C A G G T A T A A T G C T C C A G T A T G G C A T G G T A C T T C C G G
↑

If the T (*arrow*) is the first base transcribed, determine the sequence and polarity of bases in the RNA, and identify the Pribnow box and the initiator codon.

19. You have isolated a mutant that makes a temperature-sensitive rho molecule; rho functions normally at 30° C, but not at 40° C. If you grow this strain at both temperatures for a short period of time and isolate the newly synthesized RNA, what relative size RNA do you expect to find in each case?

20. Suppose you repeat the experiment in problem 19 and find the same size RNA made at both temperatures. Provide two possible explanations for this unexpected finding.

21. Why do you think most promoter regions are A-T rich? (*See also* EUKARYOTIC DNA TRANSCRIPTION)

EUKARYOTIC DNA TRANSCRIPTION

22. Would introns be more or less likely than exons to accumulate mutations through evolutionary time?

23. What would be the effect on the final protein product if an intervening sequence were removed with an extra base? one base too few?

24. What is heterogeneous nuclear messenger RNA? What are small nuclear ribonucleoproteins?

25. What product would DNA-RNA hybridization produce in a gene with five introns? no introns? Draw these hybrid molecules.

26. What are the recognition signals within the majority of introns?

27. What are the differences between group I and group II introns?

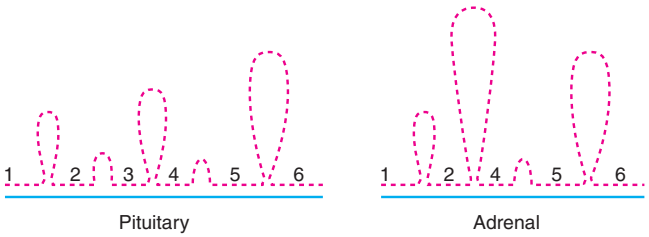
28. Diagram ribozyme functioning in a group I intron.

29. How does a spliceosome work? What are its component parts?

30. What is a transcriptional factor? an enhancer?

31. In the following drawing of a eukaryotic gene, solid red lines represent coding regions, and dashed blue lines represent introns. Draw the RNA-DNA hybrid that would result if cytoplasmic messenger RNA is hybridized to nuclear DNA.

32. RNA-DNA hybrids are formed by using messenger RNA for a given gene that is expressed in the pituitary and the adrenal glands. The DNA used in each case is the full-length gene. Based on the figure, provide an explanation for the different hybrid molecules. DNA is a dashed red line; RNA is a solid blue line.



33. Enhancers can often exert their effect from a distance; some enhancers are located thousands of bases upstream from the promoter. Propose an explanation to account for this.

UPDATED INFORMATION ABOUT THE FLOW OF GENETIC INFORMATION

34. How do prions relate to the central dogma of figure 10.39?

CRITICAL THINKING QUESTIONS

1. What are the upper limits to the size of a gene in eukaryotes?

2. Present a scenario of an activator controlling transcription in eukaryotes.

Suggested Readings for chapter 10 are on page B-7.

11

GENE EXPRESSION

Translation

STUDY OBJECTIVES

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2. To examine the genetic code 304

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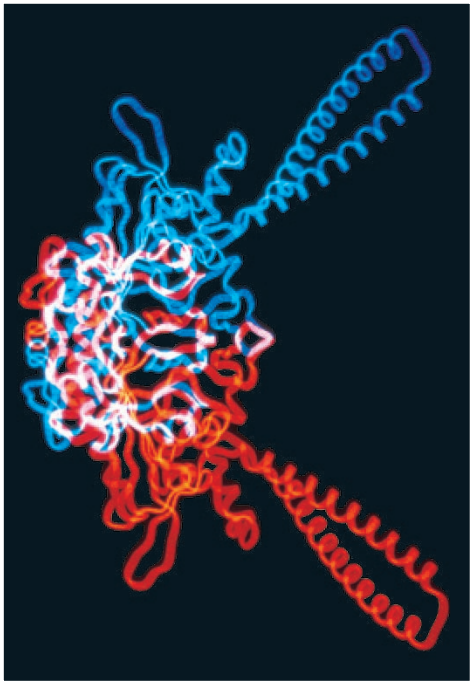


Computer-generated model of the enzyme seryl tRNA synthetase, the enzyme that charges tRNA's with the amino acid serine. (© Dr. Stephen Cusack/EMBL/SPL/Photo Researchers, Inc.)

In this chapter, we continue our discussion of gene expression, concentrating on protein biosynthesis. This process, which translates the nucleotide information in messenger RNA into amino acid sequences in proteins, is the final step of the central dogma. Nucleotide sequences in DNA are transcribed into nucleotide sequences in RNA, which are then translated into amino acid sequences in proteins.

All proteins are synthesized from only twenty naturally occurring amino acids (fig. 11.1). (There is one exception, selenocysteine, which we discuss at the end of the chapter.) These are called α-amino acids because one carbon, the α carbon, has four specific groups attached to it: an amino group, a carboxyl (acidic) group, a hydrogen, and one of the twenty different R groups (side chains), imparting the specific properties of that amino acid. (Technically, proline is termed an *imino* acid because of its structure.) Having these four groups attached imparts a property known as *chirality* on the amino acid: like left- and right-handed gloves, the mirror images cannot be superimposed. Because of optical properties, the two forms of each amino acid are referred to as D and L, in which D comes from dextrorotatory (right turning) and L comes from levorotatory (left turning). All biologically active amino acids are of the L form, and hence we need not refer to this designation. Proteins (polypeptides) are synthesized when peptide bonds form between any two amino acids (fig. 11.2). In this manner, long chains of amino acids—called *residues* when incorporated into a protein—can join, and all chains will have an amino (N-terminal) end and a carboxyl (C-terminal) end.

The sequence of polymerized amino acids determines the **primary structure** of a protein. Included in the primary structure is the formation of disulfide bridges between cysteine residues (fig. 11.3). Polypeptides can fold into several structures, the most common of which are α helices and β sheets. These folding configurations constitute the **secondary structure** of the protein. In some proteins, the folding is spontaneous; in some, it is guided by other proteins. Further folding, bringing α helices and β sheets into three-dimensional configurations, creates the **tertiary structure** of the protein (fig. 11.4). Many proteins in the active state are composed of several subunits that together make up the **quaternary structure** of the protein. Translation is the process in which the primary structure of a protein is determined from the nucleotide sequence in a messenger RNA (box 11.1).

INFORMATION TRANSFER

Before proceeding to the details of translation, a sketch of the beginning of the process may be helpful (fig. 11.5). The ribosome with its ribosomal RNA and proteins is the site of protein synthesis. The information from the gene is in the form of messenger RNA, in which each group of three nucleotides—a codon—specifies an amino acid. The amino acids are carried to the ribosome attached to transfer RNAs, and these transfer RNAs have anticodons, three nucleotides complementary to a codon, located at the end opposite the amino acid attachment site. A peptide bond will form between the two amino acids present at the ribosome, freeing one transfer RNA (at codon 1 in fig. 11.5) and lengthening the amino acid chain attached to the second transfer RNA (at codon 2 in fig. 11.5). The messenger RNA will then move one codon with respect to the ribosome, and a new transfer RNA will attach at codon 3. This cycle is then repeated, with the polypeptide lengthening by one amino acid each time. We can begin looking at the details of translation by looking at the transfer RNAs. As before, we concentrate on the prokaryotic system, noting details about eukaryotes as appropriate.

Transfer RNA

Attachment of Amino Acid to Transfer RNA

The function of transfer RNA is to ensure that each amino acid incorporated into a protein corresponds to a particular codon (a group of three consecutive nucleotides) in the messenger RNA. The transfer RNA serves this function through its structure: It has an anticodon at one end and an amino acid attachment site at the other end. The "correct" amino acid, the amino acid corresponding to the anticodon, is attached to the transfer RNA by enzymes known as aminoacyl-tRNA synthetases (e.g., arginyl-tRNA synthetase, leucyl-tRNA synthetase). A transfer RNA with an amino acid attached is said to be "charged."

An aminoacyl-tRNA synthetase joins a specific amino acid to its transfer RNA in a two-stage reaction that takes place on the surface of the enzyme. In the first stage, the amino acid is activated with ATP. In the second stage of the reaction, the amino acid is attached with a high-energy bond to the 2′ or 3′ carbon of the ribose sugar at the 3′ end of the transfer RNA (fig. 11.6). In the figure, we denote high-energy bonds, bonds that liberate a lot of free energy when hydrolyzed, as "~." Thus, during the process of protein synthesis, the energy for the formation of the peptide bond will be present where it is needed, at the point of peptide bond formation.

Component Numbers

In bacteria, there are twenty aminoacyl-tRNA synthetases, one for each amino acid. A particular enzyme recognizes a particular amino acid, as well as all the transfer RNAs that

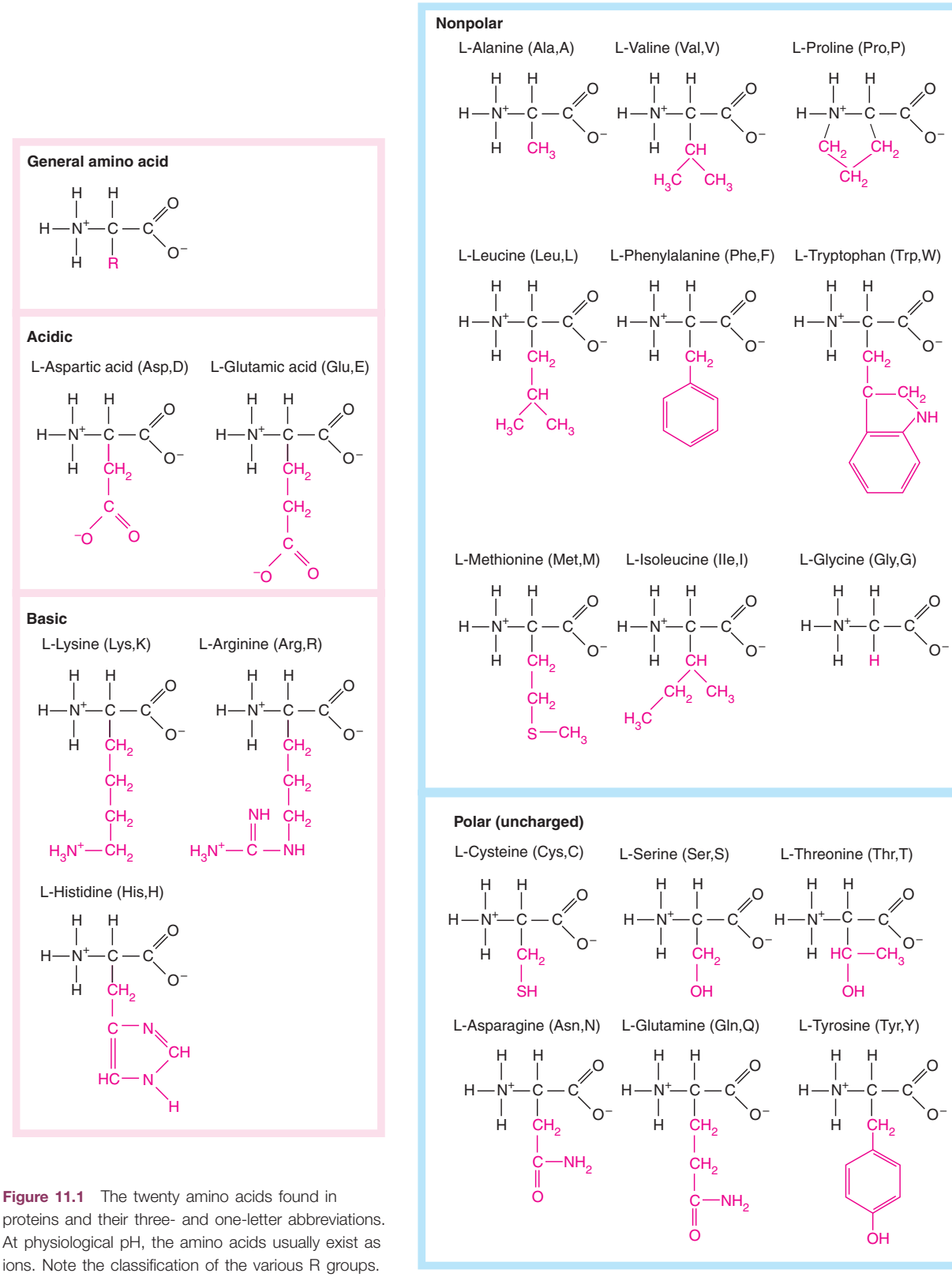


Figure 11.1 The twenty amino acids found in proteins and their three- and one-letter abbreviations. At physiological pH, the amino acids usually exist as ions. Note the classification of the various R groups.

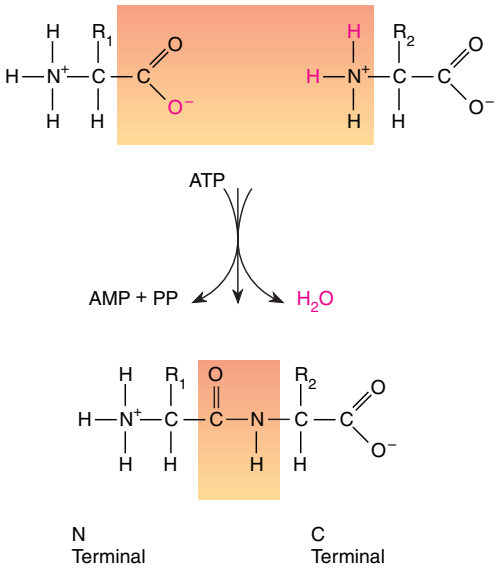


Figure 11.2 Protein synthesis: formation of a peptide bond between two amino acids. The bond is between the carboxyl group of one amino acid and the amino group of the other.

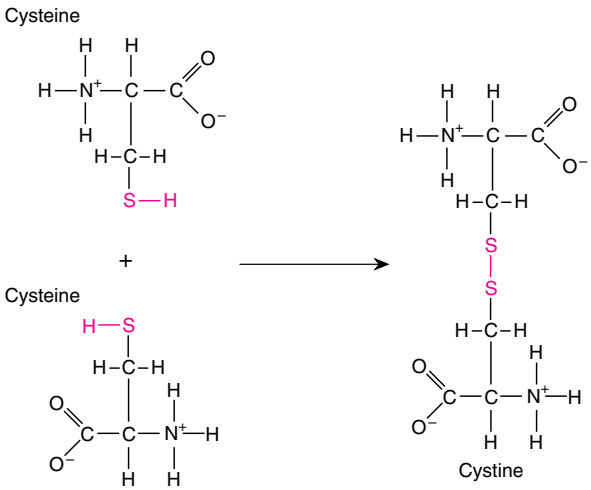


Figure 11.3 A disulfide bridge can form when two cysteines are brought into apposition. If the two amino acids are in the free form, the new structure is called *cystine.* When the two cysteines are in the same or different polypeptides, the disulfide bridge creates stability.

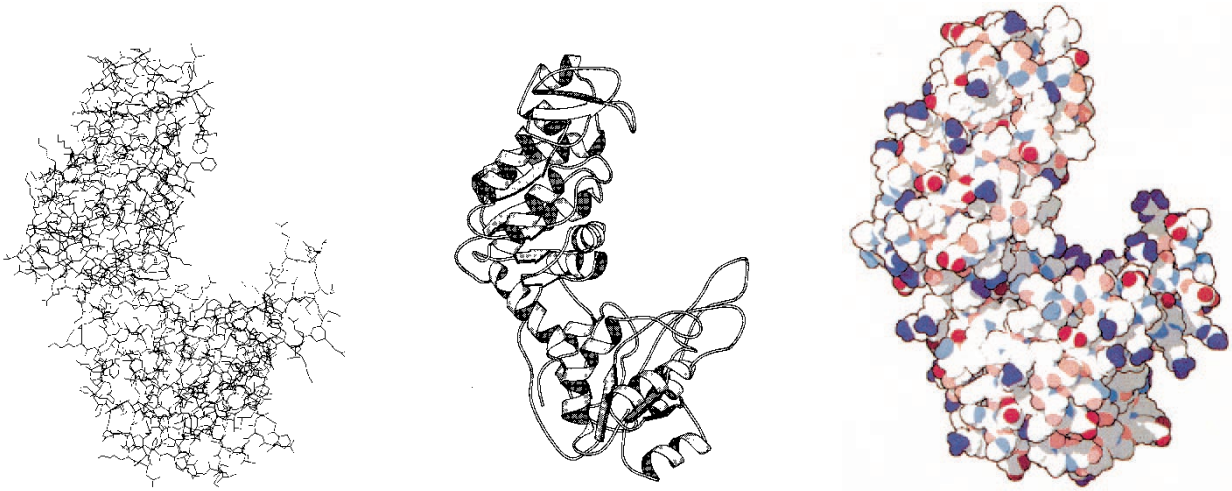


Figure 11.4 Three different ways of depicting a protein, the enzyme phosphoglycerate kinase. At *left* is a bond diagram; all the lines shown represent bonds between the various atoms of the molecule. In the *middle* is a ribbon diagram that emphasizes the secondary structure of the protein. Shown are alpha helices (spiral ribbons) and beta pleated sheets (flat arrows). Finally, on the *right* is a space-filling diagram that emphasizes the volume the molecule fills. The space-filling diagram is what the molecule would generally look like if it were magnified eight million times. (Images by David S. Goodsell, the Scripps Research Institute.)

BOX 11.1

Experimental Methods

Amino Acid Sequencing

Protein-sequencing techniques have been known since 1953, when F. Sanger worked out the complete sequence of the protein hormone insulin. The basic strategy is to purify the protein and then sequence it, beginning at one end. However, since most proteins contain too many amino acids to do this successfully, proteins are first broken into small peptides in several different ways. These peptides are sequenced, and the whole protein sequence can be determined by the overlap pattern of the sequenced subunits.

A protein can be broken into peptide fragments by many different methods, including acid and alkaline hydrolysis. For the most part, proteolytic enzymes (proteases) that hydrolyze the peptides at specific points are used. *Pepsin,* for example, preferentially hydrolyzes peptide bonds involving aromatic amino acids, methionine, and leucine; *chymotrypsin* hydrolyzes peptide bonds involving carboxyl groups of aromatic amino acids; and *trypsin* hydrolyzes bonds involving the carboxyl groups of arginine and lysine.

The proteolytic digest is usually separated into a *peptide map,* or *peptide fingerprint,* by using a two-dimensional combination of paper chromatography, electrophoresis, or column chromatography. In two-dimensional chromatography, a sample is put onto a piece of paper that is then placed in a solvent system. After an allotted time, the paper is dried, turned 90 degrees, and placed in a second solvent system for another allotted time (fig. 1). In each solvent, different peptides travel through the paper at different rates. The spots are then developed using ninhydrin, which reacts with the N-terminal amino acid and produces a colored product when heated.

The spots, which represent small peptides, can be cut out of a second, identical chromatogram that has not been sprayed with ninhydrin. These spots can then be sequenced by, for example, the Edman method, whereby the peptide is sequentially degraded

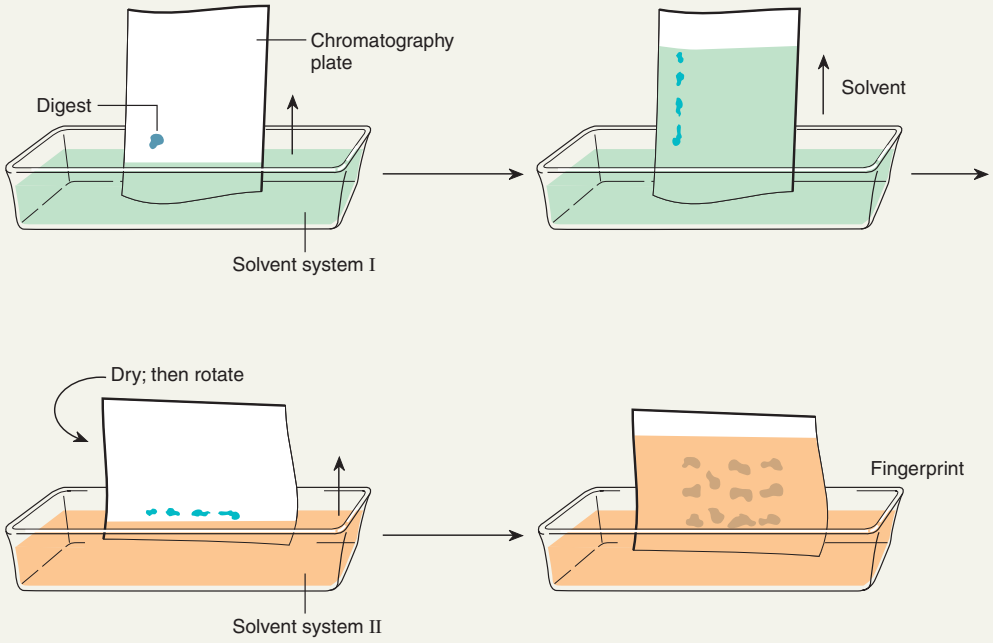


Figure 1 Two-dimensional paper chromatography of a protease digest. Chromatography is done first in one solvent system. The paper is then dried, rotated, and placed into a second solvent system. The pattern on the resulting plate is called a peptide fingerprint.

from the N-terminal end. Phenylisothiocyanate (PITC) reacts with the amino end of the peptide. When acid is added, the N-terminal amino acid is removed as a PITC derivative and can be identified. The process is then repeated until the whole peptide has been sequenced (fig. 2).

If the fingerprint pattern is worked out for two different digests of the same polypeptide, the unique sequence of the original polypeptide can be determined by overlap. In figure 3, the letters A–J represent the ten amino acids in a polypeptide. A is known to be the first (N-terminal) amino acid since the Edman method sequences peptides from this end. We can thus summarize the methodology as follows:

1. A protein is purified. If it is made up of several subunits, these subunits are separated and purified. (If disulfide bridges exist within a peptide, they must be reduced. The bridges are later determined by digestion, keeping the bridges intact, and then resequencing.)
2. Different proteolytic enzymes are used on separate subsamples so that the protein is broken into different sets of peptide fragments.
3. Two-dimensional chromatography, electrophoresis, or column chromatography can be used to isolate the peptides.
4. The Edman method of sequentially removing amino acids from the N-terminal end is used to sequence each peptide.
5. The amino acid sequence from the N- to C-terminal ends of the protein is deduced from the overlap of sequences in peptide digests generated with different proteolytic enzymes.

Today, a machine known as an amino acid sequencer (*sequenator*) can automatically sequence protein. Taking about two hours per amino acid residue, sequenators can carry out Edman degradation on polypeptides up to about fifty amino acids long.

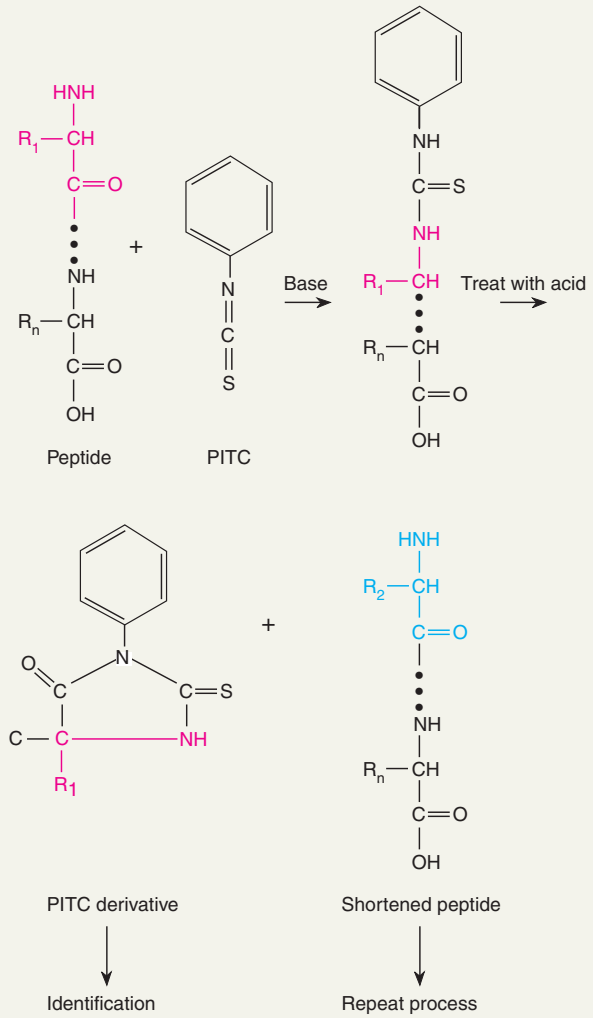


Figure 2 Isolation of amino acids from a peptide for sequencing purposes. First, the peptide reacts with PITC (phenylisothiocyanate) at the amino end. Acid treatment produces a PITC derivative of the amino-terminal amino acid and a peptide one amino acid shorter than the original. The PITC derivative can be identified. These steps are then repeated, isolating one amino acid at a time.

continued

BOX 11.1 CONTINUED

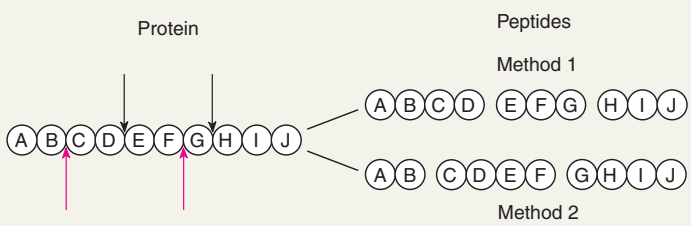


Figure 3 The overlap of peptides digested two different ways provides the sequence of the original peptide.

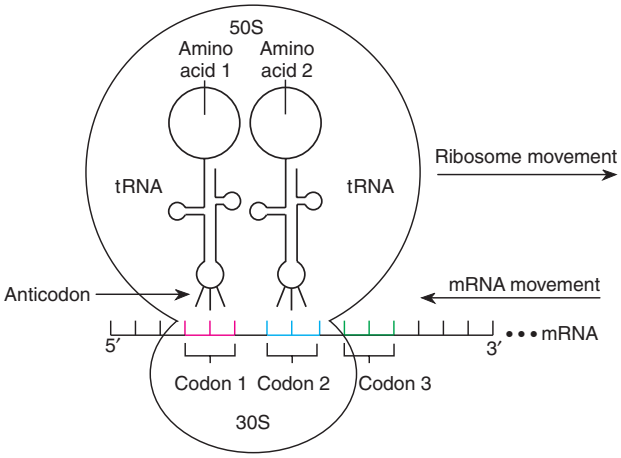


Figure 11.5 The initiation of the translation process at the ribosome. Note the two charged transfer RNAs and the messenger RNA. They are in position to form the first peptide bond between the two amino acids attached to the transfer RNAs.

code for that amino acid. In eukaryotes, there are separate sets of twenty cytoplasmic and twenty mitochondrial synthetases, all coded in the nucleus.

Aminoacyl-tRNA synthetases are a heterogeneous group of enzymes. In *E. coli,* they vary from monomeric proteins (one subunit) to tetrameric proteins, made up of two copies each of two subunits. The enzymes fall into two categories based on sequence similarity, structural features, and whether the amino acid is attached at the 2′-OH (in class I enzymes) or 3′-OH (in class II enzymes) of the 3′-terminal adenosine of the transfer RNA.

To add its appropriate amino acid to the appropriate transfer RNA, a synthetase recognizes many parts of the transfer RNA. This can be shown by experiments that introduce specific changes in transfer RNAs by site-directed mutagenesis (see chapter 13). In seventeen of the twenty *E. coli* synthetases, recognition involves part of the anticodon itself. This makes sense since the anticodon is the defining element of a transfer RNA in protein synthesis.

A synthetase can initially make errors and attach the "wrong" amino acid to a tRNA. For example, isoleucyl-tRNA synthetase will attach valine about once in 225 times. This type of error occurs because a similar, but smaller, amino acid can sometimes occupy the active site of the enzyme (compare isoleucine and valine in fig. 11.1). However, because of a proofreading step, only 1 in 270 to 1 in 800 of the errors are released intact from the enzyme. The amino acids on the rest of the incorrectly charged transfer RNAs are hydrolyzed before the transfer RNAs are released. The overall error rate is the product of the two steps; this means only about one incorrectly charged transfer RNA occurs per 60,000 to 80,000 formed.

In several cases, the number of amino acyl-tRNA synthetases in a particular organism is below twenty. For example, in some archaea, there is no cysteinyl-tRNA synthetase. However, the prolyl-tRNA synthetase activates the tRNAs for both cysteine and proline with their appropriate amino acids. Similarly, in some eubacteria, there is no glutaminyl-tRNA synthetase; the glutaminyl tRNA is charged with glutamic acid, rather than glutamine. An amido transferase enzyme then converts the glutamic acid to glutamine (see fig. 11.1).

There are sixty-four possible codons in the genetic code (four nucleotide bases in groups of three $= 4 \times 4 \times 4 = 64$). Three of these codons are used to terminate translation. Thus, sixty-one transfer RNAs are needed because there are sixty-one different nonterminator codons. About fifty transfer RNAs are known in *E. coli.* The number fifty can be explained by the wobble phenomenon, which occurs in the third position of the codon. We examine this phenomenon in the section on the genetic code. The transfer RNAs for each amino acid are designated by the convention $tRNA^{Leu}$ (for leucine), $tRNA^{His}$ (for histidine), and so on.

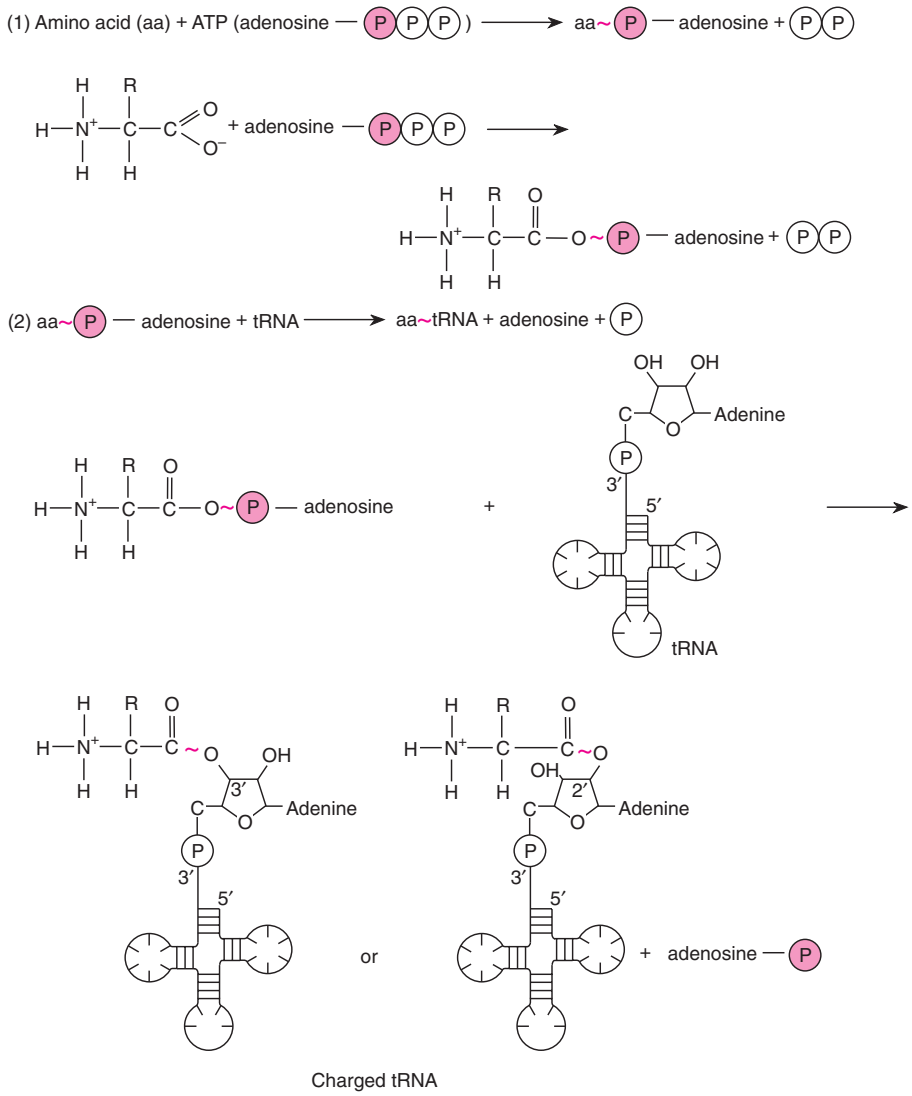


Figure 11.6 It takes a two-step process to attach a specific amino acid to its transfer RNA by an aminoacyl synthetase. High-energy bonds are indicated by ~. In the first step, an amino acid is attached to AMP with a high-energy bond. In the second step, the high-energy bond is transferred to the tRNA, which is then referred to as "charged." Depending on which class of aminoacyl-tRNA synthetase is involved, the amino acid will be attached to either the 2′ or 3′ carbon of the sugar of the 3′ terminal adenosine.

Recognition of the Aminoacyl-tRNA During Protein Synthesis

Although amino acids enter the protein-synthesizing process attached to transfer RNAs, it was theoretically possible that the ribosome recognized the amino acid itself during translation. A simple experiment was done to determine whether the amino acid or the transfer RNA was recognized.

In 1962, F. Chapeville and colleagues isolated transfer RNA with cysteine attached. They chemically converted the cysteine to alanine by using Raney nickel, a catalytic form of nickel that removes the SH group of cysteine (fig. 11.7). When these transfer RNAs were used in protein synthesis, alanine was incorporated where cysteine should have been, demonstrating that the transfer RNA, not the amino acid, was recognized during protein synthesis. The synthetase puts a specific amino acid on a specific transfer RNA; then, during protein synthesis, the anticodon on the transfer RNA—not the amino acid itself—determines which amino acid is incorporated.

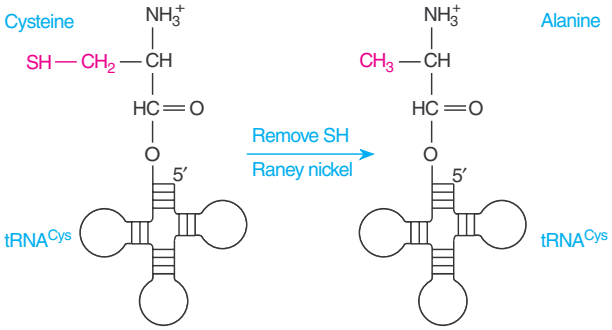


Figure 11.7 Cysteine-tRNA^{Cys} treated with Raney nickel becomes alanine-tRNA^{Cys} by the removal of the SH group of cysteine. During protein synthesis, alanine is incorporated in place of cysteine in proteins, indicating that the specificity of amino acid incorporation into proteins resides with the tRNA.

Initiation Complex

Translation can be divided into three stages: initiation, elongation, and termination. Elongation is the repetitive process of adding amino acids to a growing peptide chain. However, added complexity enters the picture in the initiation and termination of protein synthesis.

It is especially important that the translation process start precisely. Remember that the genetic code is translated in groups of three nucleotides (codons). If the reading of the messenger RNA begins one base too early or too late, the reading frame is shifted so that an entirely different set of codons is read (fig. 11.8). The protein produced, if any, will probably bear no structural or functional resemblance to the protein the gene is coded for.

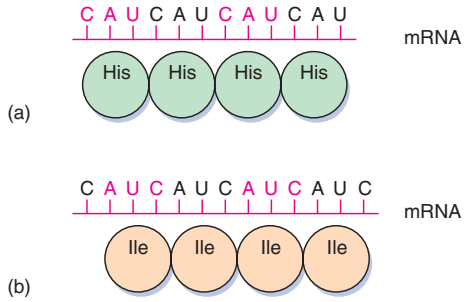


Figure 11.8 (*a*) In the normal reading of the messenger RNA, these codons are read as repeats of CAU, coding for histidine. (*b*) A shift in the reading frame of the messenger RNA causes the codons to be read as AUC repeats coding for isoleucine.

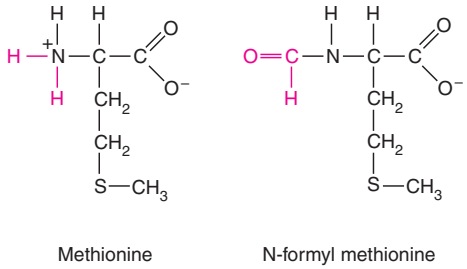


Figure 11.9 The structures of the amino acids methionine and N-formyl methionine.

Role of N-Formyl Methionine

The synthesis of every protein in *Escherichia coli* begins with the modified amino acid N-formyl methionine (fig. 11.9). However, none of the completed proteins in *E. coli* contains N-formyl methionine. Many of these proteins do not even have methionine as their first amino acid. Obviously, before a protein becomes functional, the initial amino acid is modified or removed. In eukaryotes the initial amino acid, also methionine, does not have an N-formyl group.

Methionine, with a codon of 5′-AUG-3′, known as the **initiation codon,** has two transfer RNAs with the same complementary anticodon (3′-UAC-5′) but with different structures (fig. 11.10). One of these transfer RNAs ($tRNA_f^{Met}$) serves as a part of the initiation complex. Before the initiation of translation, this transfer RNA will have its methionine chemically modified to N-formyl methionine (fMet). The other transfer RNA will not have its methionine modified ($tRNA_m^{Met}$). The translation machinery will use it to insert methionine into proteins, where called for, in all but the first position. The cell thus has a mechanism to make use of methionine in the normal way as well as to use a modified form of it to initiate protein synthesis. Because of the structure of the prokaryotic initiation transfer RNA, it can recognize AUG, GUG, and, rarely, UUG as initiation codons. In eukaryotes, CUG as well as AUG can serve as an initiation codon. Since the initiation methionine is not formylated in eukaryotes, the eukaryotic transfer RNA is designated $tRNA_i^{Met}$; there is a separate internal methionine transfer RNA, termed $tRNAm_m^{Met}$, in eukaryotes, as in prokaryotes.

Translation Initiation

The subunits of the ribosome (30S and 50S) usually dissociate from each other when not involved in translation. To begin translation, an **initiation complex** forms, consisting of the following components in prokaryotes: the 30S subunit of the ribosome, a messenger RNA, the charged N-formyl methionine tRNA (fMET-$tRNA_f^{Met}$), and

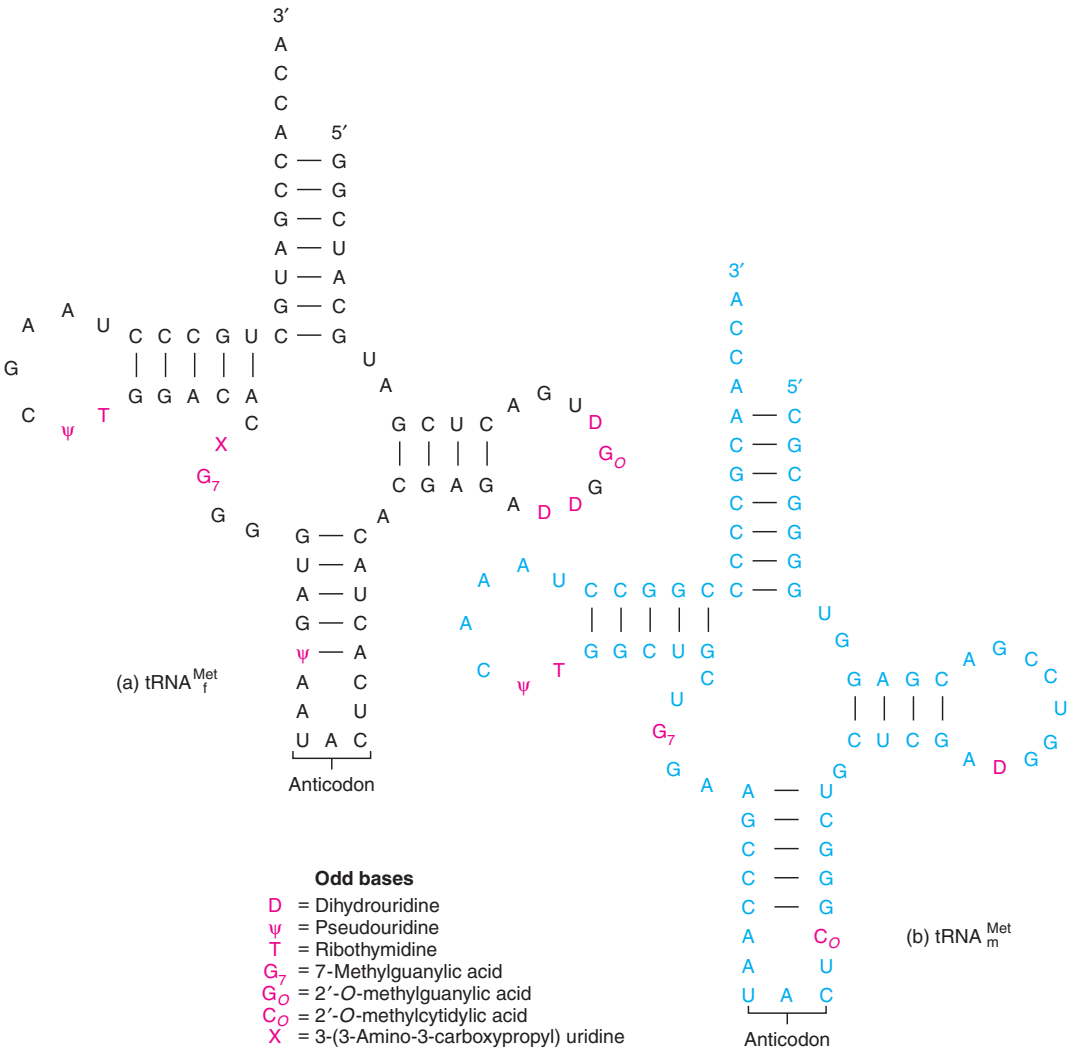


Figure 11.10 The two tRNAs for methionine in *E. coli.* (*a*) The initiator tRNA. (*b*) The interior tRNA.

three **initiation factors (IF1, IF2, IF3).** Initiation factors (as well as elongation and termination factors) are proteins loosely associated with the ribosome. They were discovered when ribosomes were isolated and then washed, losing the ability to perform protein synthesis.

The components that form the initiation complex interact in a series of steps. It is known that IF3 binds to the 30S ribosomal subunit, allowing the 30S subunit to bind to messenger RNA (fig. 11.11, *step 1*). Meanwhile, a complex forms with IF2, the charged N-formyl methionine tRNA (fMET-tRNA$_f^{Met}$) and GTP (guanosine triphosphate; fig. 11.11, *step 2*). It is IF2 that brings the initiator transfer RNA to the ribosome. IF2 binds only to the charged initiator transfer RNA, and, without IF2, the initiator transfer RNA cannot bind to the ribosome. The final step in initiation-complex formation is bringing together the first two components (fig. 11.11, *step 3*).

The hydrolysis of GTP to GDP + P_i (inorganic phosphate, PO_4^{-3}—see fig. 9.8) produces conformational changes; these changes allow the initiation complex to join the 50S ribosomal subunit to form the complete ribosome and then allow the initiation factors and GDP to be released. Frequently, the hydrolysis of a nucleoside triphosphate (e.g., ATP, GTP) in a cell occurs to release the energy in the phosphate bonds for use in a metabolic process. However, in the process of translation, the hydrolysis apparently changes the shape of the GTP so that it and the initiation factors can be released from the ribosome after the 70S particle has been formed. Thus, hydrolysis of GTP in translation is for conformational

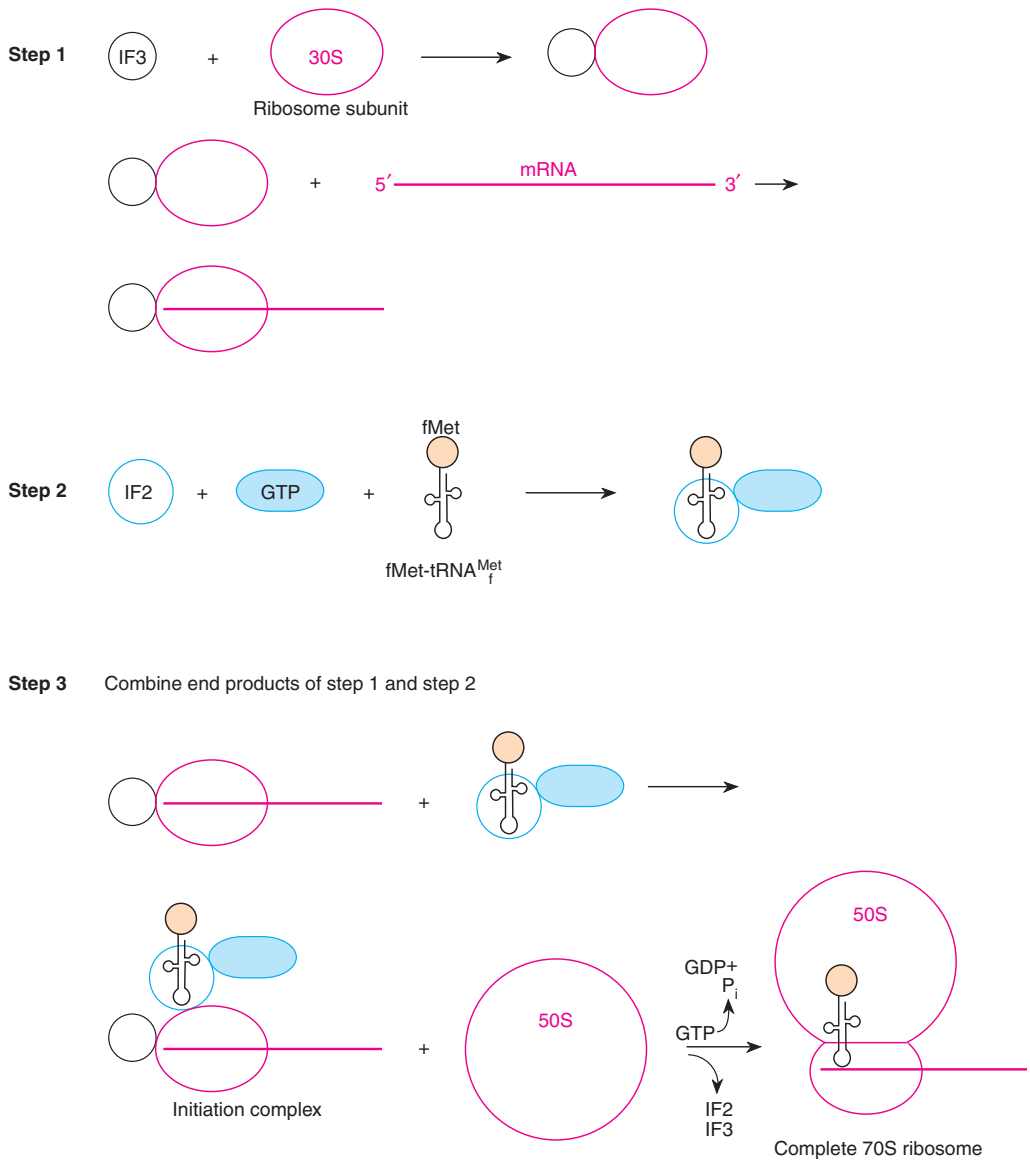


Figure 11.11 The prokaryotic 70S ribosome forms in a three-step process. In the first step, the 30S ribosomal subunit and the mRNA combine. In the second step, the initiator tRNA combines with IF2. In the final step, the components from steps 1 and 2 combine to form the initiation complex, followed by the formation of the 70S ribosome.

change rather than covalent bond formation. IF1 helps the other two initiation factors bind to the 30S ribosomal subunit or stabilizes the 30S initiation complex.

The process in eukaryotes is generally similar, but more complex. The eukaryotic initiation factor abbreviations are preceded by an "e" to denote that they are eukaryotic (eIF1, eIF2, etc.). At least eleven initiation factors are involved, including a specific cap-binding protein, eIF4E.

The ribosome apparently recognizes the prokaryotic messenger RNA through complementarity of a region at the 3′ end of the 16S ribosomal RNA and a region slightly upstream from the initiation sequence (AUG) on the messenger RNA. This idea, the **Shine-Dalgarno hypothesis** (fig. 11.12), is named after the people who first suggested it. The sequence (AGGAGGU) of complementarity between the messenger RNA and the 16S ribosomal RNA is referred to as the Shine-Dalgarno sequence. Although

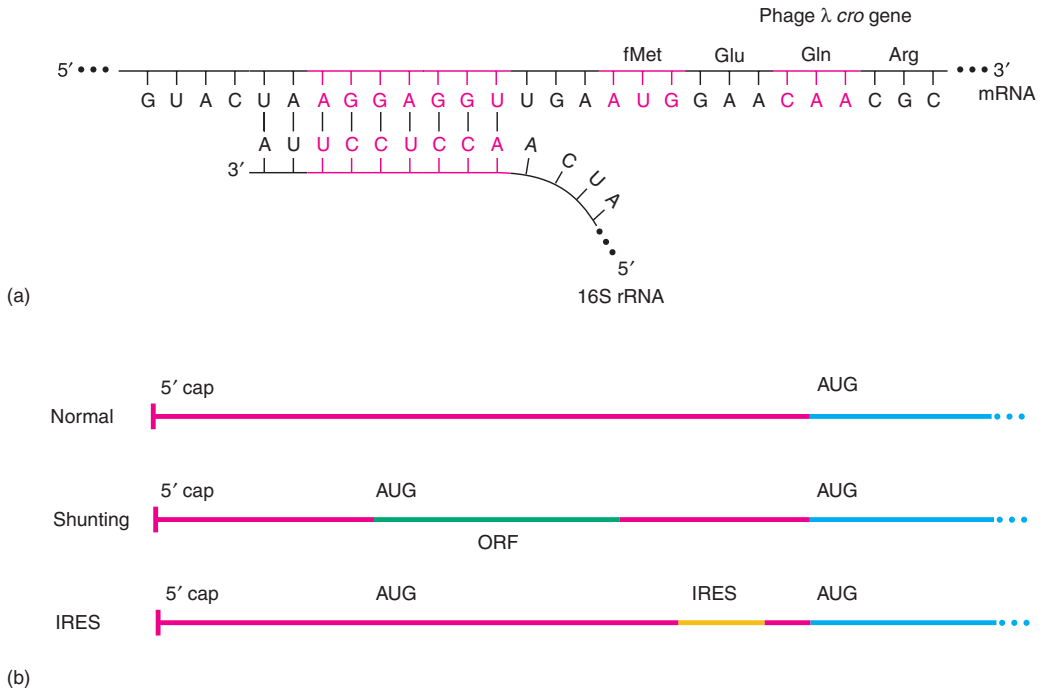


Figure 11.12 Translation initiation signals. (*a*) The Shine-Dalgarno hypothesis for prokaryotic translation. The Shine-Dalgarno sequence (AGGAGGU) is on the prokaryotic messenger RNA just upstream from the initiation codon AUG. Complementarity exists between this sequence and a complementary sequence (UCCUCCA) on the 3' end of the 16S ribosomal RNA. (*b*) Scanning, shunting, and internal ribosome entry in eukaryotic messenger RNA. The 5' untranslated region of a eukaryotic gene is shown in *red;* the beginning of the gene in *blue.* Normally, in the scanning model, the initiation codon of the gene is the first AUG encountered. In shunting, an open reading frame (ORF, *green*) may or may not be present to provide secondary structure in the messenger RNA to shunt scanning to the main gene. If the open reading frame is translated, reinitiation of translation at the same ribosome may occur at the main gene. Finally, an internal ribosome entry site (IRES, *yellow*) allows translation to begin within the messenger RNA without scanning.

there is a good deal of homology between prokaryotic and eukaryotic small ribosomal RNAs, the Shine-Dalgarno region is absent in eukaryotes. The actual mechanism for recognizing the 5′ end of eukaryotic messenger RNA appears to be based on recognition of the 5′ cap of the messenger RNA by the cap-binding protein with recruitment of other initiation factors and the small subunit of the ribosome. This is followed by the small subunit's movement down the messenger RNA. The ribosome scans the messenger RNA until it recognizes the initiation codon. This model is referred to as the **scanning hypothesis.**

In several known cases in eukaryotes, a process called **shunting** occurs, in which the first AUG does not serve as the initiation codon; rather, scanning begins, but it bypasses a region of the messenger RNA upstream of the initiation codon, called the *leader* or **5′ untranslated region (5′ UTR),** in favor of an AUG further down the messenger RNA. The cause of shunting seems to be secondary structure in the messenger RNA, upstream from the AUG codon that actually serves as the initiation codon. In some cases, very small genes, called **open reading frames (ORFs),** are present in this region of the messenger RNA and play some role in shunting. It seems also that some ORFs are translated, and then the main gene is translated by the same ribosome in a process called **reinitiation** (fig. 11.12*b*). We have seen this in the genes of some plant and animal viruses; it is a topic under current study.

Under some circumstances, eukaryotic ribosomes can initiate protein synthesis within the messenger RNA if that messenger RNA contains a sequence called an **internal ribosome entry site.** These sequences were discovered in the poliovirus RNA and in several cellular messenger RNAs. They are at least four hundred nucleotides long. Thus, although scanning accounts for the initiation of most eukaryotic messenger RNAs at their 5′ ends, some initiation can take place internally in messenger RNAs that have internal ribosome entry sites.

Aminoacyl and Peptidyl Sites in the Ribosome

When the initiator transfer RNA joins the 30S subunit of the prokaryotic ribosome with its messenger RNA attached, it fits into one of three sites in the ribosome. These sites, or cavities in the ribosome, are referred to as the aminoacyl site **(A site),** the peptidyl site **(P site),** and the exit site (**E site,** fig. 11.13) Here, we concentrate on the A and P sites, each of which contains a transfer RNA just before forming a peptide bond: the P site contains the transfer RNA with the growing peptide chain (peptidyl-tRNA); the A site contains a new transfer RNA with its single amino acid (aminoacyl-tRNA). The exit site helps eject depleted transfer RNAs after a peptide bond forms. When the complete 70S ribosome of figure 11.11 has formed, the initiation fMET-$tRNA_f^{Met}$ is placed directly into the P site (fig. 11.13), the only charged transfer RNA that can be placed directly there. The association of transfer RNA and ribosome is aided by a G-C base pairing between the 3′-CCA terminus of all transfer RNAs and a guanine in the 23S ribosomal RNA.

Elongation

Positioning a Second Transfer RNA

The next step in prokaryotic translation is to position the second transfer RNA, which is specified by the codon at the A site. The second transfer RNA is positioned in the A site of the ribosome so it is able to form hydrogen bonds between its anticodon and the second codon on the messenger RNA. This step requires the correct transfer RNA, another GTP, and two proteins called **elongation factors** (**EF-Ts** and **EF-Tu**). EF-Tu, bound to GTP, is required to position a transfer RNA into the A site of the ribosome (fig. 11.14). After the transfer RNA is positioned, the GTP is hydrolyzed to GDP + P_i. Upon hydrolysis of the GTP, the EF-Tu/GDP complex is released from the ribosome. EF-Ts is required to regenerate an EF-Tu/GTP complex. EF-Ts displaces the GDP on EF-Tu. Then a new GTP displaces EF-Ts, and now the EF-Tu/GTP complex can bind another transfer RNA. Here again, the hydrolysis of GTP changes the shape of the GTP so that the EF-Tu/GDP complex can depart from the ribosome after the transfer RNA is in place in the A site (fig. 11.15). Figure 11.16 shows the ribosome at the end of this step. EF-Tu does not bind fMet-$tRNA_f^{Met}$, so this blocked (formylated) methionine cannot be inserted into a growing peptide chain.

It takes several milliseconds for the GTP to be hydrolyzed, and another few milliseconds for the EF-Tu/GDP to actually leave the ribosome. During those two intervals of time, the codon-anticodon fit of the transfer RNA is scrutinized. If the correct transfer RNA is in place, a peptide bond forms. If not, the charged transfer RNA is released and a new cycle of EF-Tu/GTP-mediated testing of transfer RNAs begins. The error rate is only about one mistake in ten thousand amino acids incorporated into protein. The speed of amino acid incorporation is about fifteen amino acids per second in prokaryotes and about two to five per second in eukaryotes.

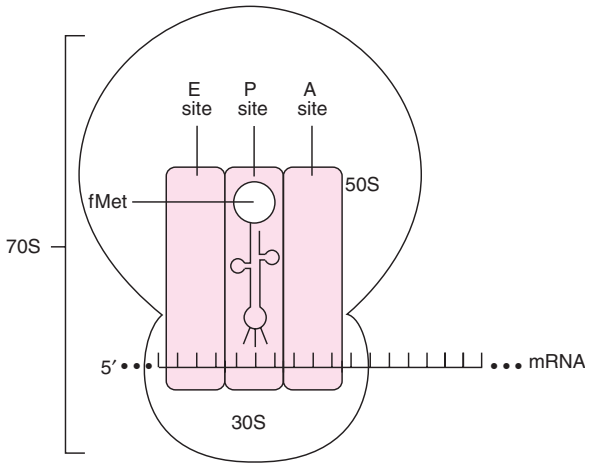


Figure 11.13 The 70S ribosome contains an A site, a P site, and an E site that can receive tRNAs. The messenger RNA runs through the bottom of the sites.

Peptide Bond Formation

The two amino acids on the two transfer RNAs are now in position to form a peptide bond between them; both amino acids are juxtaposed to an enzymatic center, **peptidyl transferase,** in the 50S subunit. This enzymatic center, an integral part of the 50S subunit, was originally believed to be composed of parts of several of the 50S proteins. Now, however, it is believed to have ribozymic activity, enzymatic activity of the ribosomal RNA of the ribosome. The enzymatic activity involves a bond transfer from the carboxyl end of N-formyl methionine to the amino end of the second amino acid (phenylalanine in fig. 11.16). Every subsequent peptide bond is identical, regardless of the amino acids involved. The energy used is contained in the high-energy ester bond between the transfer RNA in the P site and its amino acid (fig. 11.17). Immediately after the formation of the peptide bond, the transfer RNA with the dipeptide is in the A site, and a depleted transfer RNA is in the P site (box 11.2).

Translocation

The next stage in elongation is translocation of the ribosome in relation to the transfer RNAs and the messenger

RNA. Elongation factor EF-G, earlier called translocase, catalyzes the translocation process. The ribosome must be converted from the *pretranslocational state* to the *posttranslocational state* by the action of EF-G, which physically moves the messenger RNA and its associated transfer RNAs (fig. 11.18). This movement is accomplished by the hydrolysis of a GTP to GDP after EF-G enters the ribosome at the A site. After the first posttranslocational state is reached, the depleted transfer RNA in the E site is ejected, leaving the ribosome ready to accept a new charged transfer RNA in the A site. A computer-generated diagram of a ribosome with all three transfer RNA sites occupied is shown in figure 11.18*b*. In eukaryotes, three elongation factors perform the same tasks that EF-Tu, EF-Ts, and EF-G perform in prokaryotes. The factor eEF1α replaces EF-Tu, eEF1βγ replaces EF-Ts, and eEF2 replaces EF-G.

When translocation is complete, the situation is again as diagrammed in figure 11.13, except that instead of fMet-$tRNA_f^{Met}$, the P site contains the second transfer RNA ($tRNA^{Phe}$) with a dipeptide attached to it. The process of elongation is then repeated, with a third transfer RNA coming into the A site. The process repeats from here to the end (fig. 11.19), synthesizing a peptide starting from the amino (N-terminal) end and proceeding to the carboxyl (C-terminal) end. During the repetitive aspect of

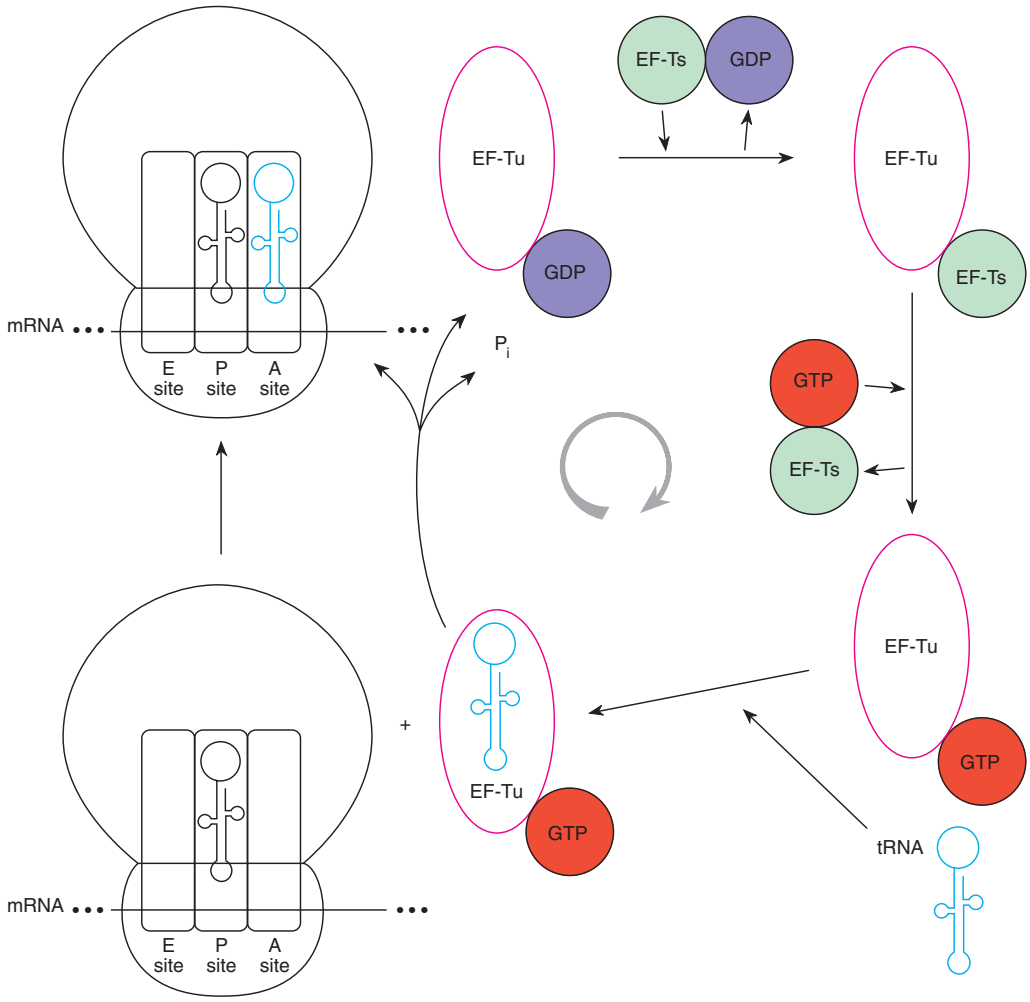


Figure 11.14 The EF-Ts/EF-Tu cycle. EF-Ts and EF-Tu are required for a transfer RNA to attach to the A site of the ribosome. At *top center,* we have EF-Tu attached to a GDP. The GDP is then displaced by EF-Ts, which in turn is displaced by GTP. A transfer RNA attaches and is brought to the ribosome. If the codon-anticodon fit is correct, the transfer RNA attaches at the A site with the help of the hydrolysis of GTP to GDP + P_i, allowing the EF-Tu to release. The EF-Tu is now back where we started. Since EF-Tu has a strong affinity for GDP, the role of EF-Ts is to displace the GDP, and later to be replaced by GTP.

BOX 11.2

Biomedical Applications

Antibiotics

Antibiotics, substances living organisms produce that are toxic to other living organisms, are of interest to us for two reasons: They have been extremely important in fighting the diseases that strike human beings and farm animals, and many are useful tools for analyzing protein synthesis. Some antibiotics impede the process of protein synthesis in a variety of ways, often poisoning bacteria selectively; the effectiveness of antibiotics normally derives from the metabolic differences between prokaryotes and eukaryotes. For example, an antibiotic that blocks a 70S bacterial ribosome without affecting an 80S human ribosome could be an excellent antibiotic. About 160 antibiotics are known.

PUROMYCIN

Puromycin resembles the 3′ end of an aminoacyl-tRNA (fig. 1). It is bound to the A site of the bacterial ribosome, where peptidyl transferase creates a bond from the nascent peptide attached to the transfer RNA in the P site to puromycin. Elongation can then no longer occur. The peptide chain is released prematurely, and protein synthesis at the ribosome terminates.

Experiments with puromycin helped demonstrate the existence of the A and P sites of the ribosome. It was found that puromycin could not bind to the ribosome if translocation factor EF-G were absent. With EF-G, translocation took place, and puromycin could then bind to the ribosome. Puromycin's ability to bind only after translocation indicates that a second site on the ribosome becomes available after translocation.

STREPTOMYCIN, TETRACYCLINE, AND CHLORAMPHENICOL

Streptomycin, which binds to one of the proteins (protein S12) of the 30S subunit of the prokaryotic ribosome, inhibits initiation of protein synthesis. Streptomycin also causes misreading of codons if chain initiation has already begun, presumably by altering the conformation of the ribosome so that transfer RNAs are less firmly bound to it. Bacterial mutants that are streptomycin resistant, as well as mutants that are streptomycin dependent (they cannot survive without the antibiotic), occur. Both types of mutants have altered 30S subunits, specifically changed in protein S12.

Tetracycline blocks protein synthesis by preventing an aminoacyl-tRNA from binding to the A site on the ribosome. Chloramphenicol blocks protein synthesis by binding to the 50S subunit of the prokaryotic ribosome, where it blocks the peptidyl transfer reaction. Chloramphenicol does not affect the eukaryotic ribosome. However, chloramphenicol, as well as several other antibiotics, is used cautiously because the mitochondrial ribosomes within eukaryotic cells are very similar to prokaryotic ribosomes. Some of the antibiotics that affect prokaryotic ribosomes thus also affect mitochondria. As was mentioned, the similarity between bacteria and mitochondria implies that mitochondria have a prokaryotic origin. (Similarities between cyanobacteria and chloroplasts also support the idea that chloroplasts have a prokaryotic origin.)

THE TROUBLE WITH ANTIBIOTICS

Over the years, antibiotics have virtually eliminated certain diseases from the industrialized world. They have also made modern surgery possible by preventing most serious infections that tend to follow operations. Antibiotics have been so successful that, in the 1980s, many pharmaceutical companies drastically cut back the development of new antibiotics. However, a disaster was in the making as we overprescribed antibiotics to people and farm animals: bacteria are not prepared to take this onslaught without fighting back.

Mutation takes place all the time at a low but dependable rate. Thus, resistant bacteria are constantly arising from sensitive strains. We can select for penicillin- and streptomycin-resistant strains of bacteria in the laboratory by allowing the antibiotic to act as a selective agent, removing all but the resistant individuals. The same sort of artificial selection that we can apply in the lab applies every time a person or animal takes an antibiotic. We may be at a point now where the ability of bacteria to develop resistance, and to pass that resistance to other strains, has put us on the verge of disaster. The process of evolution works amazingly fast in bacteria because of their ubiquity, large population sizes, and ability to transfer genetic material between individuals. We may shortly find ourselves as we were before World War II, when simple infections in hospitals were often lethal. Right now, only one antibiotic can keep the common—and potentially deadly—infectious bacterium *Staphylococcus* under control: vancomycin. Several types of disease-causing bacteria have already evolved a tolerance to vancomycin.

The answer to this potentially disastrous problem is to develop new antibiotics and reduce the irresponsible use of antibiotics in people and animals. Hopefully, the warning bell has sounded. At least a dozen new antibiotics that show promise are in the early stages of development by pharmaceutical companies.

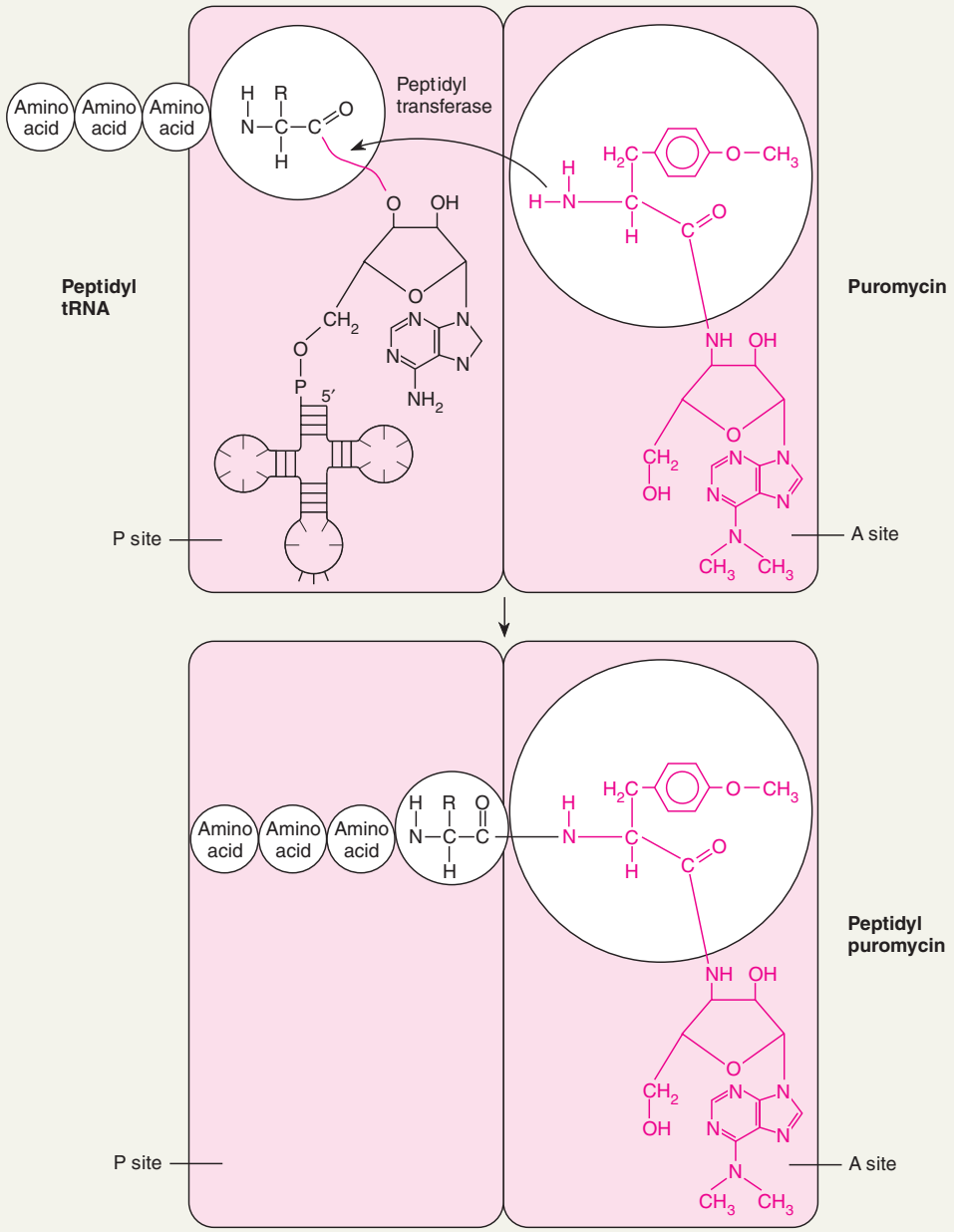
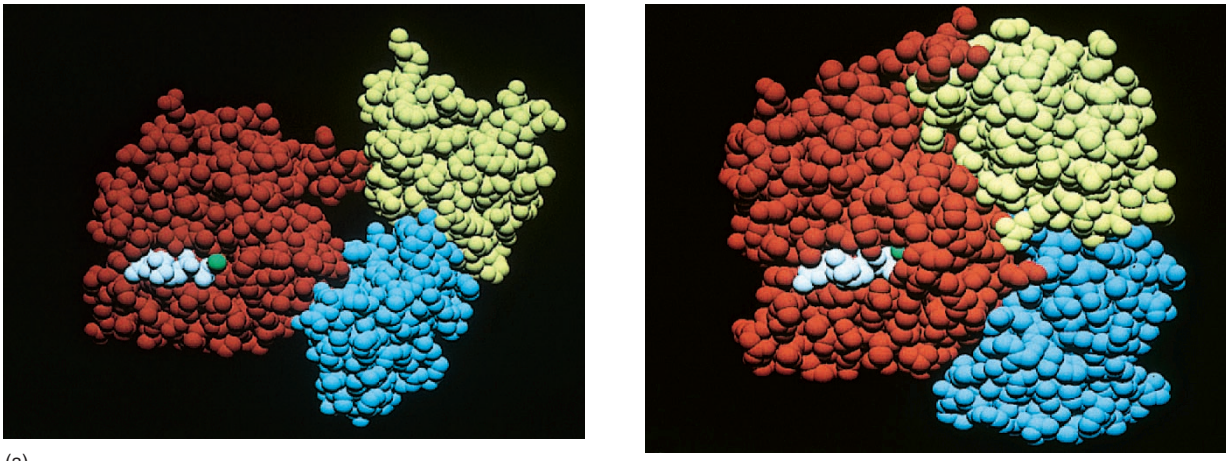


Figure 1 Puromycin is bound to the A site of the ribosome. A peptide bond then forms. Further elongation is prevented, and the chain is terminated.



(a) (b)

Figure 11.15 Space-filling model of EF-Tu bound with (*a*) GDP and (*b*) GTP, showing the change in the protein's structure. *Yellow, blue,* and *red* are domains of the protein. The GTP and GDP are in *white,* with a magnesium ion, Mg^{2+}, in *green.* When EF-Tu is bound with GDP, there is a visible hole in the molecule. The hole disappears when GTP is bound. The aminoacyl-transfer RNA is believed to bind between the *red* and *yellow* domains. (Courtesy of Rolf Hilgenfeld.)

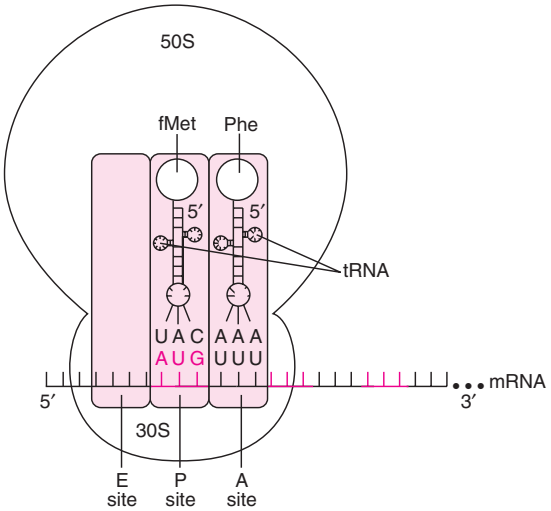


Figure 11.16 A ribosome with two transfer RNAs attached. In this case, the second codon (UUU) is for the amino acid phenylalanine. The two amino acids are next to each other.

protein synthesis, two GTPs are hydrolyzed per peptide bond: one GTP in the release of EF-Tu from the A site, and one GTP in the translocational process of the ribosome after the peptide bond has formed. In addition, every charged transfer RNA has had an amino acid attached at the expense of the hydrolysis of an ATP to AMP +PP. There is some evidence that the action of EF-Tu hydrolyzes two GTPs.

Termination

Nonsense Codons

Termination of protein synthesis in both prokaryotes and eukaryotes occurs when one of three **nonsense codons** appears in the A site of the ribosome. These codons are UAG (sometimes referred to as *amber*), UAA (*ochre*), and UGA (*opal*). ("Amber," or brown stone, is the English translation of the name Bernstein, a graduate student who took part in the discovery of UAG in R. H. Epstein's lab at the California Institute of Technology. "Ochre" and "opal" are tongue-in-cheek extensions of the first label.) In prokaryotes, three proteins called **release factors (RF)** are involved in termination, and a GTP is hydrolyzed to GDP + P_i.

When a nonsense codon enters the A site on the ribosome, a release factor recognizes it. RF1 and RF2 are class 1 release factors: They recognize stop codons and then promote hydrolysis of the bond between the terminal amino acid and its tRNA in the P site. Class 2 release factors (RF3) do not recognize stop codons, but they stimulate class 1 release factors to act. RF1 recognizes the stop codons UAA and UAG, and RF2 recognizes UAA and UGA (fig. 11.20). Both do so because they have tripep-

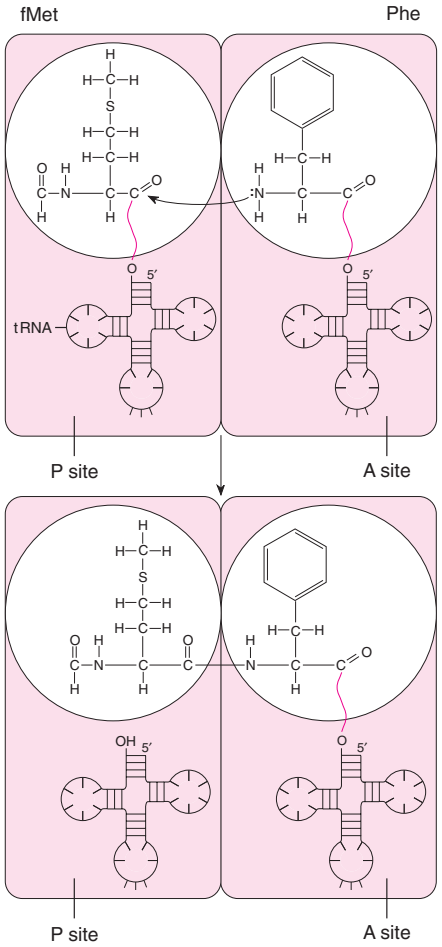


Figure 11.17 Peptide bond formation on the ribosome between N-formyl methionine and phenylalanine. The bond attaching the carboxyl end of the first amino acid to its tRNA is transferred to the amino end of the second amino acid. The first tRNA is now uncharged, whereas the second tRNA has a dipeptide attached.

tides that mimic anticodons to recognize the stop codons: proline-alanine-threonine in RF1 and serine-proline-phenylalanine in RF2. In this **molecular mimicry,** a protein mimics the shape of a nucleic acid in order to function properly.

The next base in the messenger RNA past the stop codon is usually an adenine, required for efficient termination. After the release factors act, with the hydrolysis of a GTP, the ribosome has completed its task of translating mRNA into a polypeptide. Final release of all factors and dissociation of the two subunits of the ribosome take place with the help of IF3, which rebinds to the 30S subunit, and a **ribosome recycling factor (RRF).** Table 11.1 compares prokaryotic and eukaryotic translation.

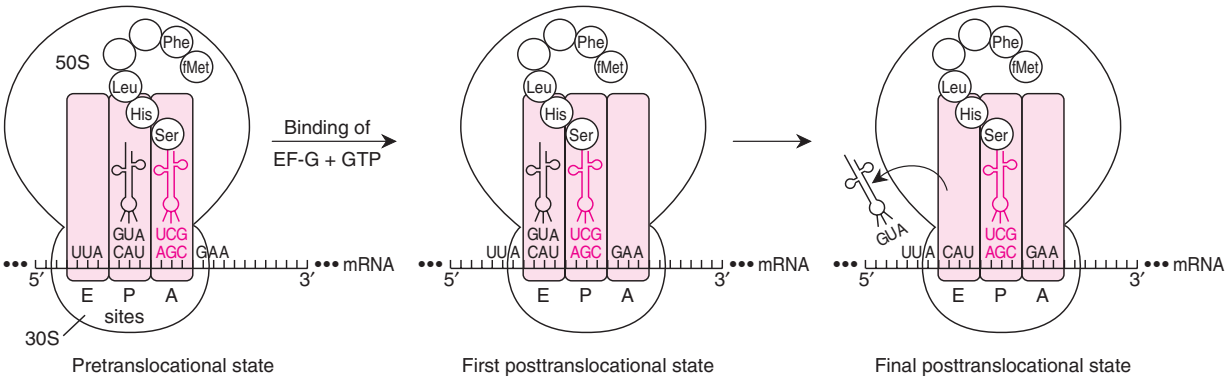
Rate and Cost of Translation

As mentioned, the average speed of protein synthesis is about fifteen peptide bonds per second in prokaryotes. Discounting the time for initiation and termination, an average protein of three hundred amino acids is synthesized in about twenty seconds (the released protein forms its final structure spontaneously or is modified with the aid of other proteins, as we shall see). An equivalent eukaryotic protein takes about 2.5 minutes to be synthesized. The energy cost is at least four high-energy phosphate bonds per peptide bond (two from an ATP during transfer RNA charging, and two from GTP hydrolysis during transfer RNA binding at the A site and translocation), or about twelve hundred high-energy bonds per protein. This cost is very high—about 90% of the energy production of an *E. coli* cell goes into protein synthesis. A high energy cost is presumably the price a living system has to pay for the speed and accuracy of its protein synthesis.

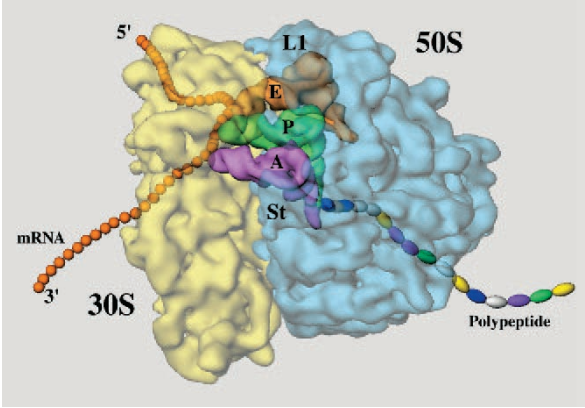
Coupling of Transcription and Translation

In prokaryotes, such as *E. coli,* in which no nuclear envelope exists, translation begins before transcription is completed. Figure 11.21 shows a length of an *E. coli* chromosome. An RNA polymerase is visible on the DNA, transcribing a gene. The messenger RNA, still being synthesized, can be seen extending away from the DNA. Attached to the messenger RNA are about a dozen ribosomes. Since translation starts at the end of the messenger that is synthesized first (5′), an initiation complex can form and translation can begin shortly after transcription begins. As translation proceeds along the messenger, its 5′ end will again become exposed, and a new initiation complex can form. The occurrence of several ribosomes translating the same messenger is referred to as a **polyribosome,** or simply a **polysome** (fig. 11.22).

In prokaryotes, most messenger RNAs contain the information for several genes. These RNAs are said to be **polycistronic** (fig. 11.23). (*Cistron,* another term for gene, is defined in chapter 12.) Each gene on the messenger RNA is translated independently: each has a Shine-Dalgarno sequence for ribosome recognition (see fig. 11.12) and an initiation codon (AUG) for fMet. The ribosome that completes the translation of the first gene may or may not continue to the second gene after dissociation. The translation of any gene follows all the steps we have outlined.



(a)

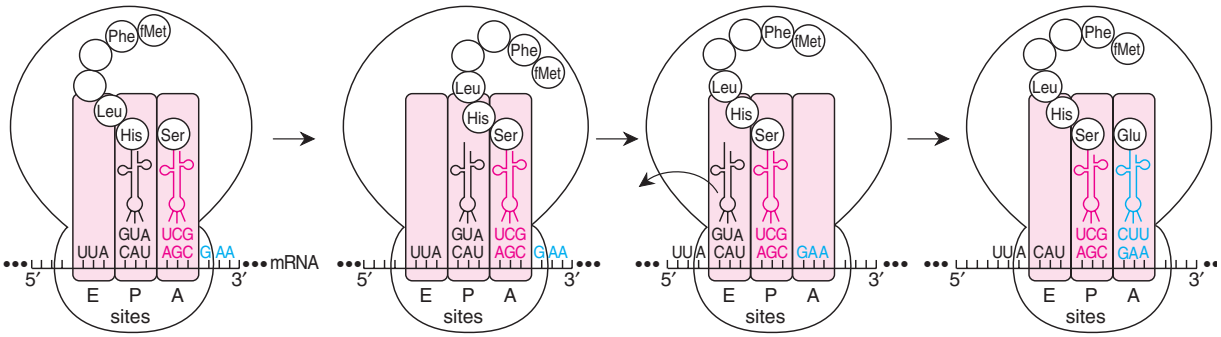


(b)

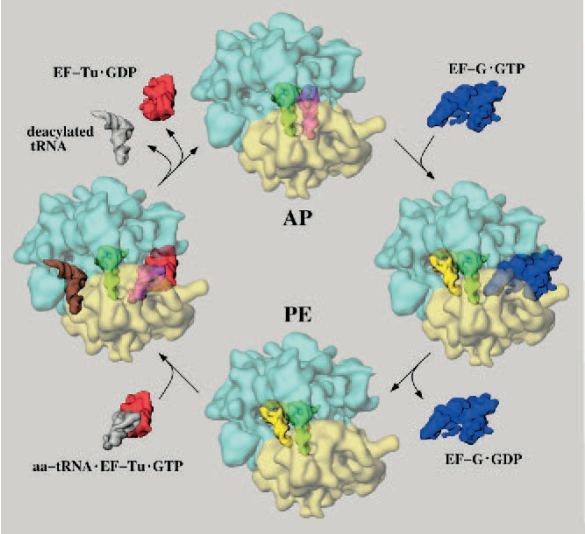
Figure 11.18 (*a*) EF-G's translocation of the ribosome converts it from a pretranslocational state (P and A sites occupied) to a posttranslocational state (E and P sites occupied). The uncharged transfer RNA in the E site is then ejected. (*b*) A see-through model of the 70S ribosome of *E. coli* with transfer RNAs in the A, P, and E sites. The structure was determined by cryoEM mapping, an electron microscopic technique using rapidly frozen specimens. The position of the messenger RNA is shown, as well as the stalk of the 50S subunit (St) and one of the polypeptides of the large subunit, L1. ([*b*] Courtesy of Joachim Frank, Howard Hughes Medical Institute.)

Table 11.1 **Some Comparisons Between Prokaryotic and Eukaryotic Translation**

	Prokaryotes	Eukaryotes
Initiation codon	AUG, occasionally GUG, UUG	AUG, occasionally GUG,CUG
Initiation amino acid	N-formyl methionine	Methionine
Initiation tRNA	$tRNA_f^{Met}$	$tRNA_i^{Met}$
Interior methionine tRNA	$tRNA_m^{Met}$	$tRNA_m^{Met}$
Initiation factors	IF1,IF2,IF3	eIF factors
Elongation factor	EF-Tu	eEF1α
Elongation factor	EF-Ts	eEF1βγ
Translocation factor	EF-G	eEF2
Release factors	RF1, RF2, RF3, RRF	eRF1, eRF3



(a)



(b)

Figure 11.19 Cycle of peptide bond formation and translocation on the ribosome. (*a*) After the peptide bond is transferred (fig. 11.17), the ribosome and messenger RNA move over one codon. Now the transfer RNA with the peptide is in the P site, and the A site is again open. In this example, the next transfer RNA that moves into the A site carries glutamic acid. (*b*) Three-dimensional model of the translocation process minus the mRNA and amino acids. The tRNA in the A site is *pale blue,* the tRNA in the P site is *green,* and the tRNA in the E site is *yellow,* then *brown* when ready to leave. Going clockwise from *a,* in which the A and P sites are occupied: EF-G translocates the ribosome after peptide bond formation and then evacuates the A site. Ef-Tu brings a new charged tRNA to the A site while the E site is emptied. ([*b*] Courtesy of Joachim Frank.)

In eukaryotes, however, almost all messenger RNAs contain the information for only one gene **(monocistronic).** Since most ribosomal recognition of eukaryotic genes depends on the 5′ cap, and since each eukaryotic messenger RNA has only one cap, usually only one polypeptide can be translated for any given messenger RNA. Exceptions occur when the messenger RNAs contain internal ribosome entry sites. Although it is certainly not the rule, the translated peptide can be modified or cleaved into smaller functional peptides. For example, in mice, a single messenger RNA codes for a protein that is later cleaved into epidermal growth factor and at least seven other related peptides. In addition, the same sequence can, in some cases, give rise to alternative proteins through alternative start codons, termination read-through, or alternative splicing.

More on the Ribosome

In the last chapter, we briefly discussed the shape and composition of the ribosomal subunits. All of the protein and RNA components have been isolated. Assembly pathways are known. We know approximately where the messenger RNA, initiation factors, and EF-Tu are located on the 30S subunit during translation (fig. 11.24; cf. fig. 11.18). We also know where peptidyl transferase activity and EF-G reside on the 50S subunit, which has a cleft leading into a tunnel that passes through the structure. At present, it seems that the nascent peptide passes through this

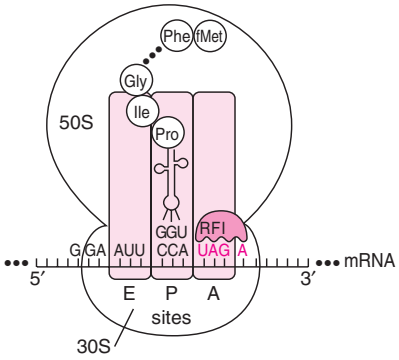


Figure 11.20 Chain termination at the ribosome. One of two release factors recognizes a nonsense codon in the A site. In this case, RF1 recognizes UAG. The complex then falls apart, releasing the peptide.

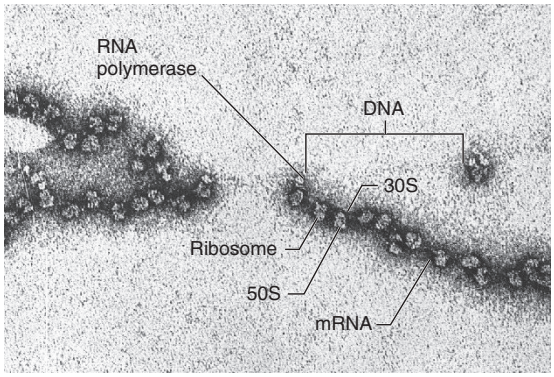
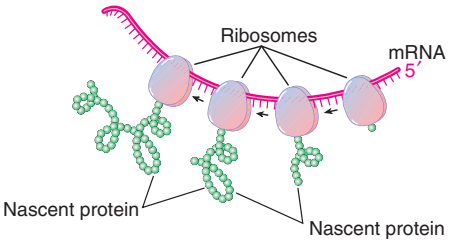
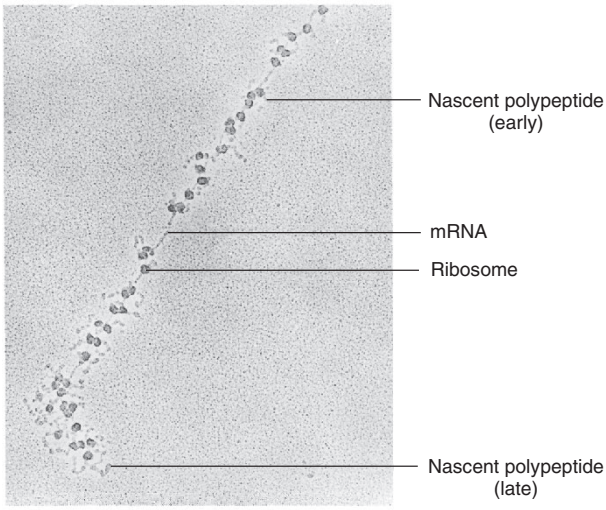


Figure 11.21 A polysome (i.e., multiple ribosomes on the same strand of mRNA). Each ribosome is approximately 250 Å units across. Also visible in this illustration are DNA and RNA polymerase. (Reproduced courtesy of Dr. Barbara Hamkalo, *International Review of Cytology,* (1972) 33:7, fig. 5. Copyright by Academic Press, Inc., Orlando, Florida.)



(a)



(b)

Figure 11.22 (*a*) Protein synthesis at a polysome. Nascent proteins exit from a tunnel in the 50S subunit. Messenger RNA is being translated by the ribosomes while the DNA is being transcribed. (*b*) A messenger RNA from the midge, *Chironomus tentans,* showing attached ribosomes and nascent polypeptides emerging from the ribosomes. Note the 5′ end of the messenger RNA at the *upper right* (small peptides). Magnification 165,000×. ([*b*] Courtesy of S. L. McKnight and O. L. Miller, Jr.)

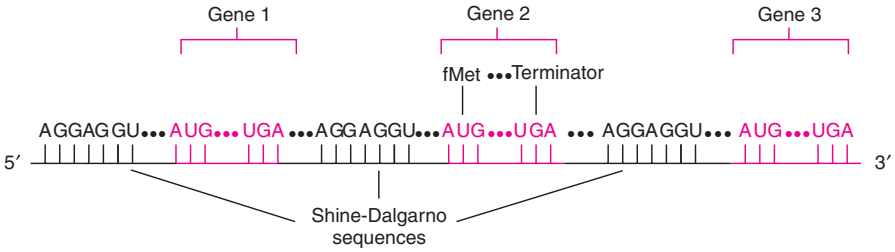


Figure 11.23 A prokaryotic polycistronic mRNA. Note the several Shine-Dalgarno sequences for ribosomal attachment and the initiation and termination codons marking each gene.

tunnel, emerging close to a membrane-binding site (fig. 11.24). The tunnel can hold a peptide length of about forty amino acids. Note that although every ribosome has a membrane-binding site, not all active ribosomes are bound to membranes.

The Signal Hypothesis

Ribosomes are either free in the cytoplasm or associated with membranes, depending on the type of protein being synthesized. Membrane-bound ribosomes, indistinguishable from free ribosomes, synthesize proteins that enter membranes. These proteins either become a part of the membrane or, in eukaryotes, either pass into membrane-bound organelles (e.g., the Golgi apparatus, mitochondria, chloroplasts, vacuoles) or are transported outside the cell membrane. The **signal hypothesis** of G. Blobel, a 1999 Nobel laureate, and his colleagues, explains the mechanism for membrane attachment. The mechanism applies to both prokaryotes and eukaryotes. Here, we describe it in mammals.

Gunter Blobel (1936–). (Courtesy of Dr. Gunter Blobel, Dept. of Cell Biology, Rockefeller University.)

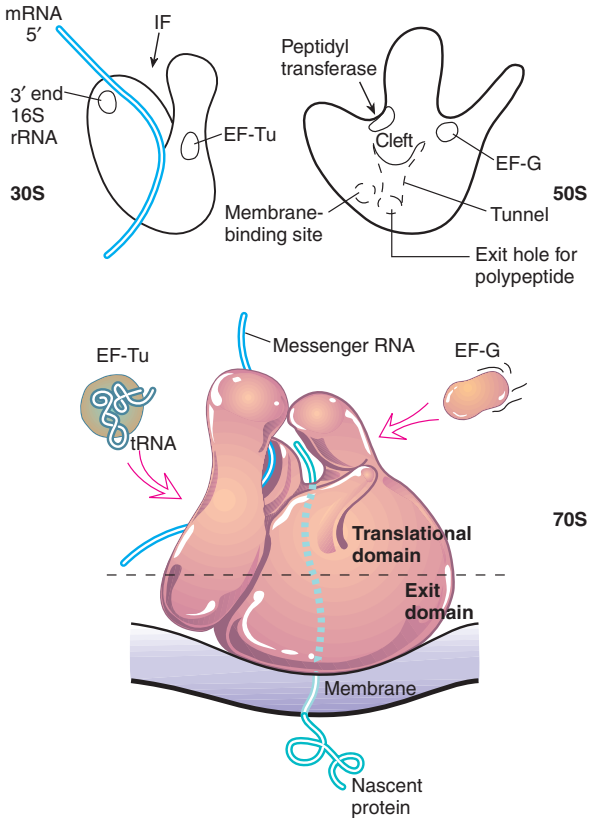


Figure 11.24 Functional sites on the prokaryotic ribosome. The ribosome is synthesizing a protein involved in membrane passage. Note the position of the messenger RNA on the 30S subunit and the cleft, tunnel, and membrane-binding site on the 50S subunit. (From C. Bernabeu and J. A. Lake, *Proceedings of the The National Academy of Sciences;* 79:3111–15, 1982. Reprinted by permission.)

The signal for membrane insertion is coded into the first one to three dozen amino acids of membrane-bound proteins. This **signal peptide** takes part in a chain of events that leads the ribosome to attach to the membrane and to the insertion of the protein. The first step occurs when the signal peptide becomes accessible outside of the ribosome. A ribonucleoprotein particle called the **signal recognition particle** (SRP), which consists of six different proteins and a 7S RNA about three hundred nucleotides long, recognizes the signal peptide. The complex of signal recognition particle, ribosome, and signal peptide then passes, or diffuses, to a membrane, where the SRP binds to a receptor called a **docking protein** (DP) or signal recognition particle receptor (fig. 11.25). During this time, protein synthesis halts. The ribosome is brought into direct contact with the membrane, and other proteins of the membrane help anchor the ribosome. Protein synthesis then resumes, with the nascent protein usually passing directly into a **translocation channel (translocon).** Once through the membrane, the signal peptide is cleaved from the protein by an enzyme called *signal peptidase.* A striking verification of this hypothesis came about through recombinant DNA techniques (chapter 13). A signal sequence was placed in front of the α-globin gene, whose protein product is normally not transported through a membrane. When this gene was translated, the ribosome became membrane bound, and the protein passed through the membrane.

Since different proteins enter different membrane-bound compartments (e.g., the Golgi apparatus), some mechanism must direct a nascent protein to its proper

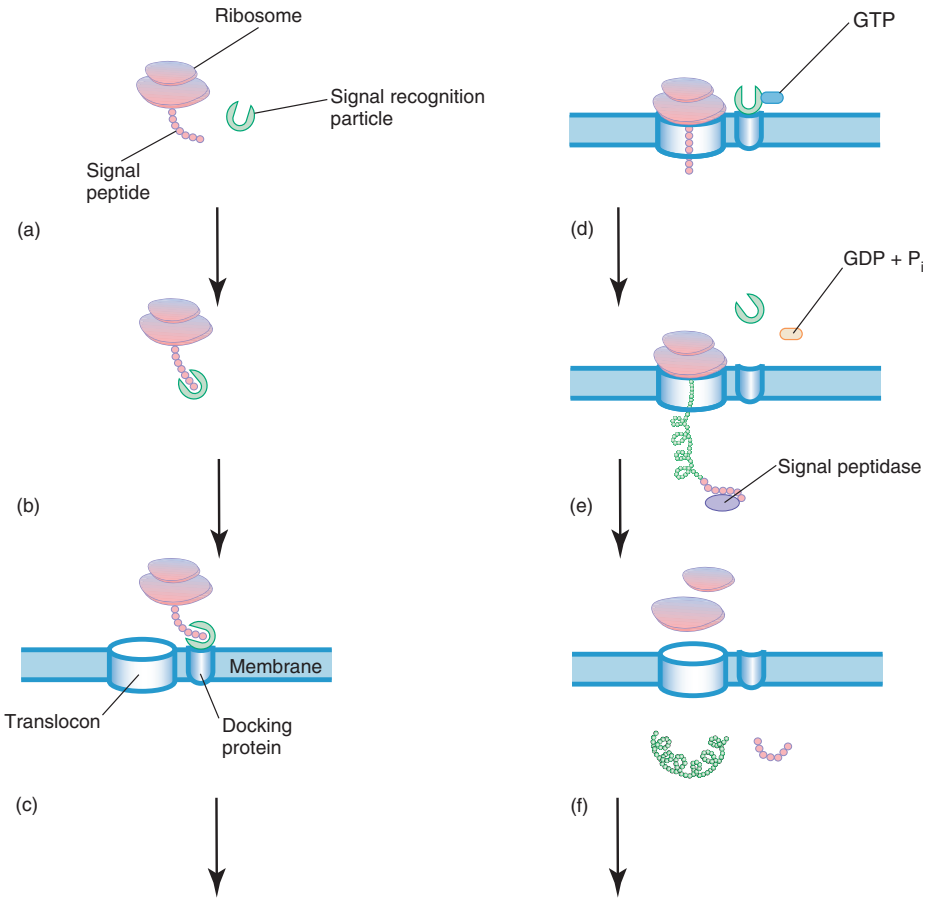


Figure 11.25 The signal hypothesis. A signal recognition particle recognizes a ribosome with a signal peptide, then draws the ribosome to a docking protein located near a translocon in the membrane. With the addition of GTP, the signal recognition particle releases the signal peptide; hydrolysis of the GTP to GDP + P_i causes the signal recognition particle to leave the docking protein. Peptide synthesis then resumes, with the newly synthesized peptide passing through the translocon in the membrane. A signal peptidase on the other side of the membrane removes the signal peptide. When translation is completed, the ribosome dissociates and drops free of the translocon.

membrane. This specificity seems to depend on the exact signal sequence and membrane-bound glycoproteins called *signal-sequence receptors*. Apparently, after the ribosome binds to the docking protein, the signal peptide interacts with a signal-sequence receptor, which presumably determines whether that protein is specific for that membrane. If it is, the remaining processes continue. If not, the ribosome may be released from the membrane.

The signal peptide does not seem to have a consensus sequence like the transcription or translation recognition boxes. Rather, similarities (at least for the endoplasmic reticulum and bacterial membrane-bound proteins) include a positively charged (basic) amino acid (commonly lysine or arginine) near the beginning (N-terminal end), followed by about a dozen hydrophobic (nonpolar) amino acids, commonly alanine, isoleucine, leucine, phenylalanine, and valine (table 11.2).

Table 11.2 The Signal Peptide of the Bovine Prolactin Protein*

NH_2 – Met Asp Ser Lys Gly Ser Ser Gln Lys Gly Ser Arg Leu Leu Leu Leu Leu Val Val Ser Asn Leu Leu Leu Cys Gln Gly Val Val Ser | Thr Pro Val...Asn Asn Cys – COOH

Source: From Sasavage et al., *Journal of Biological Chemistry,* 257:678–81, 1982. Reprinted with permission.

* The vertical line separates the signal peptide from the rest of the protein, which consists of 199 residues.

Figure 11.26 Electron micrograph of a chaperone protein (GroEL) from *E. coli.* Note the hollow, barrel shape of the protein. (Courtesy of Dr. R. W. Hendrix.)

The mitochondrion, which needs to import upwards of one thousand proteins through both inner and outer membranes, poses a specific problem. Recent research has revealed a family of translocation proteins (called Tom proteins) in the outer membrane and a different set of translocation proteins (called Tim proteins) in the inner membrane. These proteins control the passage of proteins synthesized in the cytoplasm into the mitochondrion.

The Protein-Folding Problem

Since biochemist Christian Anfinsen won a 1972 Nobel Prize for showing that the enzyme ribonuclease refolds to its original shape after denaturation in vitro, scientists have believed that the final protein shape (secondary and tertiary structure) forms spontaneously. Recently it has been shown, however, that many proteins do not normally form their final active shape in vivo without the help of proteins called **chaperones** or **molecular chaperones.** The chaperones do not provide the three-dimensional structure of the proteins they help, but rather bind to a protein in the early stages of folding to prevent unproductive folding or to allow denatured proteins to refold correctly. Like human chaperones, they prevent or undo "incorrect interactions," according to J. Ellis. That is, many proteins have a large number of different structures they could fold into. Many of these structures would have no enzymatic activity or would form functionless aggregates with other proteins. Molecular chaperones allow proteins to fold into a thermodynamically stable and functional configuration. Each cycle of refolding requires ATP energy.

A well-studied class of chaperones is known as the *chaperonins,* or Hsp60 proteins, because they are heat shock proteins about 60 kilodaltons (60,000 daltons) in size. They occur in bacteria, chloroplasts, and mitochondria. One of the best studied of these chaperonins is the protein GroE of *E. coli.* This protein in its active form is composed of two components, GroEL and GroES. GroEL (Hsp60) is made up of two disks, each composed of seven copies of a polypeptide. GroES (Hsp10) is a smaller component composed of seven copies of a small subunit. GroEL forms a barrel in which protein folding takes place (fig. 11.26). The barrel is shaped in such a way that entering proteins of a certain size make contact at interior points in either the upper or lower ring of GroEL (upper ring shown in fig. 11.27). The attachment of GroES, the cap, causes the ring to open outward at the top, stretching the protein inside. This stretching takes energy from the hydrolysis of ATP molecules located inside the rings. When GroES dissociates, the protein can fold into a new, more functional, configuration. If it doesn't, the cycle repeats.

There are several classes of molecular chaperones, proteins of different sizes and shapes that recognize different groups of proteins or protein conformations. GroEL recognizes about 300 different proteins, small enough to fit into the barrel (20–60 kilodaltons) and having hydrophobic surfaces. These include many proteins in the transcription and translation machinery of the cell. Hsp90, another heat shock protein, recognizes proteins involved in signal transduction, discussed in chapter 16. Hsp70 recognizes hydrophobic regions in polypeptide side chains, many of which extend across membranes.

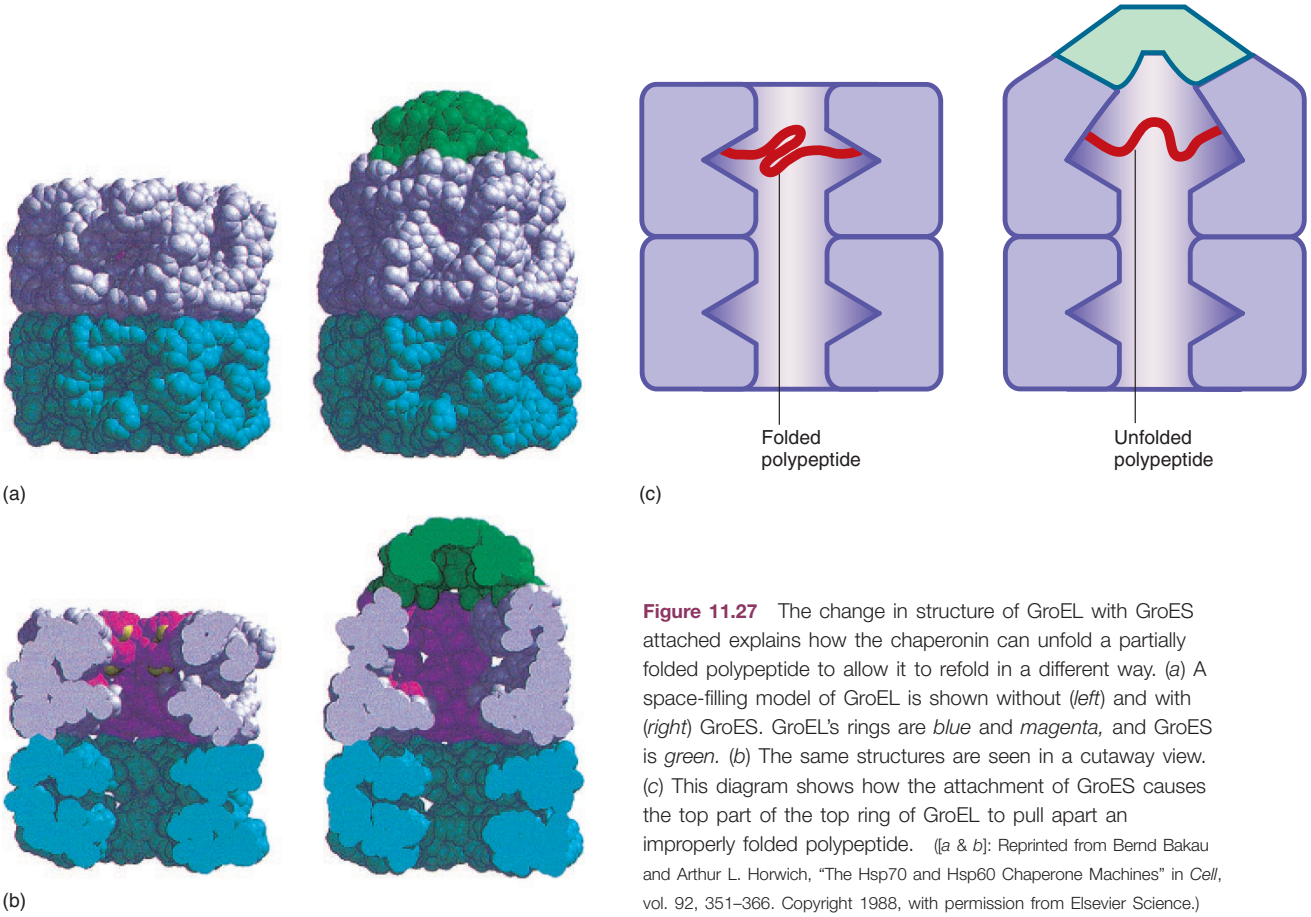


Figure 11.27 The change in structure of GroEL with GroES attached explains how the chaperonin can unfold a partially folded polypeptide to allow it to refold in a different way. (*a*) A space-filling model of GroEL is shown without (*left*) and with (*right*) GroES. GroEL's rings are *blue* and *magenta,* and GroES is *green.* (*b*) The same structures are seen in a cutaway view. (*c*) This diagram shows how the attachment of GroES causes the top part of the top ring of GroEL to pull apart an improperly folded polypeptide. ([*a* & *b*]: Reprinted from Bernd Bakau and Arthur L. Horwich, "The Hsp70 and Hsp60 Chaperone Machines" in *Cell*, vol. 92, 351–366. Copyright 1988, with permission from Elsevier Science.)

THE GENETIC CODE

Researchers in the mid-1950s assumed that the genetic code consisted of simple sequences of nucleotides specifying particular amino acids. They sought answers to questions such as: Is the code overlapping? Are there nucleotides between code words (punctuation)? How many letters make up a code word (codon)? Logic, along with genetic experiments, supplied some of the answers, but only with the rapidly improving techniques of biochemistry did they eventually decode the genetic language.

Triplet Nature of the Code

Several lines of evidence seemed to indicate that the nature of the code was triplet (three bases in messenger RNA specifying one amino acid). If codons contained only one base, they would only be able to specify four amino acids since there are only four different bases in DNA (or messenger RNA). A couplet code would have $4 \times 4 = 16$ two-base words, or codons, which is still not enough to specify uniquely twenty different amino acids. A triplet code would allow for $4 \times 4 \times 4 = 64$ codons, which are more than enough to specify twenty amino acids.

Evidence for the Triplet Nature of the Code

The experimental manipulation of mutant genes, primarily by Francis Crick and his colleagues, reinforced the triplet code concept. In these experiments, a chemical mutagen, the acridine dye proflavin, was used to cause inactivation of the rapid lysis (*rIIB*) gene of the bacteriophage T4. Proflavin inactivates the gene by either adding or deleting a nucleotide from the DNA (see chapter 12). The *rII* gene controls the plaque morphology of this bacteriophage growing on *E. coli* cells. Rapid-lysis mutants produce large plaques; the wild-type form of the gene, rII^+, results in normal plaque morphology.

Figure 11.28 shows the consequences of adding or deleting a nucleotide. From the point of addition or deletion onward, a **frameshift** causes codons to be read in different groups of three. If a deletion is combined with an addition to produce a double-mutant gene, the frameshift occurs only in the region between the two mu-

tants. If this region is small enough or does not contain coding for vital amino acids, the function of the gene may be restored. Two deletions or two insertions combined will not restore the reading frame. However, Crick and his colleagues found that the combination of three additions or three deletions did restore gene function. This finding led to the conclusion that the genetic code was triplet, because a triplet code would be put back into the reading frame by three additions or three deletions (fig. 11.29).

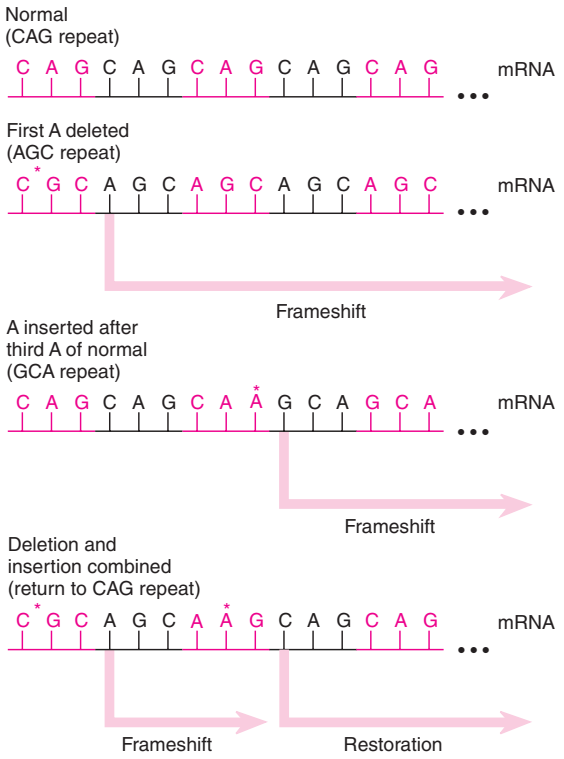


Figure 11.28 Frameshift mutations in a gene result from the addition or deletion of one or several nucleotides (any number other than a multiple of three) in the DNA. The messenger RNA shown here normally has a CAG repeat. A single-base deletion shifts the three-base reading frame to a series of AGC repeats. A later insertion restores the reading frame. *Asterisks* (*) indicate points of deletion or insertion.

Overlap and Punctuation in the Code

Questions still remained: was the code overlapping? Did it have punctuation? Several logical arguments favored a no-punctuation, nonoverlapping model (fig. 11.30). An overlapping code would be subject to two restrictions. First, a change in one base (a mutation) could affect more than one codon and thus affect more than one amino acid. But studies of amino acid sequences almost always showed that only one amino acid was changed, which argued against codon overlap. Second, certain restrictions affected which amino acids occurred next to each other in proteins. For example, the amino acid UUU coded could never be adjacent to the amino acid coded by AAA because one or both (depending on the number of bases overlapped) of the overlap codons UUA and UAA would always insert other amino acids between them. Overlap, then, seemed to be ruled out since every amino acid appears next to every other amino acid in one protein or another.

Punctuation between codons was also tentatively ruled out. The messenger RNA in the tobacco necrosis satellite virus has just about enough codons to specify its coat protein with no room left for a punctuating base or bases between each codon.

Breaking the Code

Once geneticists had figured out that the genetic code is in nonoverlapping triplets, they turned their attention to the sixty-four codons. They wondered which amino acid, for example, does ACU specify? The work was done in two stages. In the first stage, M. W. Nirenberg, S. Ochoa, and their colleagues made long artificial messenger RNAs and determined which amino acids these messenger RNAs incorporated into protein. In the second stage, specific triplet RNA sequences were synthesized. The researchers then determined the amino acid-transfer RNA complex that was bound by each sequence.

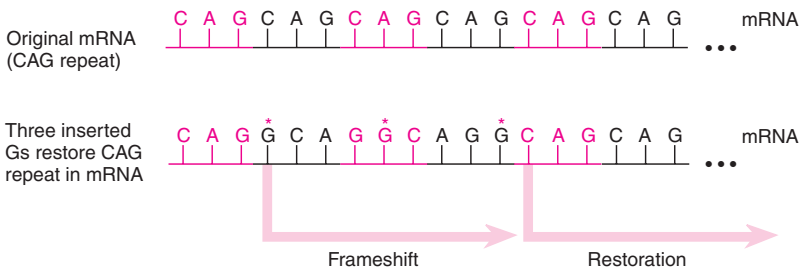
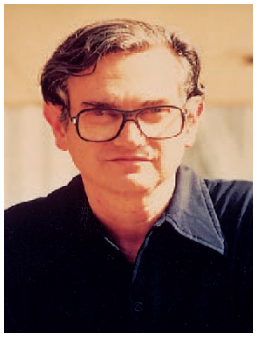


Figure 11.29 The coding frame of CAG repeats is first shifted and then restored by three additions (insertions). *Asterisks* (*) indicate insertions.

Severo Ochoa (1905–1993). (Courtesy of Dr. Severo Ochoa.)



Marshall W. Nirenberg (1934–). (Courtesy of Dr. Marshall W. Nirenberg.)

Synthetic Messenger RNAs

The ability to synthesize long-chain messenger RNAs resulted from the 1955 discovery of M. Grunberg-Manago and Ochoa of the enzyme **polynucleotide phosphorylase,** which joins diphosphate nucleotides into long-chain, single-stranded polynucleotides. Unlike a polymerase, polynucleotide phosphorylase does not need a primer on which to act. This enzyme is found in all bacteria. (Its main function in the cell is probably the reverse of its use here. It most likely serves as an exonuclease, degrading messenger RNA.) In 1961, Nirenberg and J. H. Matthei added artificially formed RNA polynucleotides of known composition to an *E. coli* ribosomal system and looked for the incorporation of amino acids into proteins.

The system just described is called a **cell-free system,** a mixture primarily of the cytoplasmic components of cells, such as *E. coli,* but missing nucleic acids and membrane components. These systems are relatively easy to create by disrupting and then fractionating whole cells. The systems hold the advantage of containing virtually all the components needed for protein synthesis except the messenger RNAs. Their disadvantages are that they are relatively short-lived (several hours) and are relatively inefficient in translation. However, an added benefit to the *E. coli* cell-free system is that it will translate, albeit inefficiently, RNAs that normally are not translated in vivo because they lack translation initiation signals. This feature allowed these scientists to use artificial messenger RNAs that contained no Shine-Dalgarno sequence for ribosomal binding.

Nirenberg and Matthei found that when the enzyme polynucleotide phosphorylase in the *E. coli* cell-free system made uridine diphosphates into a poly-U messenger RNA, phenylalanine residues were incorporated into a polypeptide. Thus, the first code word established was UUU for phenylalanine. Nirenberg and Ochoa and their associates continued the work. They found that AAA was the code word for lysine, CCC was the code word for proline, and GGG was the code word for glycine.

They then made synthetic messenger RNAs by using mixtures of the various diphosphate nucleotides in known proportions. Table 11.3 gives an example. From their experiments, it was possible to determine the bases used in many of the code words, but not their specific order. For example, cysteine, leucine, and valine are all coded by two Us and a G, but the experiment could not sort out the order of these bases (5′-UUG-3′, 5′-UGU-3′, or 5′-GUU-3′) for any one of them. Determining the order required an extra step in sophistication—that is, being able to synthesize known trinucleotides.

Synthetic Codons

Once trinucleotides of known composition could be manufactured, Nirenberg and P. Leder in 1964 developed a "binding assay." They found that isolated *E. coli* ribosomes, in the presence of high-molarity magnesium chlo-

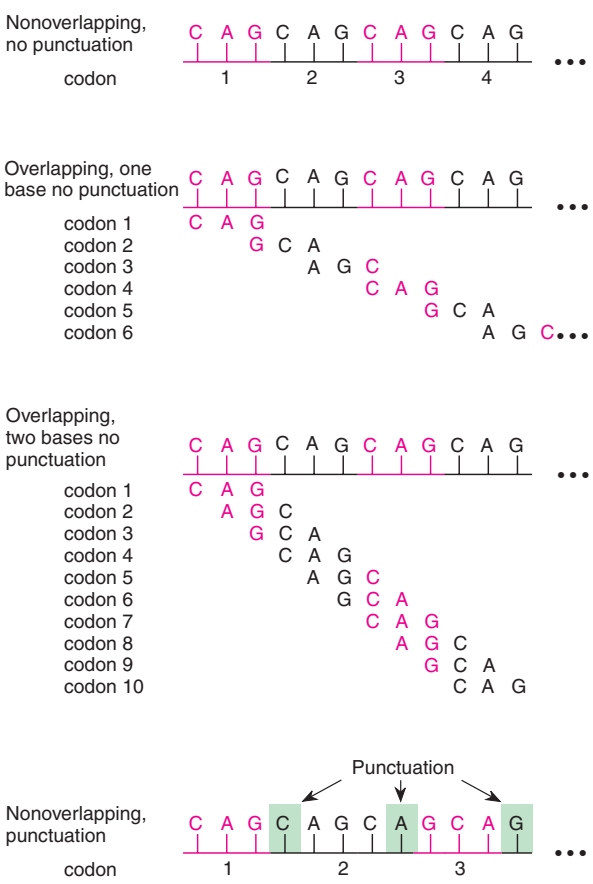


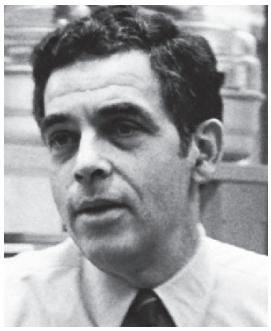
Figure 11.30 The genetic code is read as a nonoverlapping code with no punctuation (*top*). Before that was proven, it was suggested that the code could overlap by one or two bases (*middle*) or have noncoded bases (punctuation) between code words (*bottom*).

Table 11.3 **Structure of Artificial mRNA Made by Randomly Assembling Uracil- and Guanine-Containing Ribose Diphosphate Nucleotides with a Ratio of 5U:lG**

Codon	Frequency of Occurrence
UUU	$(5/6)^3 = 0.58$
UUG	$(5/6)^2(1/6) = 0.12$
UGU	$(5/6)^2(1/6) = 0.12$
GUU	$(5/6)^2(1/6) = 0.12$
UGG	$(5/6)(1/6)^2 = 0.02$
GUG	$(5/6)(1/6)^2 = 0.02$
GGU	$(5/6)(1/6)^2 = 0.02$
GGG	$(1/6)^3 = 0.005$

ride, could bind trinucleotides as if they were messenger RNAs. Also bound was the transfer RNA that carried the anticodon complementary to the trinucleotide. It was thus possible, using radioactive amino acids, to determine which messenger RNA trinucleotide coded for a particular amino acid. A given synthetic trinucleotide was mixed with ribosomes and aminoacyl-tRNAs, including one radioactively labeled amino acid. The reaction mixture was passed over a filter that would allow everything except the large trinucleotide + ribosome + aminoacyl-tRNA complex to pass through. If the radioactivity passed through the filter, it meant that the radioactive amino acid was not associated with the ribosome. The experiment was then repeated with another labeled amino acid. When the radioactivity appeared on the filter, the investigators knew that the amino acid was affiliated with the ribosome. Thus, that amino acid was coded by the selected trinucleotide codon. In other words, the radioactive amino acid was attached to a transfer RNA whose anticodon was complementary to the trinucleotide codon and thus bound at the ribosome.

Figure 11.31 shows an example. In the figure, the trinucleotide is 5′-CUG-3′. The transfer RNA with the anticodon 3′-GAC-5′ is charged with leucine. The mixture is passed through a filter. If threonine, or any other amino acid except leucine, is radioactive, the radioactivity passes through the filter. When the experiment is repeated with radioactive leucine, the leucine, and hence the radioactivity, is trapped by the filter. In a short period of time, all of the codons were deciphered (table 11.4).



Phillip Leder (1934–).
(Courtesy of Dr. Phillip Leder.)

Wobble Hypothesis

The genetic code is a **degenerate code,** meaning that a given amino acid may have more than one codon. As you can see from table 11.4, eight of the sixteen boxes contain just one amino acid per box. (A box is determined by the first and second positions; e.g., the UUX box, in which X is any of the four bases.) Therefore, for these eight amino acids, the codon need only be read in the first two positions because the same amino acid will be represented regardless of the third base of the codon. These eight groups of codons are termed **unmixed families** of codons. An unmixed family is the four codons beginning with the same two bases that specify a single amino acid. For example, the codon family GUX codes for valine. **Mixed families** code for two amino acids or for stop signals and one or two amino acids.

Six of the mixed-family boxes are split in half so that the codons are differentiated by the presence of a purine or a pyrimidine in the third base. For example, CAU and

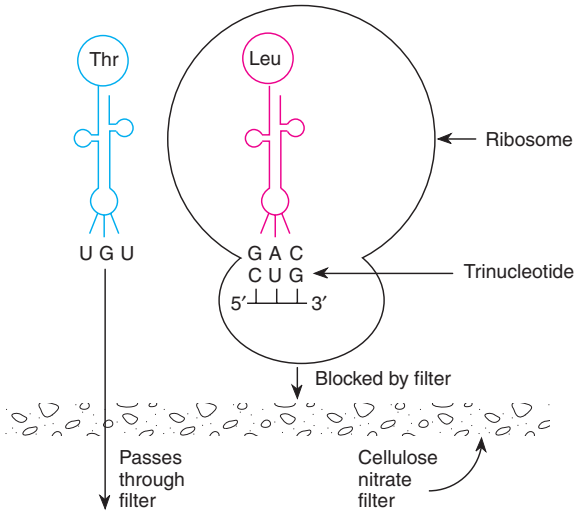


Figure 11.31 The binding assay determines the amino acid associated with a given trinucleotide codon. Transfer RNAs with noncomplementary codons pass through the membrane. Transfer RNAs with anticodons complementary to the trinucleotide bind to the ribosome and do not pass through the filter. When the transfer RNA is charged with a radioactive amino acid, the radioactivity is trapped on the filter.

Table 11.4 The Genetic Code

First Position (5′ End)	Second Position: U	Second Position: C	Second Position: A	Second Position: G	Third Position (3′ End)
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	*stop*	*stop*	A
	Leu	Ser	*stop*	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met (*start*)	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

CAC both code for histidine; in both, the third base, U (uracil) or C (cytosine), is a pyrimidine. Only two of the families of codons are split differently.

The lesser importance of the third position in the genetic code ties in with two facts about transfer RNAs. First, although there would seem to be a need for sixty-two transfer RNAs—since there are sixty-one codons specifying amino acids and an additional codon for initiation—there are actually only about fifty different transfer RNAs in an *E. coli* cell. Second, a rare base such as inosine can appear in the anticodon, usually in the position that is complementary to the third position of the codon. These two facts lead researchers to believe that some kind of conservation of transfer RNAs is occurring and that rare bases may be involved.

We should mention, to avoid confusion, that both messenger RNA and transfer RNA bases are usually numbered from the 5′ side. Thus, the number-one base of the codon is complementary to the number-three base of the anticodon (fig. 11.32). Thus, the codon base of lesser importance is the number-three base, whereas its complement in the anticodon is the number-one base.

Since the first position of the anticodon (5′) is not as constrained as the other two positions, a given base at that position may be able to pair with any of several bases in the third position of the codon. Crick characterized this ability as **wobble** (fig. 11.33). Table 11.5 shows the possible pairings that would produce a transfer RNA system compatible with the known code. For example, if an isoleucine transfer RNA has the anticodon 3′-UAI-5′, it is compatible with the three codons for that amino acid (see table 11.4): 5′-AUU-3′, 5′-AUC-3′, and 5′-AUA-3′. That is, inosine in the first (5′) position of the anticodon can recognize U, C, or A in the third (3′) position of the codon, and thus one transfer RNA complements all three codons for isoleucine.

Universality of the Genetic Code

Until 1979, scientists concluded that the genetic code was universal. That is, the codon dictionary (see table 11.4) was the same for *E. coli,* human beings, and oak trees, as well as all other species studied up to that time. The universality of the code was demonstrated, for example, by taking the ribosomes and messenger RNA from rabbit reticulocytes and mixing them with the aminoacyl-tRNAs and other translational components of *E. coli.* Rabbit hemoglobin was synthesized.

In 1979 and 1980, however, researchers noted discrepancies when sequencing mitochondrial genes for struc-

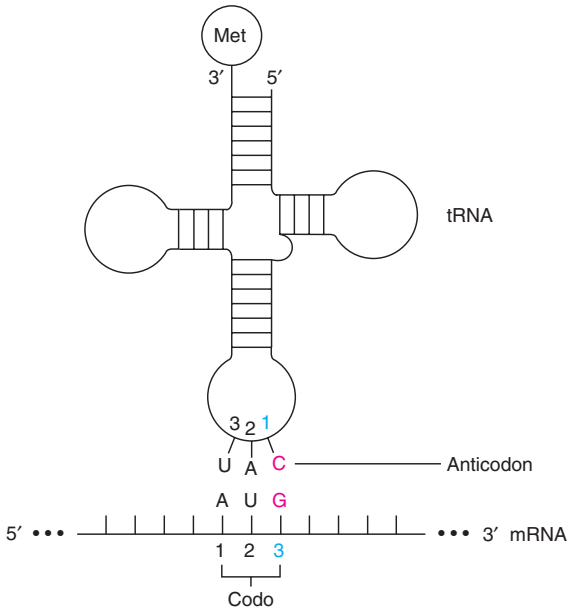


Figure 11.32 Codon and anticodon base positions are numbered from the 5′ end. The 3′ position in the codon (5′ in the anticodon) is the wobble base.

tural proteins (see chapters 13 and 17). It was discovered that there were two kinds of deviations from universality in the way mitochondrial transfer RNAs read the code. First, fewer transfer RNAs were needed to read the code. Second, there were several instances in which the mitochondrial and cellular systems interpreted a codon differently.

According to Crick's wobble rules (see table 11.5), thirty-two transfer RNAs (including one for initiation) can complement all sixty-one nonterminating codons. Unmixed families require two transfer RNAs, and mixed families require one, two, or three transfer RNAs, depending on the family. The yeast mitochondrial coding system apparently needs only twenty-four transfer RNAs. The reduction in numbers is accomplished primarily by having only one transfer RNA recognize each unmixed family

Table 11.5 **Pairing Combinations at the Third Codon Position**

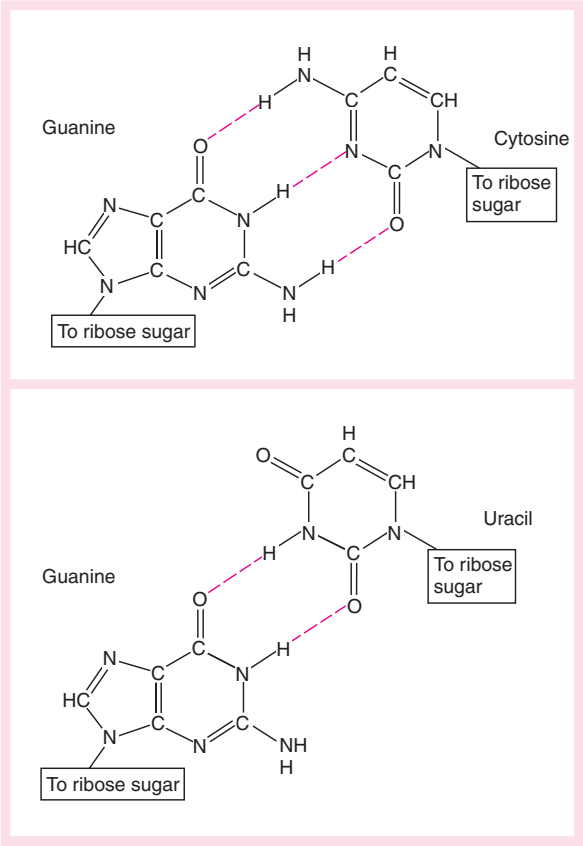
Number-one Base in tRNA (5′ End)	Number-three Base in mRNA (3′ End)
G	U *or* C
C	G
A	U
U	A *or* G
I	A, U, *or* C

(table 11.6; cf. table 11.4). Because mitochondrial transfer RNAs for unmixed families of codons have a U in the first (wobble) position of the anticodon, apparently, given the structure of the mitochondrial transfer RNAs, the U can pair with U, C, A, or G. Presumably, evolutionary pressure has minimized the number of transfer RNA genes in the DNA of the mitochondrion, in keeping with its small size. Reduction from thirty-two to twenty-four is a 25% savings. (Recent evidence suggests that mammalian mitochondria may need only twenty-two transfer RNAs.)

It has also been found that yeast mitochondria read the CUX family as threonine rather than as leucine (tables 11.4 and 11.6) and the terminator UGA (opal) as tryptophan rather than as termination. However, there appear to be differences among different groups of organisms reading the CUX family. Human and *Neurospora* mitochondria appear to read the CUX codons as leucine, just as cellular systems do. Of the groups so far analyzed, only yeast reads the CUX family as threonine. Similarly, human and *Drosophila* mitochondria read AGA and AGG as stop signals rather than as arginine (table 11.7).

In 1985, it was discovered that *Paramecium* species read the UAA and UAG stop codons as glutamine within the cell. In addition, a prokaryote (*Mycoplasma capricolum*) reads UGA as tryptophan. We do not yet know how general this finding is: scientists have scrutinized the genetic code of very few species. We can thus conclude that the genetic code seems to have universal tendencies among prokaryotes, eukaryotes, and viruses. Mitochondria, however, read the code slightly differently: different wobble rules apply, and mitochondria and cells read at least one terminator and one unmixed family of codons differently. Also, the mitochondrial discrepancies are not universal among all types of mitochondria. Further work, involving the sequencing of more mitochondrial DNAs, should elucidate the pattern of discrepancies among the mitochondria of diverse species. We also now know that not every organism reads all codons in the same way. Ciliated protozoa and a mycoplasma read some stop signals as coding for amino acids. Nuclear variants are known in the following codons: CUG, AUA, UAA, UAG, UGA, CGG, and AGA. Mitochondrial variants are known in CUX, AUA, UAA, UAG, AAA, UGA, CGX, AGA, and AGG.

One other type of variation of codon reading occurs: *site-specific variation,* in which the interpretation of a codon depends on its specific location. We are already familiar with the fact that GUG and, rarely, UUG can serve as prokaryotic initiation codons. This means that they are recognized by $tRNA_f^{Met}$. However, they are not recognized by $tRNA_m^{Met}$ (i.e., GUG and UUG are not misread internally in messenger RNAs). In some cases, two of the termination codons (UGA and UAG, but not UAA) are misinterpreted as codons for amino acids. That is, termination will not occur at the normal place, resulting in a longer-than-usual protein. In some cases, these "read-through" proteins are vital—the



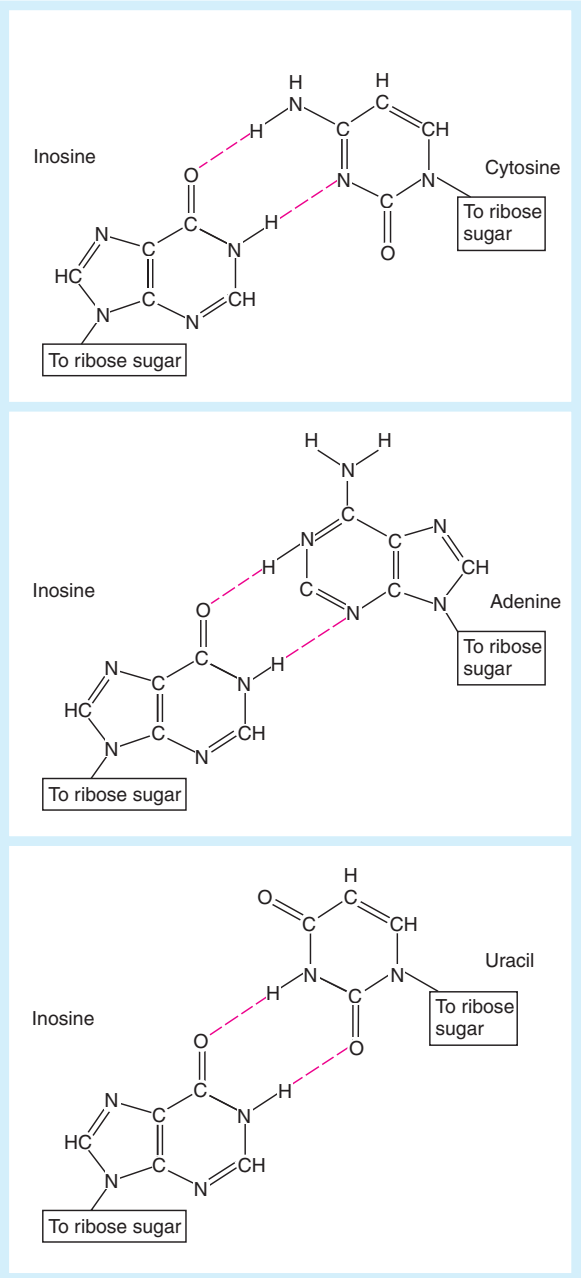


Figure 11.33 Base-pairing possibilities for guanine and inosine in the third (3′) position of a codon. In the wobble position, guanine can form base pairs with both cytosine and uracil. Inosine, in the wobble position, can pair with cytosine, adenine, and uracil.

organism depends on their existence. For example, in the phage Qβ, the coat-protein gene is read through about 2% of the time. Without this small number of read-through proteins, the phage coat cannot be constructed properly.

One last example of site-specific variation involves the amino acid selenocysteine (cysteine with a selenium atom replacing the sulfur; see fig. 11.1). Although many proteins have unusual amino acids, almost all are due to posttranslational modifications of normal amino acids. However, the amino acid selenocysteine is inserted directly into some proteins, such as formate dehydrogenase in *E. coli,* which has selenium in its active site. Selenocysteine is inserted into the protein by a novel transfer RNA that recognizes the termination codon, UGA, if that codon is involved in a particular stem-loop secondary structure in the messenger RNA. The selenocysteine transfer RNA is originally charged with a serine that is then modified to a selenocysteine. In addition to the

Table 11.6 The Genetic Code Dictionary of Yeast Mitochondria*

First Position (5′ End)	Second Position: U	Second Position: C	Second Position: A	Second Position: G	Third Position (3′ End)
U	Phe AAG	Ser AGU	Tyr AUG	Cys ACG	U
	Leu AAU		*stop*	Trp ACU	C
C	Thr GAU	Pro GGU	His GUG	Arg GCA	A
			Gln GUU		G
A	Ile UAG	Thr UGU	Asn UUG	Ser UCG	U
	Met UAC		Lys UUU	Arg UCU	C
G	Val CAU	Ala CGU	Asp CUG	Gly CCU	A
			Glu CUU		G

Source: Data from S. Bonitz, et al., "Codon recognition rules in yeast mitochondria," *Proceedings of the National Academy of Sciences* 77:3167–70, 1980.

* Anticodons (3′ → 5′) are given within boxes. (The ACU Trp anticodon is predicted.)

Table 11.7 Common and Alternative Meanings of Codons

Codon	General Meaning	Alternative Meaning
CUX	Leu	Thr in yeast mitochondria
AUA	Ile	Met in mitochondria of yeast, *Drosophila,* and vertebrates
UGA	*Stop*	Trp in mycoplasmas and mitochondria other than higher plants
AGA/AGG	Arg	*Stop* in mitochondria of yeast and vertebrates
		Ser in mitochondria of *Drosophila*
CGG	Arg	Trp in mitochondria of higher plants
UAA/UAG	*Stop*	Gln in ciliated protozoa
UAG	*Stop*	Ala or Leu in mitochondria of some higher plants

stem-loop structure 3′ (downstream) from the amber codon (UAG), a selenocysteine elongation factor (SELB) is also needed at the ribosome. This same mechanism may occur in eukaryotes, but not all of the components have yet been identified.

Evolution of the Genetic Code

It has been theorized that the genetic code has wobble in it because it originally arose from a code in which only the first two bases were needed for the small number of amino acids in use several billion years ago. As new amino acids with useful properties became available, they were incorporated into proteins by a code modified by the third base, albeit with less specificity. This view has support from the fact that codons starting with the same nucleotide come from the same biosynthetic pathway. This indicates that in early evolution, as biosynthetic pathways were extended to new amino acids, the newcomers were incorporated by use of the second and third bases of the code.

However, the question remains as to whether the genetic code is highly evolved or just a "frozen accident." In other words, is there a relationship between the codons and the amino acids they code for, or is the code just one of many random possibilities? Recent computer simulations of random codes indicate that the current genetic code is far outside the range of random in its ability to protect the organism from mutation. This suggests that the genetic code is not a frozen accident, but rather is highly evolved. Numerous examples in the current code support this view.

For example, in the unmixed codon family 5′-CUX-3′, any mutation in the third position produces another codon for the same amino acid. Wobble in the third position and codon arrangement ensures that less than half of the mutations in the third codon position result in the specification of a different amino acid.

There are also patterns in the genetic code in which the mutation of one codon to another results in an amino acid of similar properties. A high probability exists that such a mutation will produce a functional protein. All the codons with U as the middle base, for example, are for amino acids that are hydrophobic (phenylalanine, leucine, isoleucine, methionine, and valine). Mutation in the first or third positions for any of these codons still codes a hydrophobic amino acid. Both of the two negatively charged amino acids, aspartic acid and glutamic acid, have codons that start with GA. All of the aromatic amino acids—phenylalanine, tyrosine, and tryptophan (see fig. 11.1)—have codons that begin with uracil. Such patterns minimize the negative effects of mutation.

This chapter completes the discussion of the mechanics of gene expression. The next chapter deals with recombinant DNA technology, followed by several chapters concerned with the control of gene expression in both prokaryotes and eukaryotes.

SUMMARY

STUDY OBJECTIVE 1: To study the mechanism of protein biosynthesis, in which organisms, using the information in DNA, string together amino acids to form proteins 281–303

A charged transfer RNA has an anticodon at one end and a specific amino acid at the other end. The transfer RNAs are charged with the proper amino acid by aminoacyl-tRNA synthetase enzymes that incorporate the energy of ATP into amino acid-tRNA bonds. Hence, no additional source of energy is needed during peptide bond formation. During protein synthesis, the translation apparatus at the ribosome recognizes the transfer RNA. Through complementarity, the anticodon pairs with a messenger RNA codon.

An initiation complex forms at the start of translation. In prokaryotes, this complex consists of the messenger RNA, the 30S subunit of the ribosome, the initiator transfer RNA with N-formyl methionine (fMet-$tRNA_f^{Met}$), and the initiation factors IF1, IF2, and IF3. The 50S ribosomal subunit is then added and A and P sites form in the resulting 70S ribosome. The charged N-formyl methionine transfer RNA is in the P site. A GTP is hydrolyzed, and the initiation factors are released.

A transfer RNA enters the A site, which requires the involvement of elongation factors EF-Ts and EF-Tu (in *E. coli*). At least one GTP hydrolysis releases the elongation factor, EF-Tu, which had originally brought the charged transfer RNA to the ribosome. Peptidyl transferase, which appears to be a ribozymic component of the 50S ribosomal subunit, transfers the amino acid from the transfer RNA in the P site to the amino end of the amino acid on the transfer RNA in the A site.

With the help of elongation factor G (EF-G), the ribosome translocates in relation to the messenger RNA. The depleted transfer RNA is moved from the P site to the E site, where it is released; the transfer RNA with the growing peptide is moved into the P site. EF-G is then released. Elongation and translocation continue until a nonsense codon enters the A site. With the aid of the release factors RF1 and RF2, the protein is released, and the messenger RNA-ribosome complex dissociates. Eukaryotes have slightly more complex processes involving several more proteins.

Proteins pass through membranes with the help of a signal peptide synthesized at their N-terminal ends. Proteins fold into their final, functional configurations with the help of molecular chaperones, proteins that aid the folding process.

Many antibiotics interfere with translation in prokaryotes. Puromycin, streptomycin, tetracycline, and chloramphenicol all act at the ribosome. Studying the mode of action of these antibiotics has provided insights into the mechanism of the translation process.

STUDY OBJECTIVE 2: To examine the genetic code 304–312

The genetic code was first assumed to be triplet because of logical arguments regarding the minimum size of codons. With his work on deletion and insertion mutants, Crick provided evidence that the code was triplet. Part of the code was worked out initially with the synthesis of long, artificial messenger RNAs and then the synthesis of specific trinucleotide codons. Crick's wobble hypothesis accounts for the fact that fewer than sixty-one transfer RNAs can read the entire genetic code. Fewer transfer RNAs are needed because additional complementary base pairings occur in the third position (3′) of the codon.

The rule of universality of the genetic code has to be modified in light of findings regarding mitochondrial transfer RNAs; only twenty-four are needed to read the code. In addition, some sense codons are interpreted differently in mitochondrial systems; some nonmitochondrial systems read stop codons differently (a mycoplasma and ciliated protozoan); and some site-specific variation in codon reading also occurs. The structure of the code in both cells and mitochondria seems to protect the cell against a good deal of potential mutation.

SOLVED PROBLEMS

PROBLEM 1: What is the energy requirement of protein biosynthesis?

Answer: The cost of adding one amino acid to a growing polypeptide is four or five high-energy bonds: two from an ATP during the charging of the transfer RNA, and two or three from the hydrolysis of GTPs during transfer RNA binding to the A site of the ribosome and during translocation. Thus, for an average protein of three hundred amino acids, there is a cost of 1,200 to 1,500 high-energy bonds.

PROBLEM 2: What are the start and stop signals of translation?

Answer: Once a messenger RNA is attached at the ribosome, the start signal is the methionine initiation codon (usually AUG), whereas the stop signal is one of the three nonsense codons (UAA, UAG, and UGA). Binding to the ribosome in order to position the messenger RNA in relation to the A and P sites differs in prokaryotes and eukaryotes. In prokaryotes, the Shine-Dalgarno sequence allows the 16S ribosomal RNA and the messenger RNA to form hydrogen bonds, locating the beginning of the messenger RNA at the ribosome. In eukaryotes, the 5′ cap is usually recognized by the ribosome, and the ribosome then proceeds to scan the messenger RNA for the initiation codon.

PROBLEM 3: What amino acids could replace methionine if a one-base mutation occurred?

Answer: The codon for methionine (internal as well as initiation) is AUG. If the A is replaced, we would get UUG (Leu), CUG (Leu), or GUG (Val); if the U is replaced, we would get AAG (Lys), ACG (Thr), or AGG (Arg); and if the G is replaced, we would get AUA (Ile), AUU (Ile), or AUC (Ile). Hence, a one-base change in the codon for methionine could result in any of six different amino acids.

EXERCISES AND PROBLEMS*

INFORMATION TRANSFER

1. Given the following end part of a gene, which will be transcribed and then translated into a pentapeptide, provide the base sequence for its messenger RNA. Give the anticodons on the transfer RNAs by making use of wobble rules. What amino acids are incorporated? Draw the actual structure of the pentapeptide.

   3′-TACAATGGCCCTTTTATC-5′

   5′-ATGTTACCGGGAAAATAG-3′

2. Give an alternative translation mechanism that would require only one transfer RNA site on the ribosome.
3. Draw the details of a moment in time at the ribosome during the translation of the messenger RNA produced in problem 1. Include in the diagram the ribosomal sites, the transfer RNAs, and the various nonribosomal proteins involved.
4. How do prokaryotic and eukaryotic ribosomes recognize the 5′ end of messenger RNAs? Could eukaryotic messenger RNAs be polycistronic?
5. How many aminoacyl-tRNA synthetases are there? What do they use for recognition signals?
6. What are the similarities and differences among the three nonsense codons? Using the wobble rules, what are their theoretical anticodons?
7. Describe an experiment that demonstrates that the transfer RNA, and not its amino acid, is recognized at the ribosome during translation.
8. Other than the antibiotics named in the chapter, suggest five "theoretical" antibiotics that could interfere with the prokaryotic translation process.
9. How many single-base deletions are required to restore the reading frame of a messenger RNA? Give an example.
10. A "nonsense mutation" is one in which a codon for an amino acid changes to one for chain termination. Give an example. What are its consequences?
11. The reverse situation to problem 10 is a mutation from a nonsense codon to a codon for an amino acid. Give an example. What are its consequences?
12. What are the consequences when an internal methionine codon recognizes a prokaryotic initiation transfer RNA?
13. What role does EF-Ts play in elongation? EF-Tu? What are their eukaryotic equivalents?
14. What roles do RF1 and RF2 play in chain termination? What are their eukaryotic equivalents?
15. What is a signal peptide? What role does it play in eukaryotes? What is its fate?
16. Why doesn't puromycin disrupt eukaryotic translation?

*Answers to selected exercises and problems are on page A-12.

17. A peptide, fifteen amino acids long, is digested by two methods, and each segment is sequenced according to the Edman degradation technique (see box 11.1). The fifteen amino acids are denoted by the letters A through O, with F as the N-terminal amino acid. If the segments are as follows, what is the sequence of the original peptide?

Method 1: CABHLN; FGKI; OEDJM

Method 2: KICAB; JM; FG; HLNOED

18. In human hemoglobin, the β chain is 146 amino acids long. What is the minimum length of RNA needed to make this protein?

19. Part of a DNA strand to be transcribed has the following sequence:

3′-TACTAACTTACGCTCGCCTCA-5′

a. What is the sequence of RNA transcribed from this part of the strand?

b. What sequence of amino acids does the RNA produce?

THE GENETIC CODE

20. If DNA contained only the bases cytosine and guanine, how long would a code word have to be? How could we tell if this DNA were double-stranded?

21. If an artificial messenger RNA contains two parts uracil to one of cytosine, name the amino acids and the proportions in which they should be incorporated into protein.

22. What would be proved or disproved if an organism were discovered that did not follow any of the rules of the codon dictionary? Would we expect organisms from another galaxy (if they exist) to use our codon dictionary?

23. What would the genetic code dictionary (see table 11.4) look like if wobble occurred in the second position rather than the third (i.e., if an unmixed family of codons were of the form GXU)?

24. In experiments using repeating polymers, $(GCGC)_n$ incorporates alanine and arginine into polypeptides, and $(CGGCGG)_n$ incorporates arginine, glycine, and alanine. What codon can probably be assigned to glycine?

25. If poly-G is used as a messenger RNA in an incorporation experiment, glycine is incorporated into a polypeptide. If poly-C is used, proline is incorporated. If both poly-G and poly-C are used, no amino acids are incorporated into protein. Why?

26. A protein has leucine at a particular position. If the codon for leucine is CUC, how many different amino acids might appear as the result of a single-base substitution?

27. Polymers of $(GUA)_n$ result in the incorporation of only two different amino acids rather three, as for most other three-base polymers. Why?

28. The sixth amino acid in the β chain of normal human hemoglobin is glutamate. Two different mutations of this codon substitute valine and lysine. What is the likely codon for glutamate?

29. A normal protein has the following C-terminal amino acid sequence: *ser-thr-lys-leu*-COOH. A mutant is isolated with the following sequence: *ser-thr-lys-leu-leu-phe-arg*-COOH. What has probably happened to produce the mutant protein?

30. A segment of a normal protein and three different mutants appears as follows:

normal	_____gly-ala-ser-his-cys-leu-phe_____
mutant 1	_____gly-ala-ser-his
mutant 2	_____gly-ala-ser-leu-cys-leu-phe_____
mutant 3	_____gly-val-ala-ile-ala-ser_____

What is the probable sequence of bases in the normal RNA?

31. A normal protein has histidine in a given position. Four mutants are isolated and determined to have either tyrosine, glutamine, proline, or leucine in place of histidine. What are the possible codon assignments, and what codon is probably used for histidine?

CRITICAL THINKING QUESTIONS

1. How do transcriptional and translational signals interact?

2. Why is the E site necessary in translation?

Suggested Readings for chapter 11 are on page B-8.

12

DNA

Its Mutation, Repair, and Recombination

STUDY OBJECTIVES

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2. To analyze functional and structural allelism and examine the mapping of mutant sites within a gene 317
3. To verify the colinearity of gene and protein 324
4. To study mutagenesis 325
5. To investigate the processes of DNA repair and recombination 339

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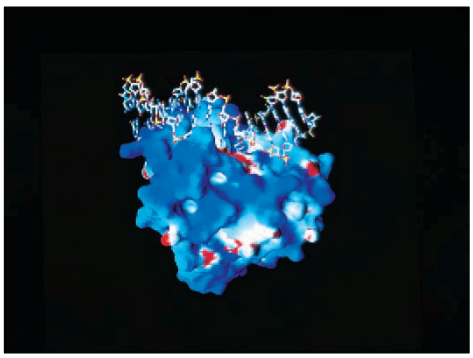


Computer-generated space-filling model of a DNA enzyme repairing damaged DNA.

(© James King-Holmes/SPL/Photo Researchers, Inc.)

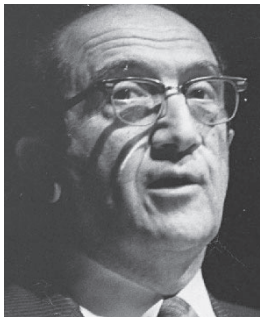
The mutation, repair, and recombination of DNA are treated together in this chapter because the three processes have much in common. The physical alteration of DNA is involved in each; repair and recombination share some of the same enzymes. We progress from mutation—the change in DNA—to repair of damaged DNA, and, finally, to recombination, the new arrangement of pieces of DNA.

MUTATION

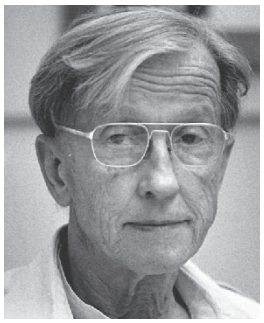
The concept of mutation (a term coined by de Vries, a rediscoverer of Mendel) is pervasive in genetics. **Mutation** is both the process by which a gene (or chromosome) changes structurally and the end result of that process. Without alternative forms of genes, the biological diversity that exists today could not have evolved. Without alternative forms of genes, it would have been virtually impossible for geneticists to determine which of an organism's characteristics are genetically controlled. Studies of mutation provided the background for our current knowledge in genetics.

Fluctuation Test

In 1943, Salvador Luria and Max Delbrück published a paper entitled "Mutations of Bacteria from Virus Sensitivity to Virus Resistance." This paper ushered in the era of bacterial genetics by demonstrating that the phenotypic variants found in bacteria are actually attributable to mutations rather than to induced physiological changes. Very little work had previously been done in bacterial genetics because of the feeling that bacteria did not have "normal" genetic systems like the systems of fruit flies and corn. Rather, bacteria were believed to respond to environmental change by physiological adaptation, a non-Darwinian view. As Luria said, bacteriology remained "the last stronghold of Lamarckism" (the belief that acquired characteristics are inherited).



Salvador E. Luria (1912–1991). (Courtesy of Dr. S. E. Luria.)



Max Delbrück (1906–1981). (Courtesy of Dr. Max Delbrück.)

What Causes Genetic Variation?

Luria and Delbrück studied the Ton^r (phage T1-resistant) mutants of a normal Ton^s (phage T1-sensitive) *Escherichia coli* strain. They used an enrichment experiment, as described in chapter 7, wherein a petri plate is spread with *E. coli* bacteria and T1 phages. Normally, no bacterial colonies grow on the plate: all the bacteria are lysed. However, if one of the bacterial cells is resistant to T1 phages, it produces a bacterial colony, and all descendants of this colony are T1 resistant. There are two possible explanations for the appearance of T1-resistant colonies:

1. Any *E. coli* cell may be induced to be resistant to phage T1, but only a very small number actually are. That is, all cells are genetically identical, each with a very low probability of exhibiting resistance in the presence of T1 phages. When resistance is induced, the cell and its progeny remain resistant.
2. In the culture, a small number of *E. coli* cells exist that are already resistant to phage T1; in the presence of phage T1, only these cells survive.

If the presumed rates of physiological induction and mutation are the same, determining which of the two mechanisms is operating is difficult. Luria and Delbrück, however, developed a means of distinguishing between these mechanisms. They reasoned as follows: If T1 resistance was physiologically induced, the relative frequency of resistant *E. coli* cells in a culture of the normal (Ton^s) strain should be a constant, independent of the number of cells in the culture or the length of time that the culture has been growing. If resistance was due to random mutation, the frequency of mutant (Ton^r) cells would depend on when the mutations occurred. In other words, the appearance of a mutant cell would be a random event. If a mutation occurs early in the growth of the culture, then many cells descend from the mutant cell, and therefore many resistant colonies develop. If the mutation does not occur until late in the growth of the culture, then the subsequent number of mutant cells is few. Thus, if the mutation hypothesis is correct, there should be considerable fluctuation from culture to culture in the number of resistant cells present (fig. 12.1).

Results of the Fluctuation Test

To distinguish between these hypotheses, Luria and Delbrück developed what is known as the **fluctuation test.** They counted the mutants both in small ("individual") cultures and in subsamples from a single large ("bulk") culture. All subsamples from a bulk culture should have the same number of resistant cells, differing only because of

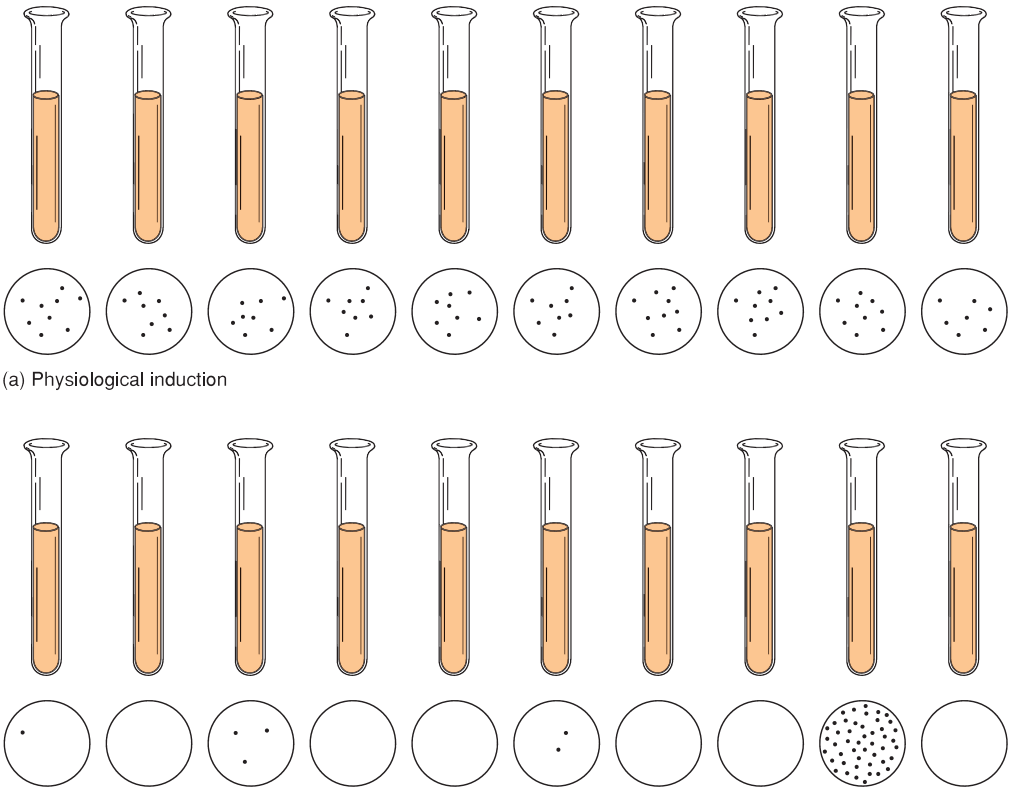


Figure 12.1 Occurrence of *E. coli* Ton^r colonies in Ton^s cultures. Ten cultures of *E. coli* cells were grown from a standard inoculum in separate test tubes in the absence of phage T1, then spread on petri plates in the presence of phage T1. The resistant cells grow into colonies on the plates. We expect a uniform distribution of resistant cells if the physiological induction hypothesis is correct (*a*) or a great fluctuation in the number of resistant cells if the random mutation hypothesis is correct (*b*).

random sampling error. If, however, mutation occurs, the number of resistant cells among the individual cultures should vary considerably from culture to culture; the number would be related to the time that the mutation occurred during the growth of each culture. If mutation arose early, there would be many resistant cells. If it arose late, there would be relatively few resistant cells. Under physiological induction, the distribution of resistant colonies should not differ between the individual and bulk cultures.

Luria and Delbrück inoculated twenty individual cultures and one bulk culture with *E. coli* cells and incubated them in the absence of phage T1. Each individual culture was then spread out on a petri plate containing a very high concentration of T1 phages; ten subsamples from the bulk culture were plated in the same way. We can see from the results (table 12.1) that there was minimal variation in the number of resistant cells among the bulk culture subsamples but a very large amount of variation, as predicted for random mutation, among the individual cultures.

If bacteria have "normal" genetic systems that undergo mutation, bacteria could then be used, along with higher organisms, to answer genetic questions. As we have pointed out, the modern era of molecular genetics began with the use of prokaryotic and viral systems in genetic research. In the next section, we turn our attention to several basic questions about the gene, questions whose answers were found in several instances only because prokaryotic systems were available.

Genetic Fine Structure

How do we determine the relationship among several mutations that cause the same phenotypic change? What are the smallest units of DNA capable of mutation and recombination? Are the gene and its protein product colinear? The answers to the latter two questions are important from a historical perspective. The answer to the first question is relevant to our current understanding of genetics.

Table 12.1 Results from the Luria and Delbrück Fluctuation Test

Individual Cultures*		Samples from Bulk Culture*	
Culture Number	Ton^r Colonies Found	Sample Number	Ton^r Colonies Found
1	1	1	14
2	0	2	15
3	3	3	13
4	0	4	21
5	0	5	15
6	5	6	14
7	0	7	26
8	5	8	16
9	0	9	20
10	6	10	13
11	107		
12	0		
13	0		
14	0		
15	1		
16	0		
17	0		
18	64		
19	0		
20	35		
Mean ($\bar{n}$)	11.4		16.7
Standard deviation	27.4		4.3

Source: From E. Luria and M. Delbrück, *Genetics,* 28: 491. Copyright © 1943 Genetics Society of America.

* Each culture and sample was 0.2 ml and contained about 2×10^7 *E. coli* cells.

Complementation

If two recessive mutations arise independently and both have the same phenotype, how do we know whether they are both mutations of the same gene? That is, how do we know whether they are alleles? To answer this question, we must construct a heterozygote and determine the **complementation** between the two mutations. A heterozygote with two mutations of the same gene will produce only mutant messenger RNAs, which result in mutant enzymes (fig. 12.2*a*). If, however, the mutations are not allelic, the gamete from the a_1 parent will also contain an a_2^+ allele, and the gamete from the a_2 parent will also contain the a_1^+ allele (fig. 12.2*b*). If the two mutant genes are truly alleles, then the phenotype of the heterozygote should be mutant. If, however, the two mutant genes are nonallelic, then the a_1 mutant will have contributed the wild-type allele at the A_2 locus, and the a_2 mutant will have contributed the wild-type allele at the A_1 locus to the heterozygote. Thus, the two mutations will complement each other and produce the wild-type. Mutations that fail to complement each other are termed **functional alleles.** The test for defining alleles strictly on this basis of functionality is termed the ***cis-trans* complementation test.**

There are two different configurations in which a heterozygous double mutant of functional alleles can form (fig. 12.3). In the *cis-trans* complementation test, only the *trans* configuration is used to determine whether the two mutations were allelic. In reality, the *cis* configuration is not tested; it is the conceptual control, in which wild-type activity (with recessive mutations) is always expected. The test is thus sometimes simply called a *trans* test. Functional alleles produce a wild-type phenotype in the *cis* configuration but a mutant phenotype in the *trans* configuration. This difference in phenotypes is called a *cis-trans* position effect.

From the terms *cis* and *trans,* Seymour Benzer coined the term **cistron** for the smallest genetic unit (length of genetic material) that exhibits a *cis-trans* position effect. We thus have a new word for the gene, one in which function is more explicit. We have, in essence, refined Beadle and Tatum's one-gene-one-enzyme hypothesis to a more accurate one-cistron-one-polypeptide concept. The cistron is the smallest unit that codes for a messenger RNA that is then translated into a single polypeptide or expressed directly (transfer RNA or ribosomal RNA).

From functional alleles, we can go one step further in recombinational analysis by determining whether two allelic mutations occur at exactly the same place in the cistron. In other words, when two mutations prove to be functional alleles, are they also **structural alleles?** The methods used to analyze complementation can be used here also. Crosses are carried out to form a mutant heterozygote (*trans* configuration) whose offspring are then tested for recombination between the two mutational sites. If no recombination occurs, then the two alleles probably contain the same

Seymour Benzer (1921–). (Courtesy of Dr. Seymour Benzer, 1970.)

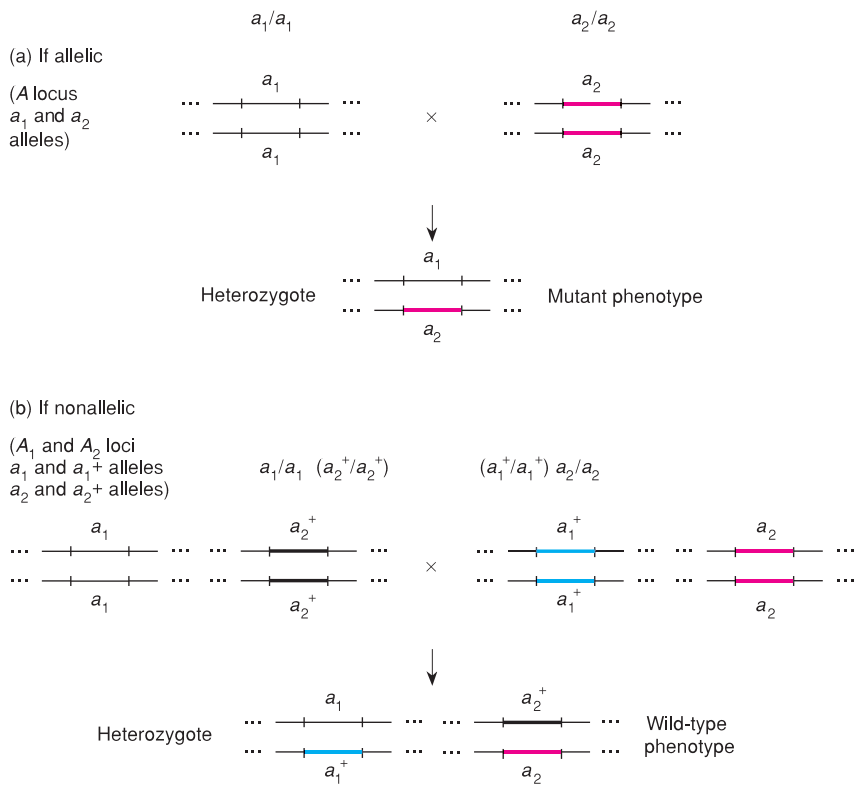


Figure 12.2 The complementation test defines allelism. Are two mutations (a_1, a_2) allelic if they affect the same trait? To find out, mutant homozygotes are crossed to form a heterozygote. (*a*) If the mutations are allelic, then both copies of the gene in the heterozygote are mutant, resulting in the mutant phenotype. (*b*) If the mutations are nonallelic, then there is a wild-type allele of each gene present in the heterozygote, resulting in the wild-type phenotype. (The two loci need not be on the same chromosome.)

structural change (involving the same base pairs) and are thus structural alleles. If a small amount of recombination occurs that generates wild-type offspring, then the two alleles are not mutations at the same point (fig. 12.4). Alleles that were functional but not structural were first termed **pseudoalleles** because it was believed that loci were made up of subloci. Fine-structure analysis led to the understanding that a locus is a length of genetic material divisible by recombination rather than a "bead on a string."

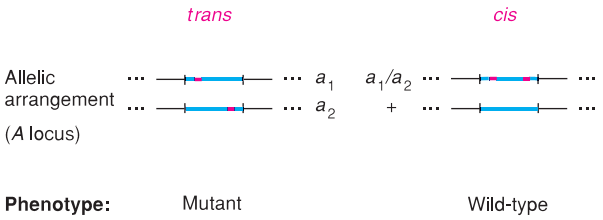


Figure 12.3 A heterozygote of two recessive mutations can have either the *trans* or *cis* arrangement. In the *trans* position, functional alleles produce a mutant phenotype. (*Red marks* represent mutant lesions.) In the *cis* position, functional alleles produce a wild-type phenotype. The *cis-trans* position effect thus reveals functional alleles.

Eye-color mutants of *Drosophila melanogaster* can be studied by complementational analysis. The white-eye locus has a series of alleles producing varying shades of red. This locus is sex linked, at about map position 3.0 on the X chromosome. (Several other eye-color loci on the X chromosome are not relevant to this cross—e.g., prune and ruby.) If an apricot-eyed female is mated with a white-eyed male, the female offspring are all heterozygous and have mutant light-colored eyes (fig. 12.5). Thus, apricot and white are functional alleles: they do not complement (table 12.2). To determine whether apricot and white are structural alleles, light-eyed females are crossed with white-eyed males, and the offspring are observed for the presence of wild-type or light-eyed males. Though their rate of appearance is less than 0.001%, this is significantly above the background mutation rate. The conclusion is that apricot and white are functional, but not structural, alleles.

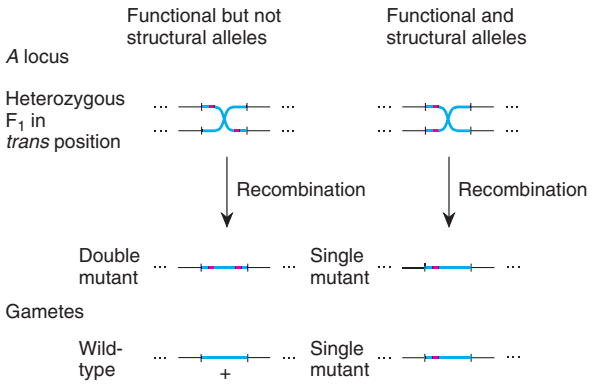


Figure 12.4 Functional alleles may or may not be structurally allelic. (*Red marks* represent mutant sites.) Functional alleles that are not also structural alleles can recombine between the mutant sites, resulting in occasional wild-type (and double mutant) offspring. Structural alleles (which are also always functional alleles) are defective at the same base pairs and cannot form either wild-type or double mutant offspring by recombination.

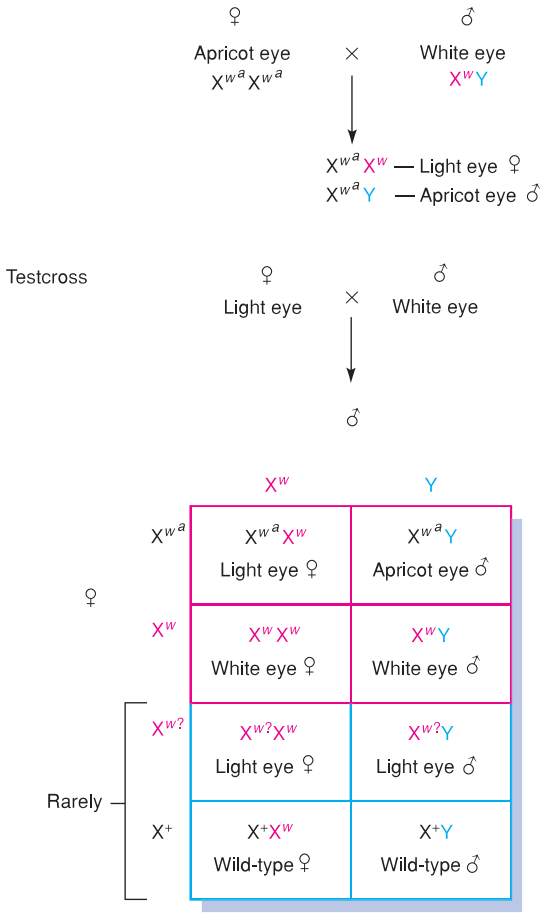


Figure 12.5 Crosses demonstrating that apricot and white eyes are functional, but not structural, alleles in *Drosophila*. Light-eyed females are heterozygous for both alleles. When testcrossed, they produce occasional offspring that are wild-type (X^+ allele) or light-eyed ($X^{w?}$ allele). This indicates a crossover between the two mutant sites (white and apricot) in the heterozygous females, producing, reciprocally, an allele with both mutational sites and the wild-type.

Fine-Structure Mapping

After Beadle and Tatum established in 1941 that a gene controls the production of an enzyme that then controls a step in a biochemical pathway, Benzer used analytical techniques to dissect the fine structure of the gene. Fine-structure mapping means examining the size and number of sites within a gene that are capable of mutation and recombination. In the late 1950s, when biochemical techniques were not yet available for DNA sequencing, Benzer used classical recombinational and mutational techniques with bacterial viruses to provide reasonable estimates on the details of fine structure and to give insight into the nature of the gene. He coined the terms **muton** for the smallest mutable site and **recon** for the smallest unit of recombination. It is now known that both muton and recon are a single base pair.

Before Benzer's work, genes were thought of as beads on a string. The very low rate of recombination between

Table 12.2 Complementation Matrix of X-Linked *Drosophila* Eye-Color Mutants

	white	prune	apricot	buff	cherry	eosin	ruby
white (*w*)	–	+	–	–	–	–	+
prune (*pn*)		–	+	+	+	+	+
apricot (w^a)			–	–	–	–	+
buff (w^{bf})				–	–	–	+
cherry (w^{ch})					–	–	+
eosin (w^e)						–	+
ruby (*rb*)							–

Note: Plus sign indicates that female offspring are wild-type; minus sign indicates that they are mutant.

sites within a gene hampered the analysis of mutational sites within a gene by means of recombination. If two mutant genes are functional alleles (involving different sites on the same gene), a distinct probability exists that we will get both mutant sites (and both wild-type sites) on the same chromosome by recombination (see fig. 12.4); but, in view of the very short distances within a gene, this probability is very low. Although it certainly seemed desirable to map sites within the gene, the problem of finding an organism that would allow fine-structure analysis remained until Benzer decided to use phage T4.

***r* II Screening Techniques.** Benzer used the T4 bacteriophage because of the growth potential of phages, in which a generation takes about an hour and the increase in numbers per generation is about a hundredfold. Actually, any prokaryote or virus should suffice, but Benzer made use of other unique screening properties of the phage that made it possible to recognize one particular mutant in about a billion phages. Benzer used *r*II mutants of T4. These mutants produce large, smooth-edged plaques on *E. coli,* whereas the wild-type produces smaller plaques whose edges are not as smooth (see fig. 7.7).

The screening system that Benzer employed made use of the fact that *r*II mutants do not grow on *E. coli* strain K12, whereas the wild-type can. The normal host strain, *E. coli* B, allows growth of both the wild-type and *r*II mutants. Thus, various mutants can be crossed by mixed infection of *E. coli* B cells, and Benzer could screen for wild-type recombinants by plating the resultant progeny phages on *E. coli* K12 (fig. 12.6), on which only a wild-type recombinant produces a plaque. It is possible to detect about one recombinant in a billion phages, all in an afternoon's work. This ability to detect recombinants occurring at such a low level of frequency allowed Benzer to see recombinational events occurring very close together on the DNA, events that would normally occur at a frequency too low to detect in fruit flies or corn.

Benzer sought to map the number of sites subject to recombination and mutation within the *r*II region of T4. He began by isolating independently derived *r*II mutants and crossing them among themselves. The first thing he found was that the *r*II region was composed of two cistrons; almost all of the mutations belonged to one of two **complementation groups.** The *A*-cistron mutations would not complement each other but would complement the mutations of the *B* cistron. The exceptions were mutations that seemed to belong to both cistrons. These mutations were soon found to be deletions in which part of each cistron was missing (table 12.3).

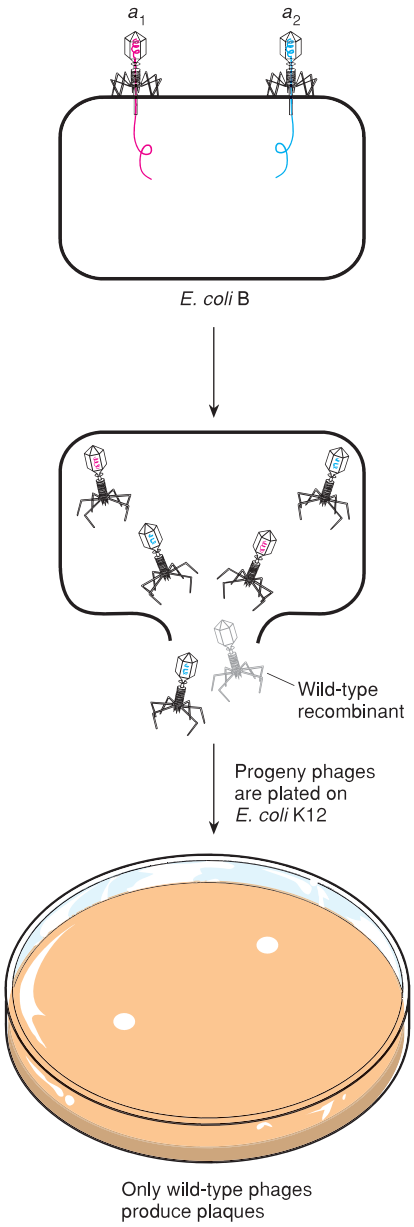


Figure 12.6 Using *E. coli* K12 and B strains to screen for recombination at the *r*II locus of phage T4. Two *r*II mutants are crossed by infecting the same B-strain bacteria with both phages. The offspring are plated on a lawn of K12 bacteria in which only wild-type phages can grow. The technique thus selects only wild-type recombinants.

Deletion Mapping. As the number of independently isolated mutations of the *A* and *B* cistrons increased, it became obvious that to make every possible pairwise cross would entail millions of crosses. To overcome this problem, Benzer isolated mutants that had partial or complete deletions of each cistron. Deletion mutations were easy to discover because they acted like structural alleles to alleles that were not themselves structurally allelic. In other words, if mutations *a, b,* and *c* are functional—but not

structural—alleles of each other, and mutation *d* is a structural allele to *a, b,* and *c,* then *d* must contain a deletion of the bases mutated in *a, b,* and *c.* Once a sequence of deletion mutations covering the *A* and *B* cistrons was isolated, a minimal number of crosses was required to localize a new mutation to a portion of one of the cistrons. A second series of smaller deletions within each region was then isolated, further localizing the mutation (fig. 12.7).

Next, each new mutant was crossed with each of the other mutants isolated in its subregion to localize the relative position of the new mutation. If the mutation was structurally allelic to a previously isolated mutation, it was scored as an independent isolation of the same mutation. If it was not a structural allele to any of the known mutations of the subregion, it was added as a new mutation point. The exact position of each new mutation within the region was determined by the relative frequency of recombination between it and the known mutations of this region (see chapter 7). Benzer eventually isolated about 350 mutations from eighty different subregions defined by deletion mutations. An abbreviated map is shown in figure 12.8.

What conclusions did Benzer draw from his work? First, he concluded that since all of the mutations in both *r*II cistrons can be ordered in a linear fashion, the original Watson-Crick model of DNA as a linear molecule was correct. Second, he concluded that reasonable inroads had been made toward saturating the map, localizing at least one mutation at every mutable site. Benzer reasoned that since many sites were represented by only one mutation, some sites must occur that were represented by zero mutations (i.e., not yet represented by a mutation). Since he had mapped about 350 sites, he calculated that there

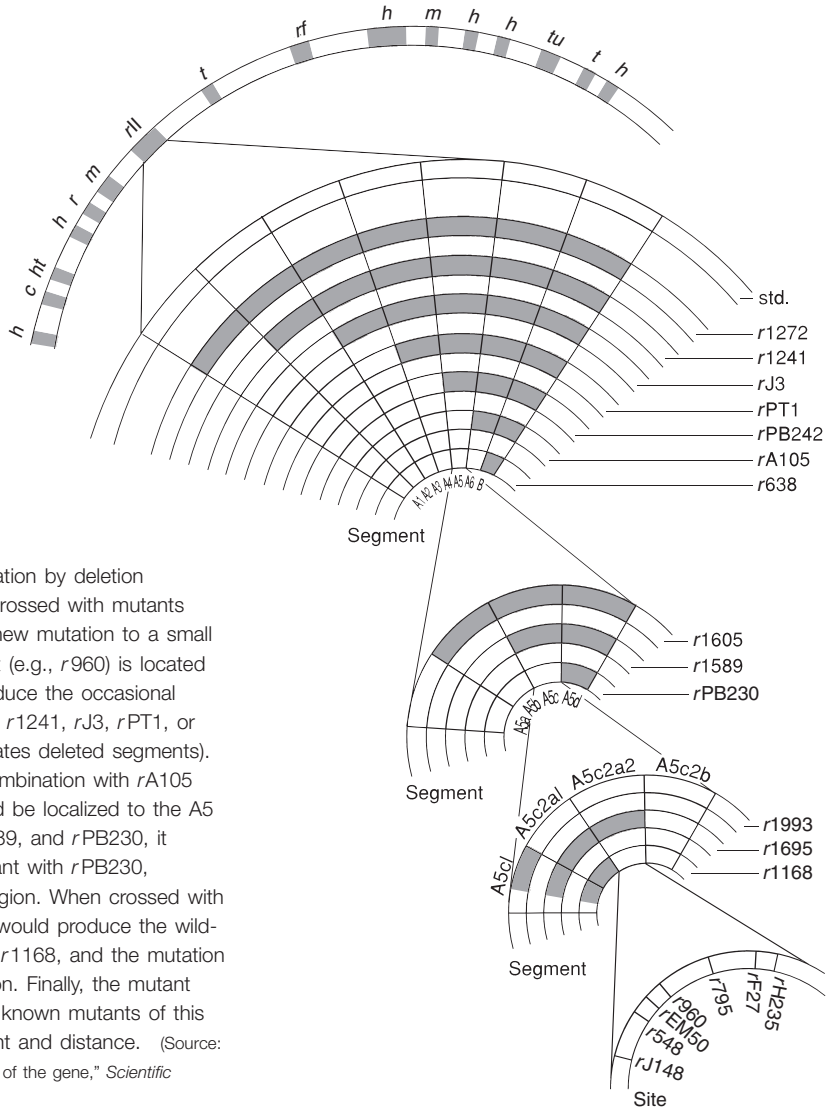


Figure 12.7 Localization of an *r*II mutation by deletion mapping. Newly isolated mutants are crossed with mutants with selected deletions to localize the new mutation to a small region of the cistron. If the new mutant (e.g., *r*960) is located in the A5c2a2 region, it would not produce the occasional wild-type by recombination with *r*1272, *r*1241, *r*J3, *r*PT1, or *r*PB242 (the *solid* part of the bar indicates deleted segments). It would produce the wild-type by recombination with *r*A105 and *r*638, and thus the mutation would be localized to the A5 region. When crossed with *r*1605, *r*1589, and *r*PB230, it would produce only the rare recombinant with *r*PB230, indicating the mutation is in the A5c region. When crossed with *r*1993, *r*1695, and *r*1168, the mutant would produce the wild-type by recombination with *r*1993 and *r*1168, and the mutation would be localized to the A5c2a2 region. Finally, the mutant would be crossed pairwise with all the known mutants of this region to determine relative arrangement and distance. (Source: Data from Seymour Benzer, "The fine structure of the gene," *Scientific American,* 206: 70–84, January 1962.)

Table 12.3 **Complementation Matrix of Ten *r*II Mutants**

	1	2	3	4	5	6	7	8	9	10
1	−	−	+	−	−	−	+	−	−	−
2		−	+	−	−	−	+	−	−	−
3			−	−	+	+	−	+	−	+
4				−	−	−	−	−	−	−
5					−	−	+	−	−	−
6						−	+	−	−	−
7							−	+	−	+
8								−	−	−
9									−	−
10										−

Note: Plus sign indicates complementation; minus sign indicates no complementation. The two cistrons are arbitrarily designated *A* and *B*. Mutants 4 and 9 must be deletions that cover parts of both cistrons. Alleles: *A* cistron: 1, 2, 4, 5, 6, 8, 9, 10; *B* cistron: 3, 4, 7, 9.

were at least another 100 sites still undetected by mutation. We now know that 450 sites is an underestimate. However, since the protein products of these cistrons were not isolated, there were no independent estimates of the number of nucleotides in these cistrons (number of amino acids times three nucleotides per codon). Thus, although Benzer had not saturated the map with mutations, he certainly had made respectable progress in dissecting the gene and demonstrating that it was not an indivisible unit, a "bead on a string."

Hot Spots. Benzer also looked into the lack of uniformity in the occurrence of mutations (note two major "hot spots" at B4 and A6c of fig. 12.8). Presuming that all base pairs are either AT or GC, this lack of uniformity was unexpected. Benzer suggested that spontaneous mutation is not just a function of the base pair itself, but is affected by the surrounding bases as well. This concept still holds.

To recapitulate, Benzer's work supports the model of the gene as a linear arrangement of DNA whose nucleotides are the smallest units of mutation. The link between any adjacent nucleotides can break in the recombinational process. The smallest functional unit, determined by a complementation test, is the cistron. Mutagenesis is not uniform throughout the cistron, but may depend on the particular arrangement of bases in a given region.

Intra-Allelic Complementation

Benzer warned that certainty is elusive in the complementation test because sometimes two mutations of the same functional unit (cistron) can result in partial activity. The

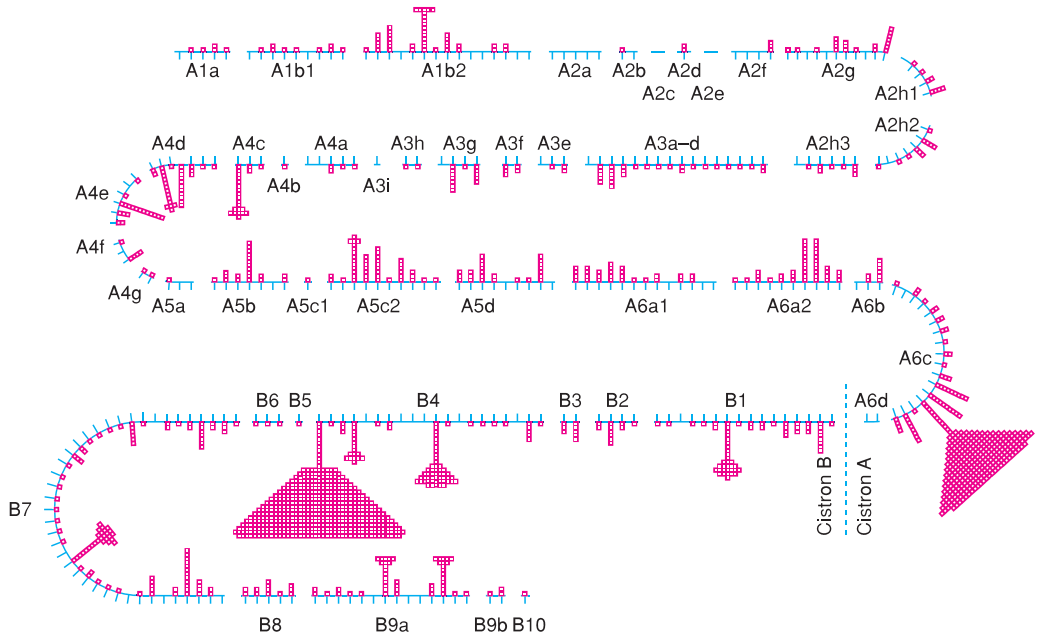


Figure 12.8 Abbreviated map of spontaneous mutations of the *A* and *B* cistrons of the *r*II region of T4. Each square represents one independently isolated mutation. Note the "hot spots" at A6c and B4. (From Seymour Benzer, "On the topography of the genetic fine structure", *Proceedings of the National Academy of Sciences USA* 47:403–15, 1961. Reprinted by permission.)

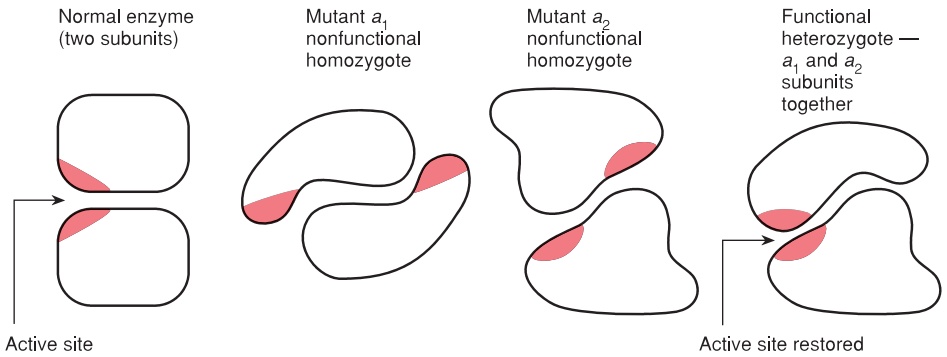


Figure 12.9 Intra-allelic complementation. With certain mutations, it is possible to get enzymatic activity in a heterozygote for two nonfunctional alleles, if the two polypeptides form a functional enzyme. (Active site is shown in *color.*)

problem can be traced to the interactions of subunits at the polypeptide level. Some proteins are made up of subunits, and it is possible that certain mutant combinations produce subunits that interact to restore the enzymatic function of the protein (fig. 12.9). This phenomenon is known as **intra-allelic complementation.** With this in mind, geneticists routinely use the complementation test to determine functional relationships among mutations.

Colinearity

Next we look at the colinearity of the gene and the polypeptide. Benzer's work established that the gene was a linear entity, as Watson and Crick had proposed. However, Benzer could not demonstrate the colinearity of the gene and its protein product. To do this, it is necessary to show that for every mutational change in the DNA, a corresponding change takes place in the protein product of the gene. Colinearity would be established by showing that nucleotide and amino acid changes occurred in a linear fashion and in the same order in the protein and in the cistron.

Ideally, Benzer himself might have solved the colinearity issue. He was halfway there, with his 350 or so isolated mutations of phage T4. However, Benzer did not have a protein product to analyze; no mutant protein had been isolated from *r*II mutants. In the midst of competition to find just the right system, Charles Yanofsky of Stanford University and his colleagues emerged in the mid-1960s with the required proof, showing that the order of a polypeptide's amino acids corresponded to the nucleotide sequence in the gene that specified it. Yanofsky's success rested with his choice of an amenable system, one using the enzymes from a biochemical pathway.

Yanofsky did his research on the tryptophan biosynthetic pathway in *E. coli.* The last enzyme in the pathway, tryptophan synthetase, catalyzes the reaction of indole-3-glycerol-phosphate plus serine to tryptophan and 3-phosphoglyceraldehyde. The enzyme itself is made of four subunits specified by two separate cistrons, with each polypeptide present twice.

Yanofsky and his colleagues concentrated on the *A* subunit. They mapped *A*-cistron mutations with transduction (see chapter 7) using the transducing phage P1. They first tested each new mutant against a series of deletion mutants to establish the region where the mutation was. Then they crossed mutants for a particular region among themselves to establish relative positions and distances.

The protein products of the bacterial genes were isolated using electrophoresis and chromatography to establish the fingerprint patterns of the proteins (see chapter 11). Assuming a single mutation, a comparison of the mutant and the wild-type fingerprints would show a difference of just one polypeptide spot (fig. 12.10), avoiding the need to sequence the entire protein. The mutant amino acid was

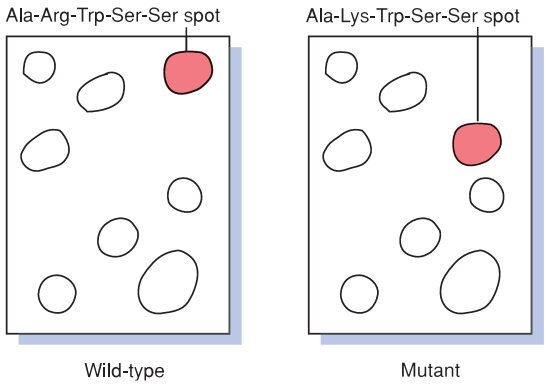


Figure 12.10 Difference in "fingerprints" between mutant and wild-type polypeptide digests. The single spot that differs in the mutant can be isolated and sequenced, eliminating the need to sequence the whole protein.

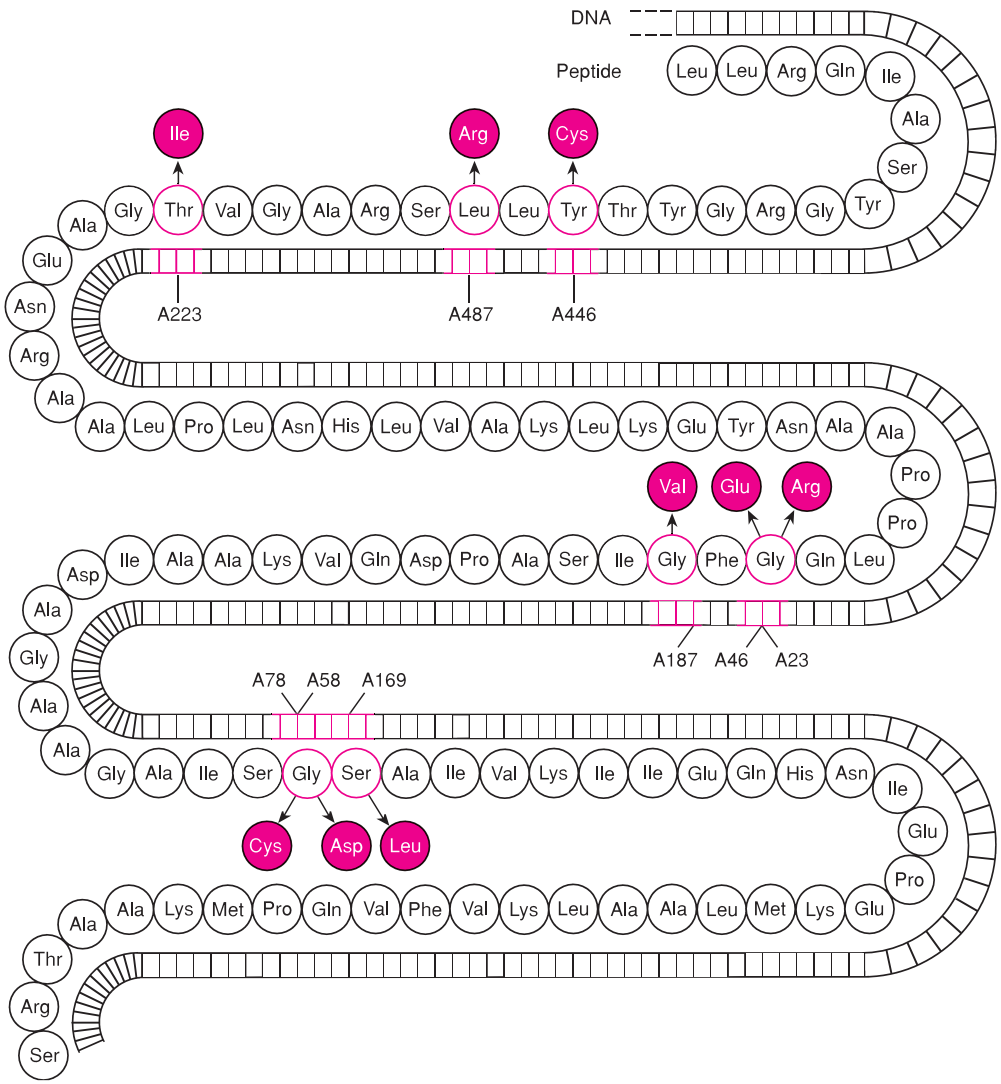


Figure 12.11 Amino acid sequence of the carboxyl terminal end of the tryptophan synthetase *A* protein and its DNA. Mutations are shown on the DNA (e.g., A446), as are the changed amino acids of those mutations (in *color*). DNA and protein changes are colinear.

identified by analysis of just this one spot. Figure 12.11 shows the details of nucleotide and amino acid changes for nine of the mutations in this 267-amino acid protein.

We can see from this figure that nine mutations in the linear *A* cistron of tryptophan synthetase are colinear with nine amino acid changes in the protein itself. In two cases, two mutations mapped so close as to be almost indistinguishable. In both cases, the two mutations proved to be in the same codon: the same amino acid position was altered in each (A23–A46, A58–A78). Thus, exactly as predicted and expected, colinearity exists between gene and protein. Brenner and his colleagues, using head-protein mutants of phage T4, independently confirmed this work at the same time.

Spontaneous Versus Induced Mutation

H. J. Muller won the Nobel Prize for demonstrating that X rays can cause mutations. This work was published in 1927 in a paper entitled "Artificial Transmutation of the Gene." At about the same time, L. J. Stadler induced mutations in barley with X rays. The basic impetus for their work was the fact that mutations occur so infrequently that genetic research was hampered by the inability to

Hermann J. Muller (1890–1967). (Courtesy of National Academy of Sciences.)

Lewis J. Stadler (1896–1954). (*Genetics*, 41, 1956: frontispiece.)

obtain mutants. Muller exposed flies to varying doses of X rays and then observed their progeny. He came to several conclusions. First, X rays greatly increased the occurrence of mutations. Second, the inheritance patterns of X-ray-induced mutations and the resulting phenotypes of organisms were similar to those that resulted from natural, or "spontaneous," mutations.

Mutation Rates

The **mutation rate** is the number of mutations that arise per cell division in bacteria and single-celled organisms, or the number of mutations that arise per gamete in higher organisms. Mutation rates vary tremendously depending upon the length of genetic material, the kind of mutation, and other factors. Luria and Delbrück, for example, found that in *E. coli* the mutation rate per cell division of Ton^s to Ton^r was 3×10^{-8}, whereas the mutation rate of the wild-type to the histidine-requiring phenotype (His^+ to His^-) was 2×10^{-6}. The rate of **reversion** (return of the mutant to the wild-type) was 7.5×10^{-9}. The mutation and reversion rates differ because many different mutations can cause the His phenotype, whereas reversion requires specific, and hence less probable, changes to correct the His phenotype back to the wild-type. The lethal mutation rate in *Drosophila* is about 1×10^{-2} per gamete for the total genome. This number is relatively large because, as with His, many different mutations produce the same phenotype (lethality, in this case).

Point Mutations

The mutations of primary concern in this chapter are **point mutations,** which consist of single changes in the nucleotide sequence. (In chapter 8 we discussed chromosomal mutations, changes in the number and visible structures of chromosomes.) If the change is a replacement of some kind, then a new codon is created. In many cases, this new codon, upon translation, results in a new amino acid. As discussed in chapter 11, one of the outcomes of redundancy in the genetic code is partial protection of the cell from the effects of mutation; common amino acids have the most codons, similar amino acids have similar codons, and the wobble position of the codon is the least important position in translation. However, when base changes result in new amino acids, new proteins appear. These new proteins can alter the morphology or physiology of the organism and result in phenotypic novelty or lethality.

Frameshift Mutation

A point mutation may consist of replacement, addition, or deletion of a base (fig. 12.12). Point mutations that add

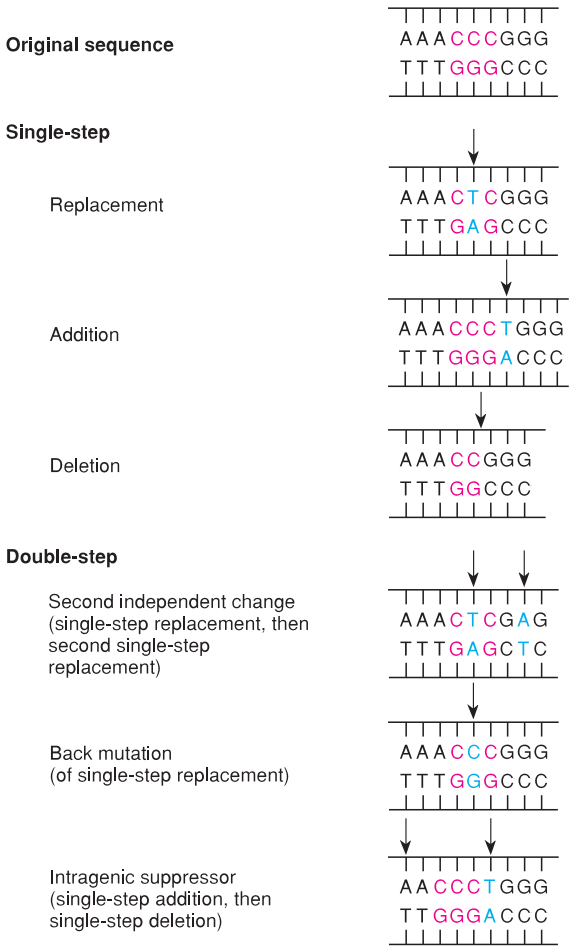


Figure 12.12 Types of DNA point mutations. Single-step changes are replacements, additions, or deletions. A second point mutation in the same gene can result either in a double mutation, reversion to the original, or intragenic suppression. In this case, intragenic suppression is illustrated by the addition of one base followed by the nearby deletion of a different base.

or subtract a base are, potentially, the most devastating in their effects on the cell or organism because they change the reading frame of a gene from the site of mutation onward (fig. 12.13). A frameshift mutation causes two problems. First, all the codons from the frameshift on will be different and thus yield (most probably) a useless protein. Second, stop-signal information will be misread. One of the new codons may be a nonsense codon, which causes translation to stop prematurely. Or, if the translation apparatus reaches the original nonsense codon, it is no longer recognized as such because it is in a different reading frame, and therefore, the translation process continues beyond the end of the gene.

Back Mutation and Suppression

A second point mutation in the same gene can have one of three possible effects (see fig. 12.12). First, the mutation can result in either another mutant codon or in one codon that has experienced two changes. Second, if the change is at the same site, the original sequence can be returned, an effect known as **back mutation:** the gene then becomes a revertant, with its original function restored. Third, **intragenic suppression** can take place. Intragenic suppression occurs when a second mutation in the same gene masks the occurrence of the original mutation without actually restoring the original sequence. The new sequence is a double mutation that appears to have the original (unmutated) phenotype. In figure 12.12, a T addition is followed by an A deletion that substitutes the AACCCT sequence for the original AAACCC. These sequences, when transcribed (UUGGGA, UUUGGG), are codons for leucine-glycine and phenylalanine-glycine, respectively. Intragenic suppression occurs whether the new codons are for different amino acids or the same amino acids, as long as the phenotype of the organism is reverted approximately to the original. Suppressed mutations can be distinguished from true back mutations either by subtle differences in phenotype, by genetic crosses, by changes in the amino acid sequence of a protein, or by DNA sequencing.

Conditional Lethality

A class of mutants that has been very useful to geneticists is the conditional-lethal mutant, a mutant that is lethal under one set of circumstances but not under another set. **Nutritional-requirement mutants** are good examples (see chapter 7). **Temperature-sensitive mutants** are conditional-lethal mutants that have made it possible for geneticists to work with genes that control vital functions of the cell, such as DNA synthesis. Many temperature-sensitive mutants are completely normal at 25° C but cannot synthesize DNA at 42° C. Presumably, temperature-sensitive mutations result in enzymes with amino acid substitutions that cause protein denaturation to occur at temperatures above normal. Thus, the enzyme has normal function at 25° C, the **permissive temperature,** but is nonfunctional at 42° C, the **restrictive temperature.**

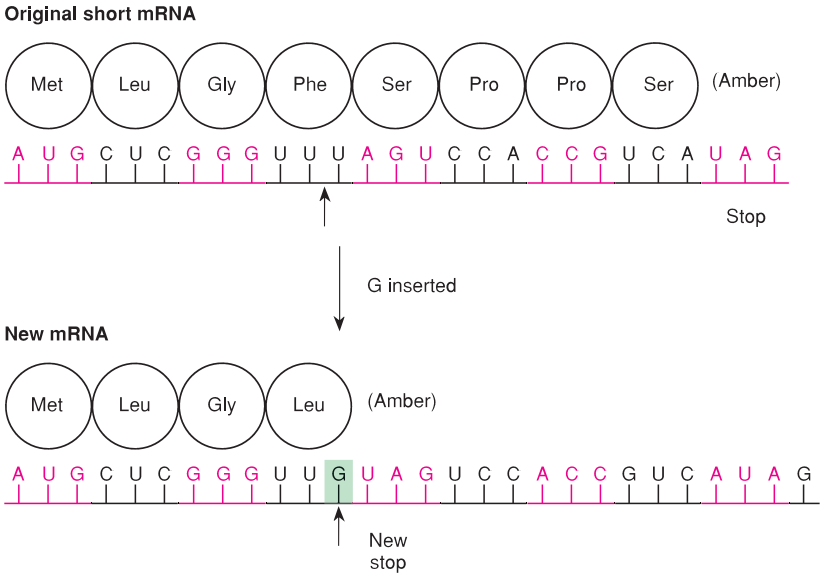


Figure 12.13 Possible effects of a frameshift mutation. The insertion of a single base results in the creation of a new stop sequence (*amber*). The result will be premature termination of translation.

The interesting thing about most conditional-lethal mutants of *E. coli* that cannot synthesize DNA at the restrictive temperature is that they have a completely normal DNA polymerase I. From this information, we infer that polymerase I is not the enzyme *E. coli* normally uses for DNA replication. When an organism with a conditional mutation of polymerase I was isolated, it was able to replicate its DNA normally, but unable to repair damage to the DNA. This led to the conclusion that polymerase I is primarily involved in repair rather than replication of DNA. Conditional-lethal mutants thus allow genetic analysis on genes otherwise impossible to study.

Spontaneous Mutagenesis

Watson and Crick originally suggested that mutation could occur spontaneously during DNA replication if pairing errors occurred. If a base of the DNA underwent a proton shift into one of its rare tautomeric forms **(tautomeric shift)** during the replication process, an inappropriate pairing of bases would occur. Normally, adenine and cytosine are in the amino (NH_2) form. Their tautomeric shifts are to the imino (NH) form. Similarly, guanine and thymine go from a keto (C=O) form to an enol (COH) form (fig. 12.14). Table 12.4 shows the new

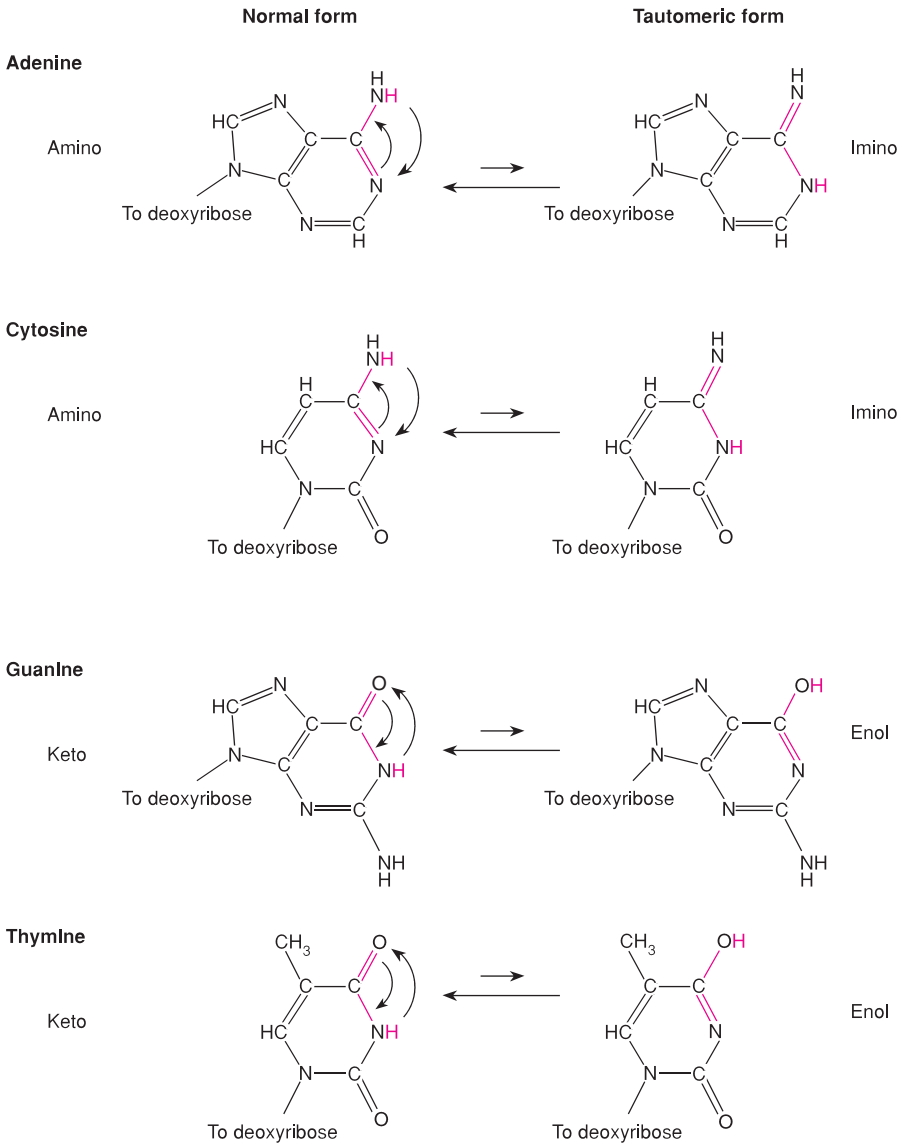


Figure 12.14 Normal and tautomeric forms of DNA bases. Adenine and cytosine can exist in the amino, or the rare imino, forms; guanine and thymine can exist in the keto, or rare enol, forms.

Table 12.4 **Pairing Relationships of DNA Bases in the Normal and Tautomeric Forms**

Base	In Normal State Pairs with	In Tautomeric State Pairs with
A	T	C
T	A	G
G	C	T
C	G	A

base pairings that would occur following tautomeric shifts of the DNA bases. Figure 12.15 illustrates the molecular structure of one of these tautomeric pairings.

During DNA replication, a tautomeric shift in either the incoming base (*substrate transition*) or the base already in the strand (*template transition*) results in mispairing. The mispairing will be permanent and result in a new base pair after an additional round of DNA replication. The original strand is unchanged (fig. 12.16).

In the example in figure 12.16, the replacement of one base pair maintains the same purine-pyrimidine relationship: AT is replaced by GC and GC by AT. In both examples, a purine-pyrimidine combination is replaced by a purine-pyrimidine combination. (Or, more specifically, a purine replaces another purine: guanine replaces

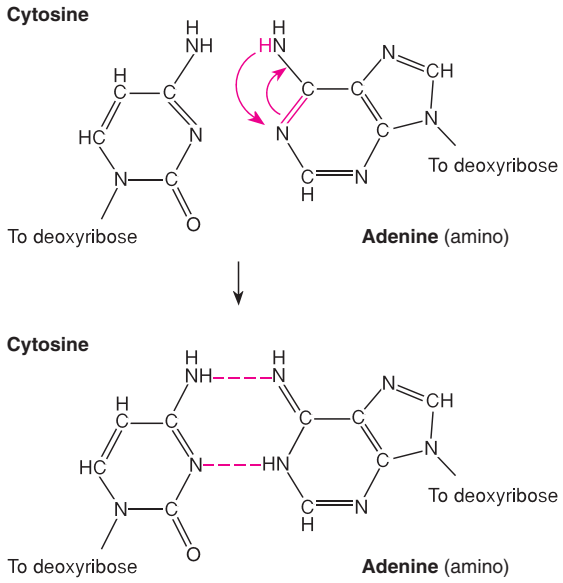


Figure 12.15 Tautomeric forms of adenine. In the common amino form, adenine does not base-pair with cytosine; in the tautomeric imino form, it can.

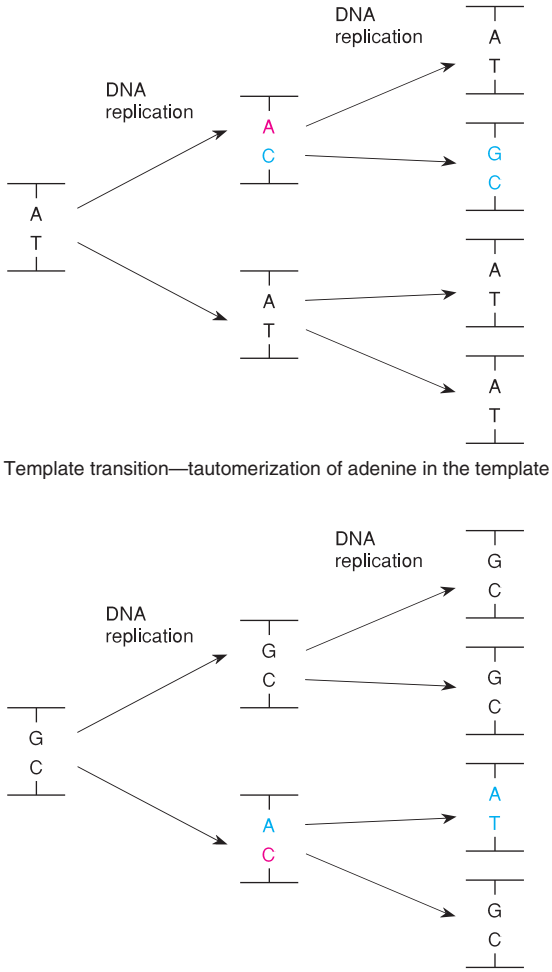


Figure 12.16 Tautomeric shifts result in transition mutations. The tautomerization can occur in the template base or in the substrate base. Tautomeric shifts are shown in *red;* the resulting transition in *blue.* The transition shows up after a second generation of DNA replication.

adenine in the first example and adenine replaces guanine in the second.) The mutation is referred to as a **transition mutation:** a purine (or pyrimidine) replaces another purine (or pyrimidine) through a transitional state involving a tautomeric shift. When a purine replaces a pyrimidine or vice versa, it is referred to as a **transversion mutation.**

Transversions may arise by a combination of two events, a tautomerization and a base rotation. (We saw base rotations in the formation of Z DNA in chapter 9.) For example, an AT base pair can be converted to a TA base pair (a transversion) by an intermediate AA pairing

(fig. 12.17). Adenine can pair with adenine if one of the bases undergoes a tautomeric shift while the other rotates about its base-sugar (glycosidic) bond (fig. 12.18). The normal configuration of the base is referred to as the *anti* configuration; the rotated form is the *syn* configuration. Since we now believe that as many as 10% of bases may be in the *syn* configuration at any moment, the transversion mutagenesis rate should be about 10% of the transition mutagenesis rate, a value not inconsistent with current information.

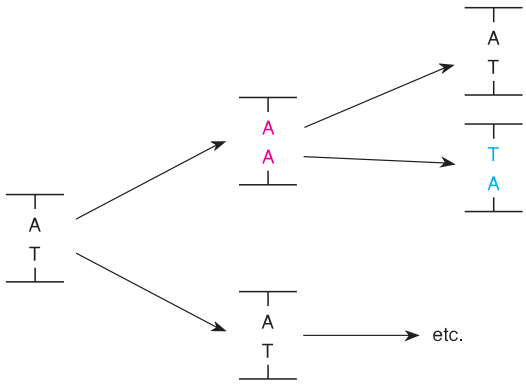


Figure 12.17 A model for transversion mutagenesis. An AT base pair can be converted to a TA base pair (a transversion) by way of an intermediate AA base pair. One of the *red* bases is in the rare tautomeric form, while the other is in the *syn* configuration. After a second round of DNA replication, one DNA duplex will have a transversion at that point (*blue*).

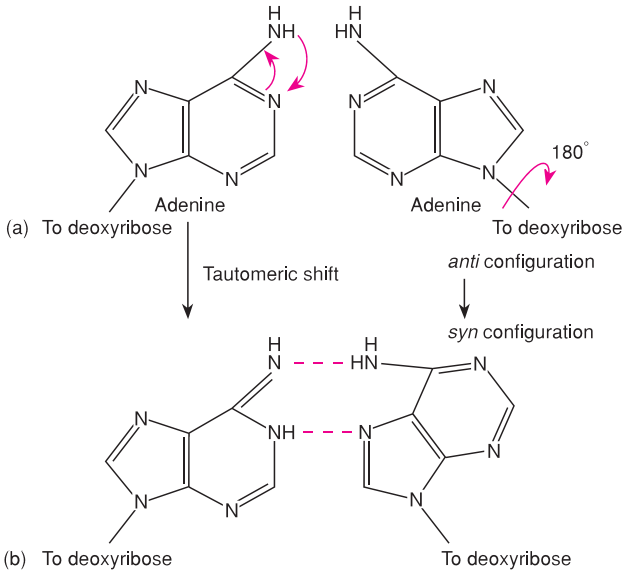


Figure 12.18 Transversion mutagenesis. An AA base pair can form if one base undergoes a tautomeric shift while the other rotates about its glycosidic (sugar) bond. In (*a*), both bases are in their normal configurations; no hydrogen bonding occurs. In (*b*), hydrogen bonds are possible.

Some base-pair mutations can have serious results. If guanine undergoes an oxidation to 8-oxoguanine (fig. 12.19), it pairs with adenine. A GC base pair is converted to a TA base pair through an 8-oxoguanine-adenine intermediate. This transversion has been found to be common in cancers.

Since 1953, when Watson and Crick first described the structure of DNA, tautomerization has been accepted as the obvious source of most transition mutations. However, recent structural data has cast some doubt on this assumption. X-ray crystallography and nuclear magnetic resonance (NMR) studies indicate that both bases in transition mismatches may be in their normal forms. Other mechanisms, similar to wobble base pairing (see chapter 11), may be responsible for most transition mutations. These studies also indicate that some transversions result from direct purine-purine or pyrimidine-pyrimidine base pairing during DNA synthesis. Much work needs to be done to clarify the nature of spontaneous mutagenesis.

Chemical Mutagenesis

Muller demonstrated that X rays can cause mutation. Certain chemical and temperature treatments can also cause mutation. Determining the mode of action of various chemical mutagens has provided insight into the mutational process as well as the process of carcinogenesis (box 12.1). In addition, knowing how chemical mutagens act has allowed geneticists to produce large numbers of specific mutations at will (box 12.2).

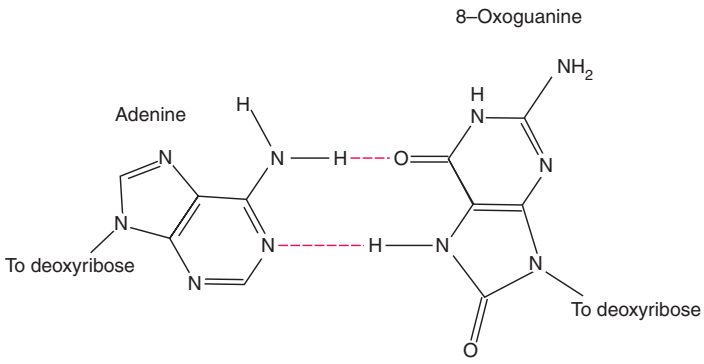


Figure 12.19 The structure of 8-oxoguanine, which base pairs with adenine, converting a GC base pair to a TA base pair—a transversion. (After C. Mol, et al., "DNA repair mechanisms for the recognition and removal of damaged DNA bases," *Annual Review of Biophysics and Biomolecular Structure,* 28:101–28,1999, figure 6.)

Transitions

Transitions are routinely produced by base analogues. Two of the most widely used base analogues are the pyrimidine analogue 5-bromouracil (5BU) and the purine analogue 2-aminopurine (2AP; fig. 12.20). The mutagenic mechanisms of the two are similar. The 5-bromouracil is incorporated into DNA in place of thymine; it acts just like thymine in DNA replication and, since it doesn't alter the hydrogen bonding, should induce no mutation. However, it seems that the bromine atom causes 5-bromouracil to tautomerize more readily than thymine does. Thus, 5-bromouracil goes from the keto form (fig. 12.20) to the enol form more readily than thymine. Transitions frequently result when the enol form of 5-bromouracil pairs with guanine.

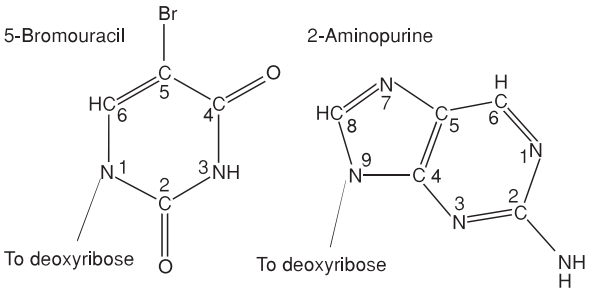


Figure 12.20 Structure of the base analogues 5-bromouracil (5BU) and 2-aminopurine (2AP).

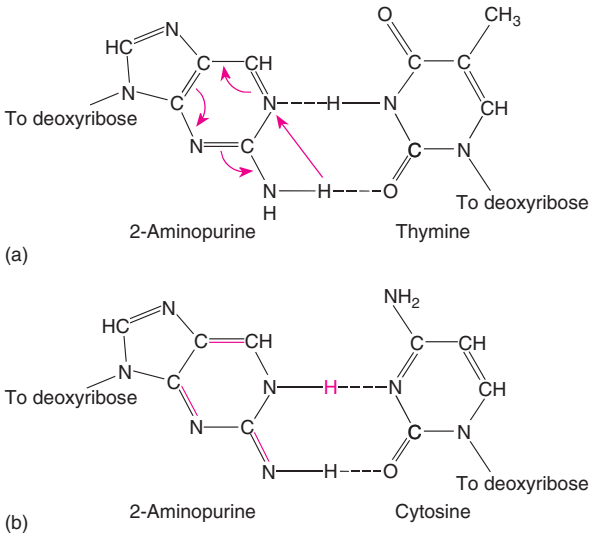


Figure 12.21 Two possible base pairs with 2-aminopurine. (*a*) In the normal state, 2-aminopurine acts like adenine and pairs with thymine. (*b*) In the rare state, 2-aminopurine acts like guanine and forms complementary base pairs with cytosine.

The 2-aminopurine is mutagenic by virtue of the fact that it can, like adenine, form two hydrogen bonds with thymine. When in the rare state, it can pair with cytosine (fig. 12.21). Thus, at times it replaces adenine, and at other times guanine. It promotes transition mutations.

Nitrous acid (HNO_2) also readily produces transitions by replacing amino groups on nucleotides with keto groups ($—NH_2$ to $=O$). The result is that cytosine is converted to uracil, adenine to hypoxanthine, and guanine to xanthine. As figure 12.22 shows, transition

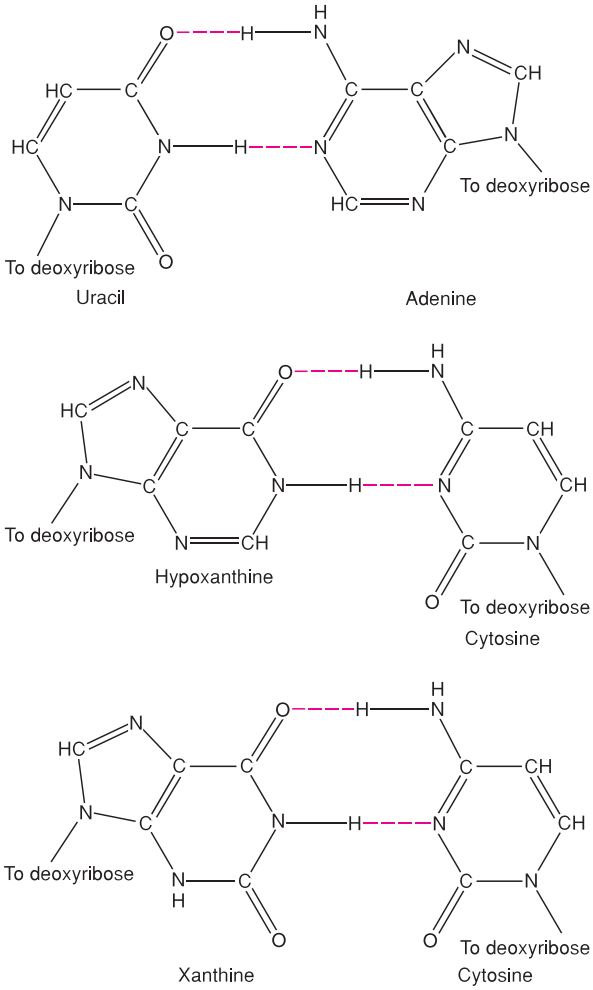


Figure 12.22 Nitrous acid converts cytosine to uracil, adenine to hypoxanthine, and guanine to xanthine. Uracil pairs with adenine, whereas its progenitor, cytosine, normally pairs with thymine; hypoxanthine pairs with cytosine, whereas its progenitor, adenine, normally pairs with thymine; and xanthine pairs with cytosine—the same base that guanine, its progenitor, pairs with. Thus, only the first two pairings result in transition mutations.

BOX 12.1

Biomedical Applications

The Ames Test for Carcinogens

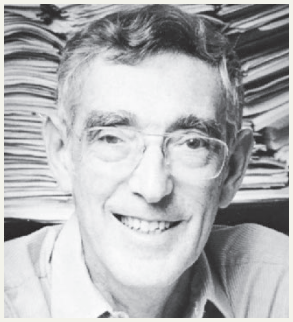
Which chemicals cause cancer in human beings? It is difficult to determine whether any substance is a carcinogen. Tests for carcinogenicity usually involve administering the substance in question to laboratory rats or mice to determine whether the substance actually causes cancer in these animals. Even these tests, however, are not absolute predictors of cancer in people. Tests of this nature are very expensive ($1 million to $2 million each) and time-consuming (three to four years). Since more than fifty thousand different chemical compounds are used in industry, with thousands more added each year, the challenge of making the working environment as well as the general environment safe seems overwhelming. We can, however, make a preliminary determination about the cancer-causing properties of any substance very quickly because of the relationship between mutagenicity and carcinogenicity. Many substances in the environment that can cause cancer also cause mutations. Both kinds of effects are related to DNA damage.

Bruce Ames, at the University of California at Berkeley, developed a routine screening test for mutagenicity. Substances that prove positive in this test are suspected of being carcinogens and would have to be tested further to determine their potential to cause cancer in mammals.

Ames worked with a strain of *Salmonella typhimurium* that requires histidine to grow. This strain will not grow on minimal medium. However, the strain will grow if a mutagen is added to the medium, causing the defective gene in the histidine pathway to revert to the wild-type. (Mutagens inducing gross chromosomal damage, such as deletions or inversions, will not be detected.) Under normal circumstances, there is a background mutation rate; a certain number of *Salmonella* cells revert spontaneously, and therefore a certain number of colonies will grow on the minimal medium. A mutagen, however, increases the number of colonies that can grow on minimal medium. This procedure is, therefore, a rapid, inexpensive, and easy test for mutagenicity.

To improve this test's ability to detect carcinogens, Ames added a supplement of rat liver extract to the medium. It is known that, although many substances are themselves not carcinogens, the breakdown of these substances in the liver creates substances that are carcinogenic. Rat liver enzymes act on a substance the same way human livers do, converting a noncarcinogenic primary substance into a possible carcinogen. The liver enzymes can also make a mutagen nonmutagenic.

Other short-term tests are in use that effectively duplicate the Ames test. These include tests for mutagenicity in mouse lymphoma cells and two tests in Chinese hamster ovary cells: a test for chromosomal aberrations and a test for sister-chromatid exchanges. None of these tests surpasses the Ames test, which has scored better than 90% correct when tested with hundreds of known carcinogens. Thousands of other substances have been subjected to this test; many have proven to be mutagenic. These substances are usually withdrawn from the workplace or home environment. From time to time, we read that a certain substance is believed to be carcinogenic and is being removed from grocery store shelves. Examples have included hair dyes, food preservatives, food-coloring agents, and artificial sweeteners. Many of these first were suspected after they failed the Ames test.



Bruce Ames (1928–).
(Courtesy Dr. Bruce Ames.)

BOX 12.2

Experimental Methods

In Vitro Site-Directed Mutagenesis

One way of studying the way that proteins work is to change the sequence of amino acids in the protein. For example, if a scientist were working on the active site of a particular enzyme, he or she could learn how the enzyme modifies its substrates by changing one or a few amino acids. Changes could be made, for example, in order to study the role of shape or charge on the functioning of the enzyme. Advances in recombinant DNA techniques have made it possible for a research scientist to create exactly the changes he or she wants in a protein.

To begin with, the gene for the protein or enzyme must be cloned so that it can be manipulated (see chapter 13). Once cloned, deletions are easy to create with restriction endonucleases (described in chapter 13). If a particular endonuclease cuts the gene in two places, the intervening segment can be spliced out (fig. 1*a*; see chapter 13). If the endonuclease cuts only once, exonucleases can digest the ends of the cut, extending the deletion away from the cut in both directions (fig. 1*b*). Insertions can be created by either cutting the gene and repairing the single-stranded ends (fig. 1*c*) or by creating an oligonucleotide (a linker) with the desired sequence and inserting the linker at the site of an endonuclease cut (fig. 1*d*).

Far more impressive, however, is the ability to change a single specific codon in order to replace any amino acid in the protein with any other amino acid. The process involves directed mutagenesis using artificially created oligonucleotides.

Basically, a short sequence of DNA (an oligonucleotide) is synthesized complementary to a region of the cloned gene, but with a change in one or more bases of a codon to specify a different amino acid. That oligonucleotide is then hybridized with the single-stranded form of the clone (fig. 2). Although one or more

continued

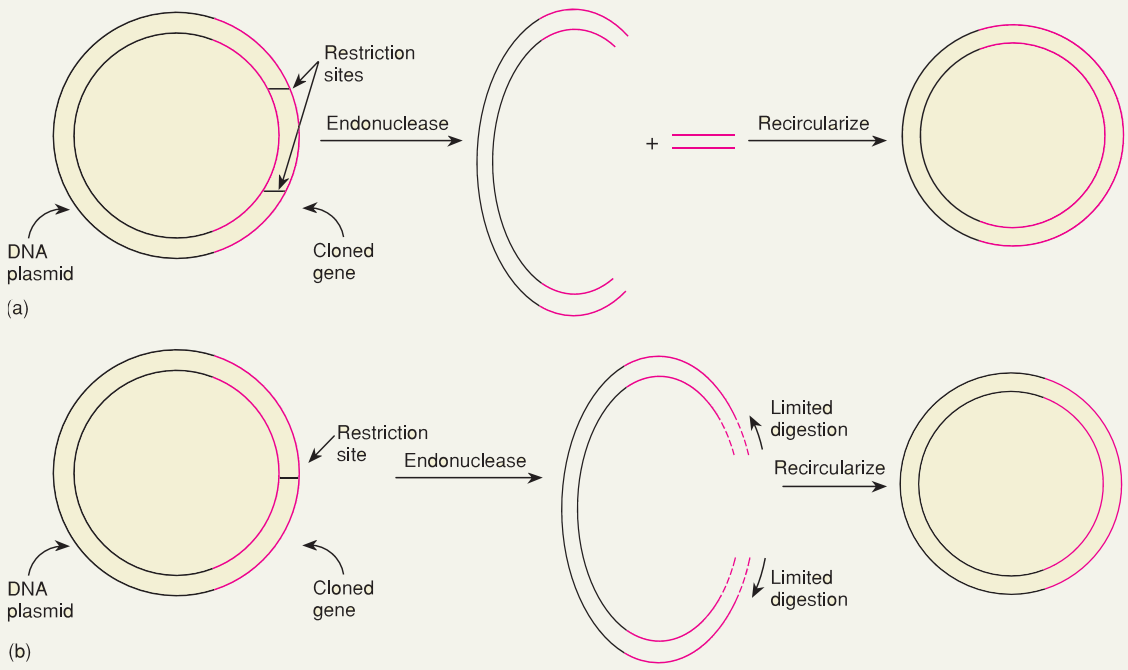


Figure 1 A cloned gene can be mutated in several ways. (*a*) If a restriction endonuclease has two sites in the gene, the intermediate piece can be spliced out. (*b*) If the endonuclease has only one site, the gene can be opened at that site, and limited digestion by exonucleases will delete part of the gene.

continued

BOX 12.2 CONTINUED

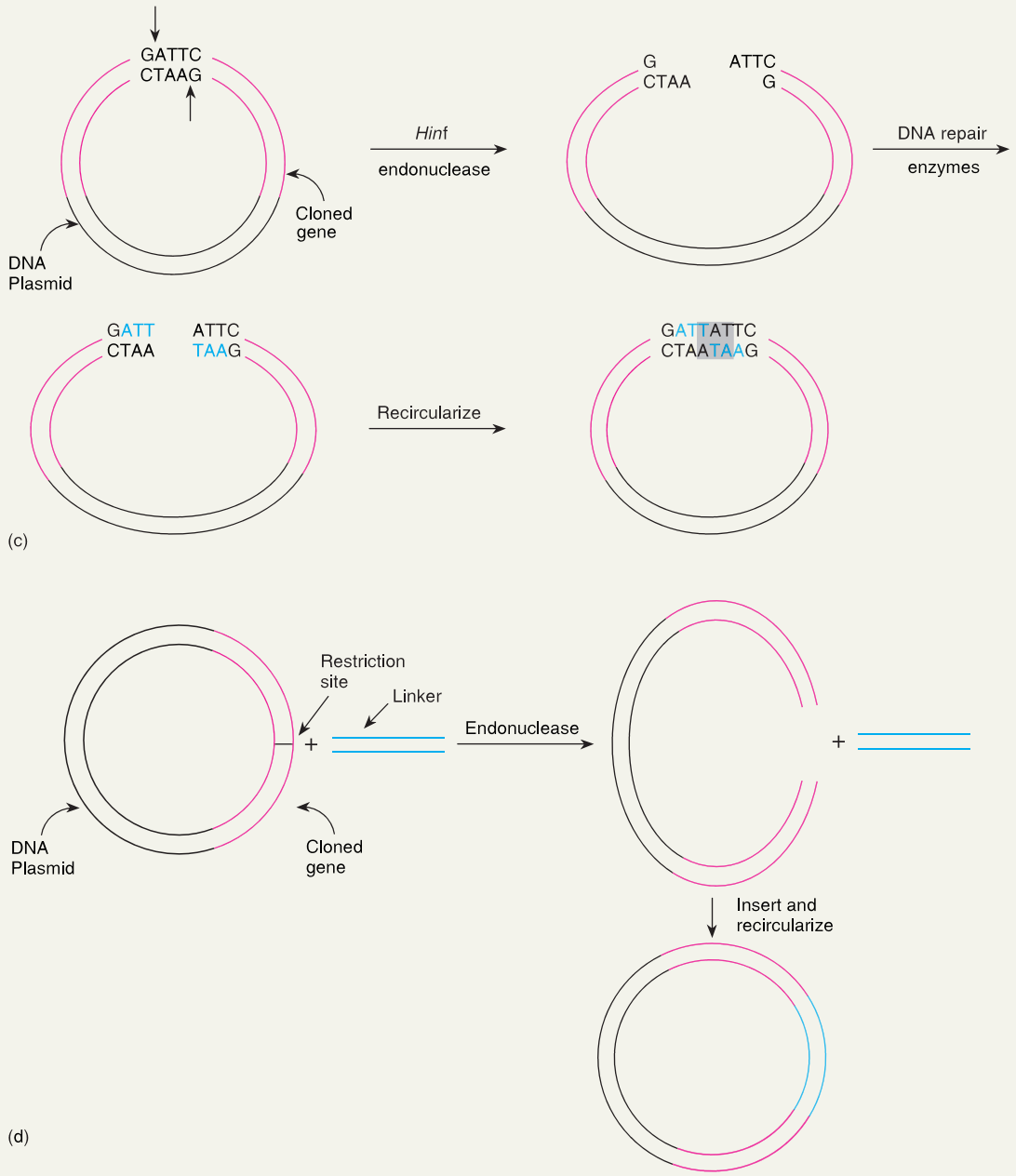


Figure 1—*continued* (*c*) If an endonuclease has an offset region between its splice points of three or six nucleotides (one or two codons), that length can be inserted by repairing the single-stranded ends after cutting by the endonuclease. The resulting blunt ends can be spliced together. (Note that actually an ATT region has been converted to an ATTATT region. If reading codons along the DNA, the actual insertion is of a TAT codon.) (*d*) A linker of any length (usually the length of a specific number of codons) can be inserted at a restriction site.

bases will not match, hybridization can usually be facilitated by adjusting the pH or ionic strength of the solution. The hybridized oligonucleotide is then used as a primer for DNA replication; the whole plasmid is replicated, resulting in hybrid DNA. In subsequent DNA replications of the hybrid, both the original gene and the mutated DNA will be produced. The latter can be isolated by appropriate selection methods; it is a plasmid with a cloned gene that has the exact mutation the researcher wanted. Using techniques of this type, geneticists have made many advancements in understanding exactly how various components of an enzyme contribute to its function.

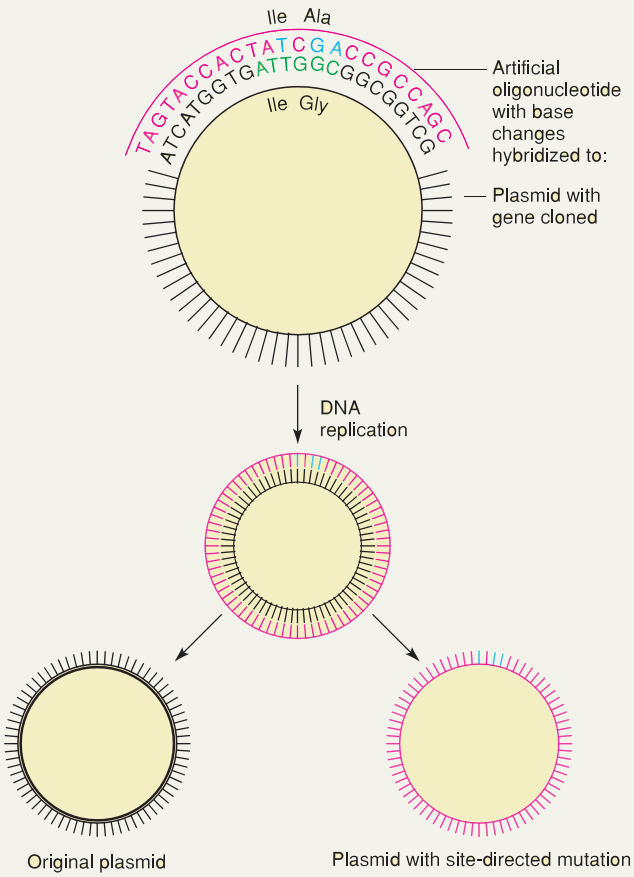


Figure 2 Site-directed mutagenesis can involve any nucleotide(s). In this case, an inserted gene with an Ile-Gly sequence is converted, at the direction of the investigator, to an Ile-Ala sequence. A single-stranded form of the plasmid is isolated. A synthetically prepared oligonucleotide (twenty-three bases in this example) is added. It can be made to hybridize at the complementary site despite differing by three bases. Then DNA replication is carried out using the oligonucleotide configuration as a primer. After the strands of the duplex are separated, the investigators can isolate the original plasmid as well as the mutated plasmid. (Note that the investigators changed two codons, although they changed only one amino acid, because they also wanted to introduce an Alu site at that point for future studies.) (From J. E. Villafranca, et al., "Directed mutagenesis of dihydrofolate reductase," *Science* 222:782–88. Copyright 1983 by the AAAS.)

mutation results from two of the changes. Uracil pairs with adenine instead of guanine, thus leading to a UA base pair in place of a CG base pair; hypoxanthine (H) pairs with cytosine instead of thymine, the original base paired with adenine. Thus, in this case, an HC base pair replaces an AT base pair. Both of these base pairs (UA and HC) are transition mutations. Xanthine, however, pairs with cytosine just as guanine does. Thus, the replacement of guanine with xanthine does not cause changes in base pairing.

Like nitrous acid, heat can also deaminate cytosine to form uracil and thus bring about transitions (CG to TA). Apparently, heat can also bring about transversions by an unknown mechanism.

Transversions

Ethyl methane sulfonate ($CH_3SO_3CH_2CH_3$) and ethyl ethane sulfonate ($CH_3CH_2SO_3CH_2CH_3$) are agents that cause the removal of purine rings from DNA. The multistep process begins with the ethylation of a purine ring and ends with the hydrolysis of the glycosidic (purine-deoxyribose) bond, causing the loss of the base. These sites where this happens are referred to as AP (apurinic-apyrimidinic) sites. If the AP site is not repaired, any of the four DNA bases could be inserted into the new strand opposite the gap (fig. 12.23). If thymine is placed in the newly formed strand, then the original base pair is restored; insertion of cytosine results in a transition mutation; insertion of either adenine or guanine results in a transversion mutation. Of course, the gap is still there, and it continues to generate new mutations each generation until it is repaired. During DNA replication in *E. coli,* the polymerase tends to place adenine opposite the gap more frequently than it places other bases.

Insertions and Deletions

The molecules of the acridine dyes, such as proflavin and acridine orange (fig. 12.24), are flat. Presumably, they initiate mutation by inserting into the DNA double helix, causing the helix to buckle in the region of insertion, possibly leading to base additions and deletions during DNA replication. Crick and Brenner used acridine-induced mutations to demonstrate both that the genetic

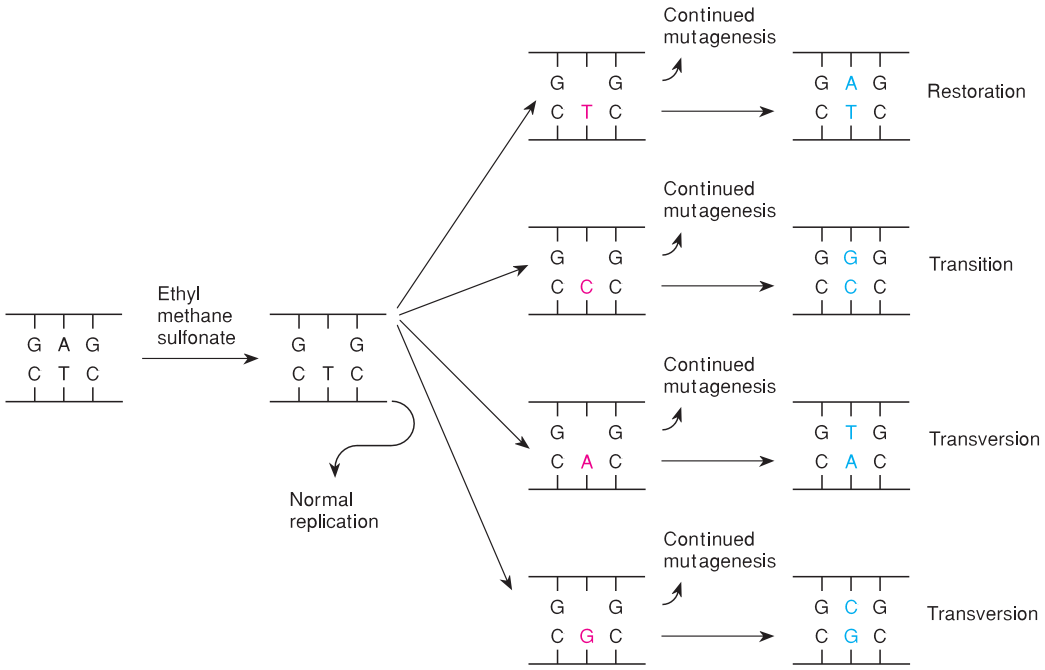


Figure 12.23 Four possible outcomes after treatment of DNA with an alkylating agent, which removes the purine—adenine in this example. The bases shown in *red* are the four bases that DNA polymerase may insert opposite the gap. After another round of DNA replication, the gap remains to generate further mutations. The inserted base forms a base pair (*blue*), which can be a restoration or a transition or transversion mutation.

code was read from a fixed point and that it was triplet (chapter 11).

Misalignment Mutagenesis

Additions and deletions in DNA can also come about by misalignment of a template strand and the newly formed (progeny) strand in a region containing a repeated sequence. For example, in figure 12.25 we expect the progeny strand to contain six adjacent adenines because the template strand contains six adjacent thymines. Misalignment of the progeny strand results in seven consecutive adenines: six thymines replicated, plus one already replicated but misaligned. Misalignment of the template strand results in five consecutive adenines because one thymine is not available in the template. Regions with long runs of a particular base may be very mutation prone. They may explain the "hot spots" observed by Benzer (see fig. 12.8) and others.

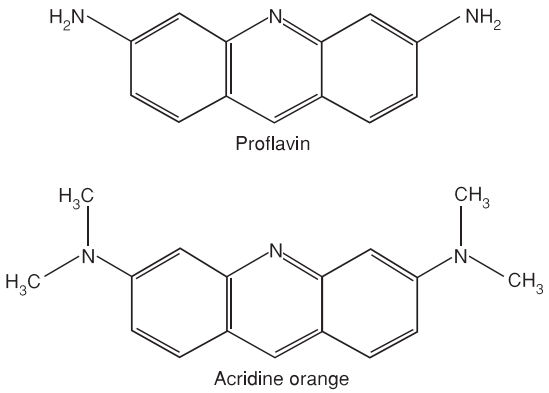


Figure 12.24 Structure of two acridine dyes: proflavin and acridine orange.

Intergenic Suppression

When a critical mutation occurs in a codon, several routes can still lead to survival of the individual; simple reversion and intragenic suppression are two that we have already considered. A third route is through **intergenic suppression**—restoration of the function of a mutated gene by changes in a different gene, called a **suppressor gene.** Suppressor genes are usually transfer RNA genes. When mutated, intergenic suppressors change the way in which a codon is read.

Suppressor genes can restore proper reading to nonsense, missense, and frameshift mutations. **Nonsense mutations** convert a codon that originally specified an amino acid into one of the three nonsense

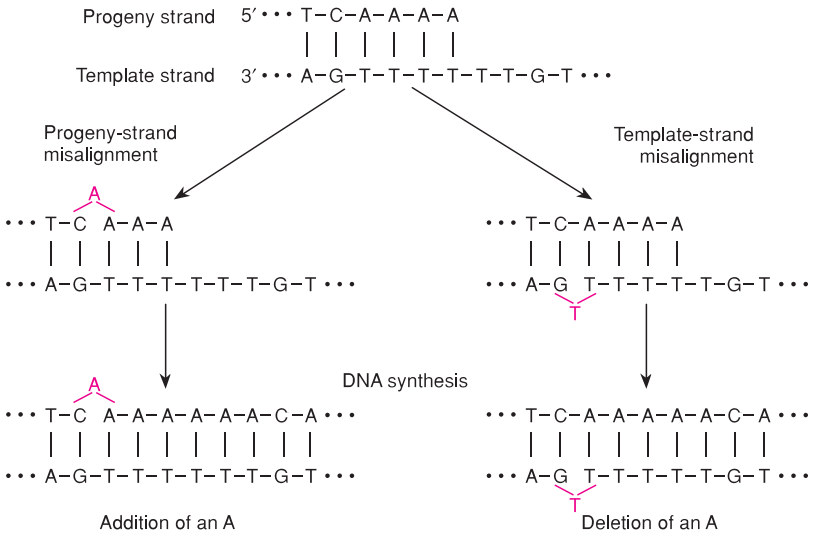
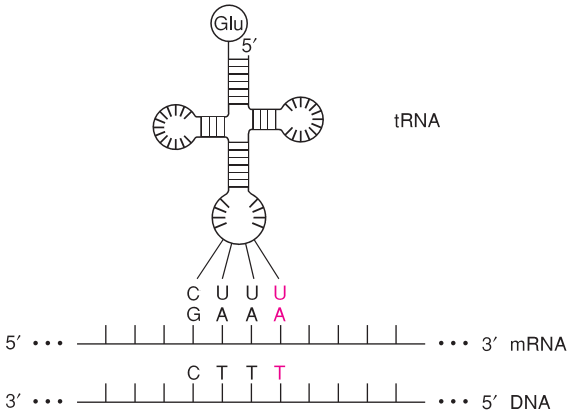


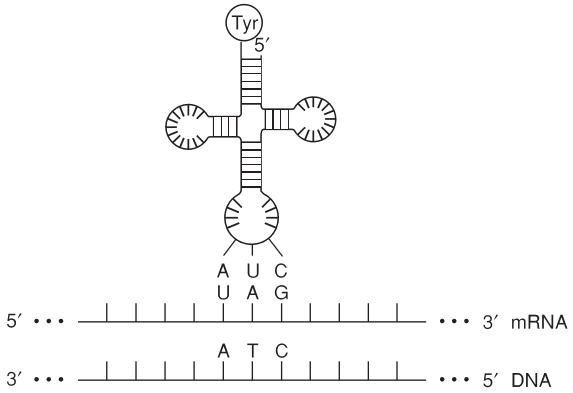
Figure 12.25 Misalignment of a template or progeny strand during DNA synthesis. If the progeny strand is misaligned after DNA replication has begun, the resulting progeny strand will have an additional base. If the template strand is misaligned during DNA replication, the resulting progeny strand will have a deleted base. These changes will show up after another round of DNA replication. (From J.W. Drake, B.W. Glickman, and L.S. Ripley, "Updating the Theory of Mutation," *American Scientist,* 71:621–630, 1983. Reprinted with permission of American Scientist, magazine of Sigma Xi, The Scientific Research Society.)

codons. **Missense mutations** change a codon so that it specifies a different amino acid. Frameshift mutations, by additions or deletions of nucleotides, cause an alteration in the reading frame of codons. A frameshift mutation, caused by the insertion of a single base, can be suppressed by a transfer RNA that has an added base in its anticodon (fig. 12.26*a*). It reads four bases as a codon and thus restores the original reading frame.

The transfer RNA produced by a nonsense suppressor gene reads the nonsense codon as if it were a codon for an amino acid; an amino acid is placed into the protein, and reading of the messenger RNA continues. At least three suppressors of the mutant amber codon (UAG) are known in *E. coli.* One suppressor puts tyrosine, one puts glutamine, and one puts serine into the protein chain at the point of an amber codon. Normally, tyrosine transfer RNA has the anticodon 3′-AUG-5′. The suppressor transfer RNA that reads amber as a tyrosine codon has the anticodon 3′-AUC-5′, which is complementary to amber. Hence, a mutated tyrosine transfer RNA reads amber as a tyrosine codon (fig. 12.26*b*).



(a) Frameshift suppression



(b) Nonsense suppression

Figure 12.26 Frameshift and nonsense suppression by mutant transfer RNAs. In (*a*), a thymine has been inserted into DNA, resulting in a frameshift. However, a transfer RNA with four bases in the codon region reads the inserted base as part of the previous codon in the messenger RNA. The frameshift thus does not occur. In (*b*), an *amber* mutation (UAG), which normally results in chain termination, is read as tyrosine by a mutant tyrosine transfer RNA that has the anticodon sequence complementary to the *amber* codon.

If the amber nonsense codon is no longer read as a stop signal, then won't all the genes terminating in the amber codon continue to be translated beyond their ends, causing the cell to die? In the tyrosine case, two genes for tyrosine transfer RNA were found; one contributes the major fraction of the transfer RNAs, and the other, the minor fraction. It is the minor-fraction gene that mutates to act as the suppressor. Thus, most messenger RNAs are translated normally, and most amber mutations result in premature termination, although a sufficient number are translated (suppressed) to ensure the viability of the mutant cell. In general, intergenic suppressor mutants would be eliminated quickly in nature because they are inefficient—the cells are not healthy. In the laboratory, we can provide special conditions that allow them to be grown and studied.

Mutator and Antimutator Mutations

Whereas intergenic suppressors represent mutations that "restore" the normal phenotype, mostly through mutation of transfer RNA loci, **mutator** and **antimutator mutations** cause an increase or decrease in the overall mutation rate of the cell. They are frequently mutations of DNA polymerase, which, as you remember, not only polymerizes DNA nucleotides 5′ → 3′ complementary to the template strand, but also checks to be sure that the correct base was put in (they proofread). If, in the proofreading process, the polymerase discovers an error, it can correct this error with its 3′ → 5′ exonuclease activity. Mutator and antimutator mutations sometimes involve changes in the polymerase's proofreading ability (exonuclease activity).

Phage T4 has its own DNA polymerase with known mutator and antimutator mutants. Mutator mutants are very poor proofreaders (they have low exonuclease-to-polymerase ratios), and thus they introduce mutations throughout the phage genome. Antimutator mutants, however, have exceptionally efficient proofreading ability (high exonuclease-to-polymerase ratios) and, therefore, result in a very low mutation rate for the whole genome (box 12.3).

BOX 12.3

Experimental Methods

Adaptive Mutation

Although we discuss evolution in detail at the end of the book, note here that we view mutation as a random process, not one that occurs because a cell "needs" a particular mutation. For example, Luria and Delbrück's work showed that the mutation in *E. coli* for resistance to phage T1 occurred randomly before exposure to the phage, not because the cells would benefit from the mutation.

Because of the entrenched dogma that mutations occur through random processes, the scientific community was startled when, in 1988, John Cairns—a highly respected senior scientist—and colleagues reported a new observation—**adaptive,** or **directed, mutation,** occurring when the cell needed it. Their system was the *lacZ* gene in *E. coli.* Cells that could not use lactose as an energy source ($lacZ^-$) were plated on a medium in which lactose was the sole energy source. Some mutants already there of course produced colonies ($lacZ^+$). The expectation was that no new mutations would occur over time because the $lacZ^-$ cells would have either died or stopped metabolizing. Unexpectedly, Cairns and colleagues found that more and more colonies appeared over time, coming from cells that had mutated to $lacZ^+$. As a control, they looked for revertants of other genes not involved with lactose metabolism. These mutations did not occur in a directed manner.

Scientists were extremely skeptical of this work for two reasons. First, it seemed to fly in the face of our common understanding of the mutational process. Second, there were no obvious explanations for how this could occur. Numerous articles were published refuting the notion of adaptive mutations and suggesting other explanations for the results. These explanations included artifacts of miscounting cells to mutants that were extremely slow growing, but there all the time.

In the past several years, other scientists have found at least a half dozen similar results in other organisms and other genes. The debate is ongoing, but work published in mid-1994 seems to have recast it into the realm of methods of mutagenesis rather than non-Darwinian processes. Several scientists found that the "directed" mutations seem to be of a certain type, mainly single nucleotide deletions within runs of the same nucleotide: for example, a deletion of a C in a CCCC sequence. This type of error happens during DNA replication and could be the result of a repair deficiency. That is, under extreme duress, the cells may be going into a "hypermutational" mode, or selection may favor hypermutable genotypes in which repair mechanisms are shut down in order to intentionally create lots of errors in the DNA. Any errors that do not alleviate the problem result in cell death, a death that was inevitable anyway; however, some errors will correct the problem ($lacZ^-$ to $lacZ^+$). Those cells will survive. One recent study indicated that a subpopulation of about 0.06% of the population was hypermutable, with a mutation rate about 200 times that of the normal cells. That group of cells could account for the adaptive mutations. Other scientists found that when the locus of importance was on a plasmid, the adaptive mutation could occur by increased replication of the plasmid. (Currently, the favored term is *adaptive mutation,* rather than *directed mutation,* indicating that the mutations are useful to the cell, not that some unknown process directs them.)

This controversy, although not necessarily resolved, has actually brought out the best in the scientific method: A skeptical scientific community tried its best to refute an unreasonable observation. Other scientists then repeated the observation and extended it, making it more worthy of further study. Finally, further work has given us reasonable mechanisms that not only require no rejection of the original concept of random mutation, but actually give us hypotheses to test further.

DNA REPAIR

Radiation, chemical mutagens, heat, enzymatic errors, and spontaneous decay constantly damage DNA. For example, it is estimated that several thousand DNA bases are lost each day in every mammalian cell due to spontaneous decay. Some types of DNA damage interfere with DNA replication and transcription. In the long evolutionary challenge to minimize mutation, cells have evolved numerous mechanisms to repair damaged or incorrectly replicated DNA. Many enzymes, acting alone or in concert with other enzymes, repair DNA. Repair systems are generally placed in four broad categories: damage reversal, excision repair, double-strand break repair, and

postreplicative repair. Enzymes that process repair steps have been conserved during evolution. That is, enzymes found in *E. coli* have homologues in yeast, fruit flies, and human beings. However, eukaryotic systems are almost always more complex.

Damage Reversal

Ultraviolet (UV) light causes linkage, or **dimerization,** of adjacent pyrimidines in DNA (fig. 12.27). Although cytosine-cytosine and cytosine-thymine dimers are occasionally produced, the principal products of UV irradiation are thymine-thymine dimers. These can be repaired in several different ways. The simplest is to reverse the dimerization process and restore the original unlinked thymines.

In *E. coli,* an enzyme called DNA photolyase, the product of the *phr* gene (for **photoreactivation**), binds to dimerized thymines. When light shines on the cell, the enzyme breaks the dimer bonds with light energy. The enzyme then falls free of the DNA. This enzyme thus reverses the UV-induced dimerization. Another example of an enzyme that performs direct DNA repair is O^6-mGua DNA methyltransferase, which removes the methyl groups from O^6-methylguanine, the major product of DNA-methylating agents (fig. 12.28). Although other repair mechanisms seem to be present in all organisms, photoreactivation is not; it is apparently absent in human beings.

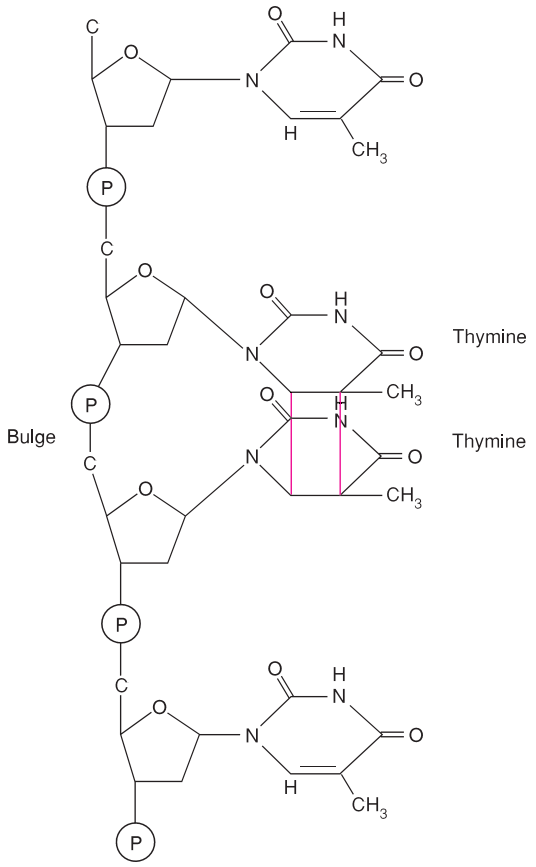


Figure 12.27 UV-induced dimerization of adjacent thymines in DNA. The *red lines* represent the dimer bonds in the adjacent thymines.

Excision Repair

Excision repair refers to the general mechanism of DNA repair that works by removing the damaged portion of a DNA molecule. Various enzymes can sense damage or distortion in the DNA double helix. During excision repair, bases and nucleotides are removed from the damaged strand. The gap is then patched using complementarity with the remaining strand. We can broadly categorize these systems as base excision repair and nucleotide excision repair, which includes mismatch repair. We will discuss only the major repair pathways; others exist. Presumably, redundancy in repair has been selected for because of the critical need to keep DNA intact and relatively mutation free.

Base Excision Repair

A base can be removed from a nucleotide within DNA in several ways: by direct action of an agent such as radiation, by spontaneous hydrolysis, by an attack of oxygen free radicals, or by **DNA glycosylases,** enzymes that sense damaged bases and remove them. Currently, at least five DNA glycosylases are known. For example, uracil-DNA glycosylase, the product of the *ung* gene in

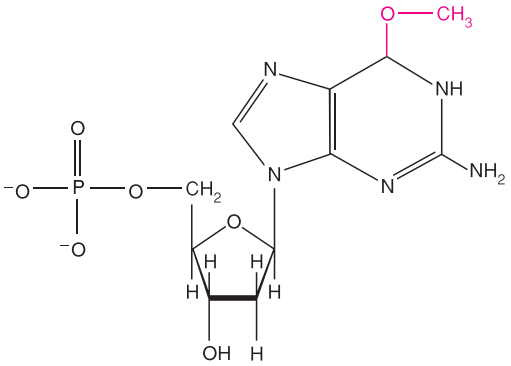


Figure 12.28 The structure of O^6-methylguanine. The *red color* shows the modification of guanine, in which the normal configuration is a double-bonded oxygen (keto form).

E. coli, recognizes uracil within DNA and cleaves it out at the base-sugar (glycosidic) bond. The resulting site is called an AP (apurinic-apyrimidinic) site, because of the lack of a purine or pyrimidine at the site (see fig. 12.23). An **AP endonuclease** then senses the minor distortion of the DNA double helix and initiaties excision of the single AP nucleotide in a process known as **base excision repair.** The AP endonuclease nicks the DNA at the 5′ side of the base-free AP site. A DNA polymerase then inserts a nucleotide at the AP site; an exonuclease, lyase, or phosphodiesterase enzyme then removes the base-free nucleotide. (Lyases are enzymes that can break C-C, C-O, and C-N bonds.) DNA ligase then closes the nick (fig. 12.29). The replacement of just one base occurs 80–90% of the time. In the remaining 10–20% of cases, several nucleotides may be removed, depending probably on which DNA polymerase (I or III) first repairs the site (fig. 12.29). In mammals, DNA polymerase β performs two roles in base excision repair: It both inserts a new base where the AP site was and also eliminates the AP nucleotide residue by exonuclease activity.

One question that concerned scientists was how the glycosylases gain access to the inappropriate or damaged bases within the double helix. Recently, it has been demonstrated that these enzymes remove the inappropriate or damaged bases by first flipping them out of the interior of the double helix in a process called **base flipping.** For example, the enzyme in human beings that recognizes 8-oxoguanine in DNA (see fig. 12.19), 8-oxoguanine DNA glycosylase, flips the base out to excise it. Base flipping seems to be a common mechanism in repair enzymes that need access to bases within the double helix (fig. 12.30).

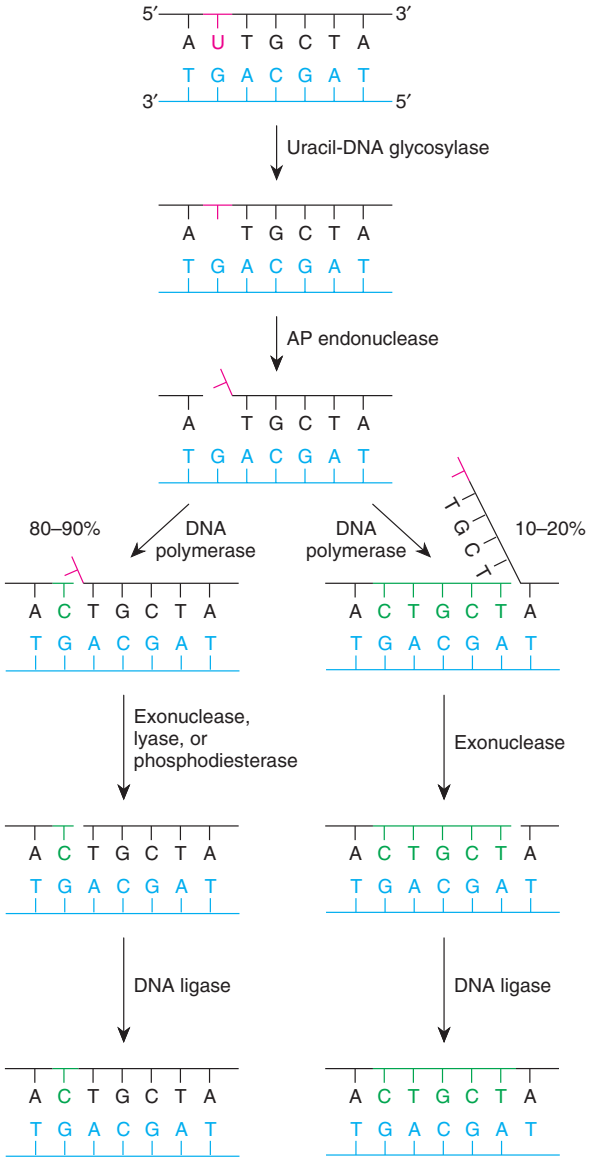
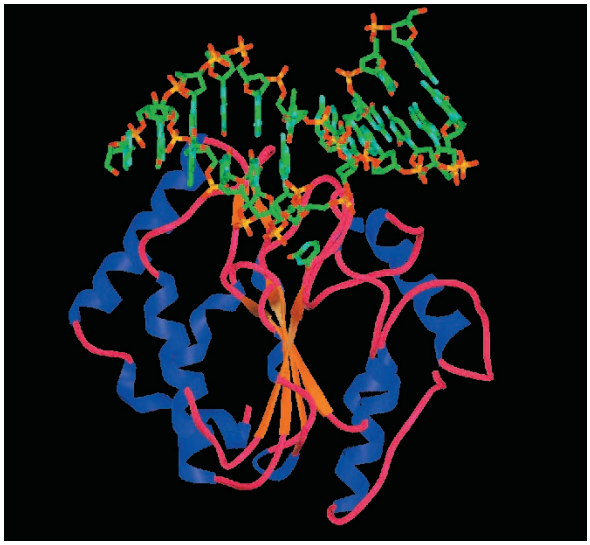


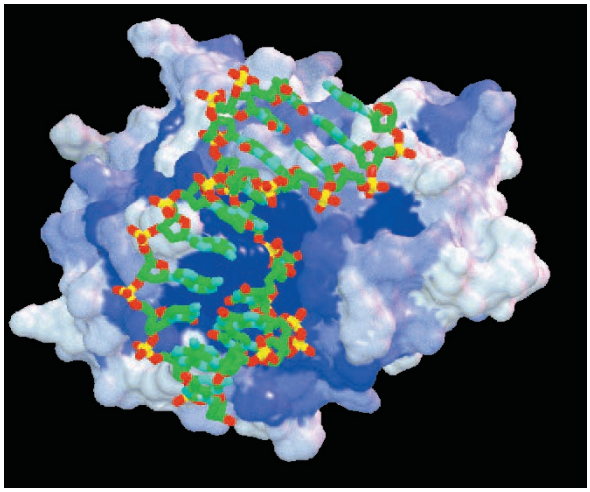
Figure 12.29 Mechanism of base excision repair. In this case, a uracil-DNA glycosylase enzyme removes a uracil (*red*) from DNA. An AP (apurinic-apyrimidinic) endonuclease nicks the DNA on the 5′ side of the base-free site. Between 80 and 90% of the time, a DNA polymerase will replace the single nucleotide (*green*); an exonuclease, lyase, or phosphodiesterase will remove the base-free nucleotide. The final nick is sealed with DNA ligase. Between 10 and 20% of the time, the DNA polymerase will extend polymerization beyond the single nucleotide. In those cases, an exonuclease and DNA ligase finish the repair. (From T. Lindahl, "The Croonian Lecture, 1996: Endogenous Damage to DNA," *Philosophical Transactions of the Royal Society of London,* B351, pp. 1529–1538, figure 6, 1996.)

Nucleotide Excision Repair

Whereas base excision repair is initiated by glycosylases and usually involves the replacement of only one nucleotide residue, **nucleotide excision repair** is initiated by enzymes that sense distortions in the DNA backbone and replace a short stretch of nucleotides. For example, six enzymes in *E. coli* excise a short stretch of DNA containing thymine dimers if the dimerization is not reversed by photoreactivation. Two copies of the protein product of the *uvrA* gene (for ultraviolet light—UV—repair) combine with one copy of the product of the *uvrB* gene to form a $UvrA_2UvrB$ complex that moves along the DNA, looking for damage (fig. 12.31). (The complex has 5′ to 3′ helicase activity.) When the complex finds damage such as a thymine dimer, with moderate to large distortion of the DNA double helix, the $UvrA_2$ dimer dissociates, leaving the UvrB subunit alone. This causes the DNA to bend and attracts the protein product of the *uvrC* gene, UvrC. The UvrB subunit first



(a)



(b)

Figure 12.30 Two views of the human enzyme, uracil-DNA glycosylase, bound to DNA that has a uracil present. In (*a*), the uracil-containing nucleotide residue has been flipped out and the uracil cleaved; in (*b*), the uracil-containing residue has been flipped out, but the uracil has not yet been cleaved. In both, DNA is a *green* stick figure with *red* oxygen, *yellow* phosphorus, and *blue* nitrogen atoms. In (*a*), the enzyme is shown as a ribbon diagram; note the cleaved uracil bound to the ribbon. In (*b*), the enzyme is shown as a molecular surface; the uracil-containing nucleotide is flipped out by the *purple* knob just left of and below center of the structure with the flipped-out residue to the right. (From S. Parikh, C. Mol, and J. Tainer, "Base excision repair enzyme family portrait" in *Structure*, 1997, 5:1543–1550, fig 1 a&b, p. 1544. Courtesy of J.A. Tainer, The Scripps Research Institute.)

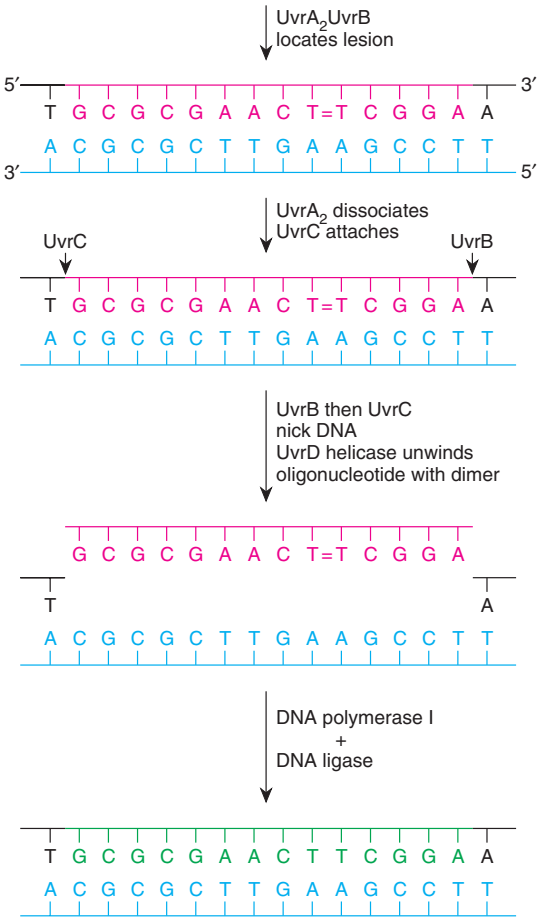


Figure 12.31 Nucleotide excision repair. A lesion in DNA (a thymine dimer) is located by a protein made of two copies of UvrA and one of UvrB. Then, the UvrA subunits detach, and UvrC attaches on the 5′ side of the lesion. UvrB nicks the DNA on the 3′ side and UvrC on the 5′ side of the lesion; UvrD helicase unwinds the oligonucleotide containing the lesion (*red*). DNA polymerase I and DNA ligase then repair the patch (*green*).

nicks (hydrolyzes) the DNA four to five nucleotides on the 3′ side of the lesion; next, the UvrC subunit nicks the DNA eight nucleotides on the 5′ side of the lesion. (The three components, UvrA, UvrB, and UvrC, are together called the *ABC excinuclease,* for excision endonuclease.) The enzyme helicase II, the product of the *uvrD* gene, then removes the twelve- to thirteen-base oligonucleotide as well as UvrC. DNA polymerase I fills in the gap and, in the process, evicts the UvrB, and DNA ligase closes the remaining nick (fig. 12.31). This is another relatively simple system designed to detect helix distortions and repair them.

Like base excision repair, nucleotide excision repair is present in all organisms. In yeast, approximately twelve genes are involved, many in what is called the RAD3 group. In human beings, twenty-five proteins are involved; they remove twenty-seven to twenty-nine nucleotides, as compared to twelve to thirteen in *E. coli.*

Transcription and nucleotide excision repair are linked in eukaryotes. Transcription factor TFIIH (see chapter 11) is involved in repair of UV damage; it has helicase activity and is found in both processes. Since it has been shown that genes that are actively being transcribed are preferentially repaired, we can now envision a model in which transcription, when blocked by a DNA lesion like a thymine dimer, signals the formation of a repair complex, using TFIIH in both processes. In prokaryotes, RNA polymerase dissociates from the DNA in this circumstance, losing the nascent transcript. This would be inefficient in eukaryotes, whose genes are much longer and more expensive to transcribe; for example, the human dystrophin gene, defective in the disease Duchenne muscular dystrophy, is 2.4 million bases long and takes almost eight hours to transcribe. We believe that eukaryotic RNA polymerase II backs up when stalled at a DNA lesion and continues after the lesion is repaired, without losing the transcript. Much active research is going on in this area.

In human beings, the autosomal recessive trait *xeroderma pigmentosum* is caused by an inability to repair thymine dimerization induced by UV light. Persons with this trait freckle heavily when exposed to the UV rays of the sun, and they have a high incidence of skin cancer. There are seven complementation groups (loci *XPA-XPG*) whose protein products are involved in the first steps of nucleotide excision repair and whose defects cause xeroderma pigmentosum in human beings. One of them, *XPD,* is a component of TFIIH.

Excision repair triggered by mismatches is referred to as **mismatch repair,** which encompasses about 99% of all DNA repairs. As DNA polymerase replicates DNA, some errors are made that the proofreading polymerase does not correct. For example, a template G can be paired with a T rather than a C in the progeny strand. The GT base pair does not fit correctly in the DNA duplex. The mismatch repair system, which follows behind the replicating fork, recognizes this problem. This system, whose members in *E. coli* are specified by the *mutH, mutL, mutS,* and *mutU* genes, is responsible for the removal of the incorrect base by an excision repair process. (The genes are called *mut* for mutator because mutations of these genes cause high levels of spontaneous mutation in the cells. The *mutU* gene is also known as *uvrD.*) The mismatch repair enzymes initiate the removal of the incorrect base by nicking the DNA strand on one side of the mismatch.

You might wonder how the mismatch repair system recognizes the progeny, rather than the template, base as the wrong one. After all, in a mismatch, there are no defective bases—theoretically, either partner could be the "wrong" base. In *E. coli,* the answer lies in the methylation state of the DNA. DNA methylase, the product of the *dam* locus, methylates 5′-GATC-3′ sequences, which are relatively common in the DNA of *E. coli,* at the adenine residue. Since the mismatch repair enzymes follow the replication fork of the DNA, they usually reach the site of mismatch before the methylase does. Template strands will be methylated, whereas progeny strands, being newly synthesized, will not be. Thus, the methylation state of the DNA cues the mismatch repair enzymes to eliminate the progeny-strand base for repair. After the methylase passes by, both strands of the DNA are methylated, and the methylation cue is gone.

In figure 12.32, we present one model of mismatch repair. The MutS protein, in the form of a homodimer—two

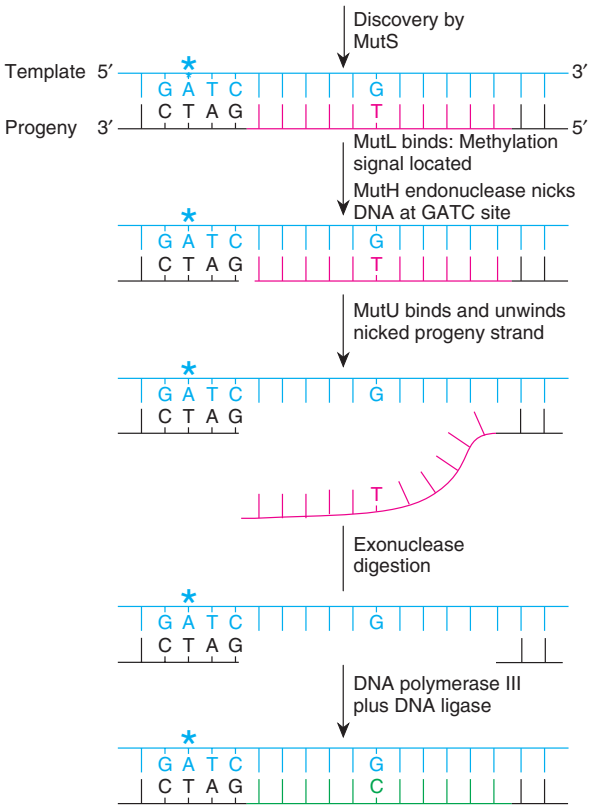


Figure 12.32 Mismatch repair. The MutS protein discovers mismatches; MutL binds and the MutH endonuclease nicks the progeny strand at the 3′-CTAG-5′ sequence. MutU helicase unwinds the nicked oligonucleotide with the mismatch (*red*). Exonuclease digestion, followed by DNA polymerase III and DNA ligase repair, completes the operation.

copies of the same protein—finds the mismatch. MutL, also in the form of a homodimer, then binds, and together they find the methylation signal. They also activate the endonuclease MutH, which then nicks the unmethylated strand at the 3′-CTAG-5′ recognition site, which can be one thousand to two thousand bases away from the mismatch. At the recognition site, the MutS-MutL tetramer loads the helicase MutU (UvrD), which then unwinds the nicked strand. Any one of at least four different exonucleases then attacks the unwound oligonucleotide. DNA polymerase III then repairs the gap, and DNA ligase seals it. This sequence of events highlights a common theme in DNA repair: Once a lesion is found, the damaged DNA has some protein bound to it until the repair is finished.

Our understanding of DNA damage and repair helps provide an answer to an evolutionary question—Why does DNA have thymine while RNA has uracil? If we live in an RNA world, in which RNA evolved first, why don't DNA and RNA both contain uracil? One answer is that a common damage to cytosine, spontaneous deamination, results in uracil. If uracil were a normal base in DNA, the conversion of cytosine to uracil by deamination would not leave any clue to a mismatch repair system that a mutation had occurred. Thus, thymine replaces uracil in DNA, since thymine is not confused with any other normal base in DNA by common spontaneous changes. In fact, cytosine, guanine, adenine, and thymine are not converted simply to any other of the bases in DNA. Hence, changes of these bases leave clues for the repair systems.

Double-Strand Break Repair

Some damage to DNA, such as that caused by ionizing radiation, is capable of breaking both strands of the double helix. When that happens, the cell uses one of two mechanisms to repair the broken ends: It can simply bring the ends back together (a process called *nonhomologous end joining*), or it can use a mechanism that relies on the nucleotide sequences of a homologous piece of DNA, such as a sister chromatid or a homologous chromosome. That method is called *homology-directed recombination.*

In nonhomologous end joining, a protein called Ku, a heterodimer of Ku70 and Ku80, binds to broken chromosomal ends. It then recruits a protein kinase (PK_{CS}); their interaction and the interaction with other proteins is stabilized by a scaffold protein called XRCC4 (for X-ray cross complementation group 4). The complex directs the annealing of the broken ends by DNA ligase IV. No particular sequence information is used, and if more than two broken ends are present, incorrect attachments can take place (e.g., translocations). The second method, homology-directed recombination, involves a second piece of DNA homologous to the broken piece. The method is very similar to our current model of DNA recombination and is discussed in the section entitled "Recombination" later in the chapter.

Postreplicative Repair

When DNA polymerase III encounters certain damage in *E. coli,* such as thymine dimers, it cannot proceed. Instead, the polymerase stops DNA synthesis and, leaving a gap, skips down the DNA to resume replication as far as eight hundred or more bases away. If allowed to remain, this gap will result in deficient and broken DNA. Since part of one strand is absent and the other has damage, there appears to be no viable template for replicating new DNA. However, the cell has two mechanisms to repair this gap: one uses polymerases that can replicate these lesions, and the other is a repair process that uses homologous DNA.

Originally, several proteins were known to facilitate the replication of DNA with lesions; they were believed to interact with the polymerase to make it capable of using damaged DNA as a template. We now know that these proteins are, in fact, polymerases that have the ability to replicate damaged DNA. In *E. coli,* polymerase V can copy damaged DNA. In yeast, polymerases η and ζ, also called REV3/7 and RAD30 polymerases, respectively, can also copy damaged DNA. Some of these polymerases are relatively error free; polymerase V and polymerases η put adenine-containing nucleotides opposite dimerized thymines. However, polymerases ζ and the *E. coli* polymerase IV, which also appears during times of damage, are error prone in their replicative roles. One possible reason for this is that the error-prone polymerases developed by evolutionary processes: They create mutations at a time when the cell might need variability. That is, DNA damage can occur when the environment is stressful for the cell; variability might help the cell survive. As we will see later, the cell can sense DNA damage and act appropriately.

In addition to using repair polymerases, the cell can use a second repair mechanism to replicate damaged DNA when the polymerase leaves a gap. A replication fork creates two DNA duplexes. Thus, an undamaged copy of the region with the lesion exists on the other daughter duplex. A group of enzymes, with one specified by the *recA* locus having central importance, repairs the gap. Since the repair takes place at a gap created by the failure of DNA replication, the process is called **postreplicative repair.** The *recA* locus was originally discovered and named in the recombination process. In fact, postreplicative repair is sometimes called recombinational repair, and it shares many enzymes with recombination.

The RecA Protein

The RecA protein has two major properties. First, it coats single-stranded DNA (fig. 12.33) and causes that coated, single-stranded DNA to invade double-stranded DNA (fig. 12.34). By invasion, we mean that the single-stranded DNA attempts to form complementary base pairs with the antiparallel strand of the double-stranded DNA while displacing the other strand of that double helix. A mechanism for this activity, assuming two sites on the enzyme, appears in figure 12.35. RecA continues to move the single-stranded DNA along the double-stranded DNA until a region of homology is found. The second major property of the RecA protein is that, when stimulated by the presence of single-stranded DNA, it causes autocatalysis of another repressor, called LexA, and thus initiates several sequences of reactions.

The RecA protein is responsible for filling a postreplicative gap in newly replicated DNA with a strand from the undamaged sister duplex. Gap-filling

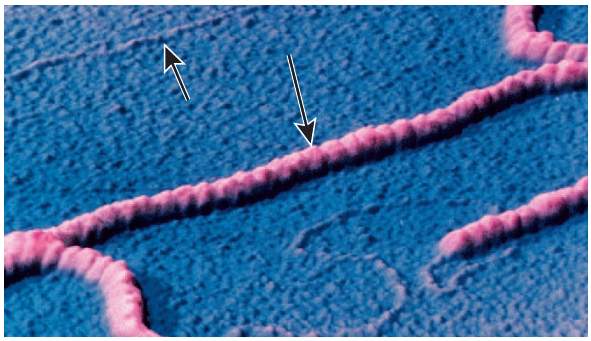


Figure 12.33 Scanning tunneling microscope picture of single-stranded DNA coated with RecA protein (*large arrow*). The *small arrow* indicates uncoated, double-stranded DNA. (In fig. 12.35, we show how the very large, coated DNA can invade the very small, uncoated DNA.) (© Science VU-IBMRL/Visuals Unlimited.)

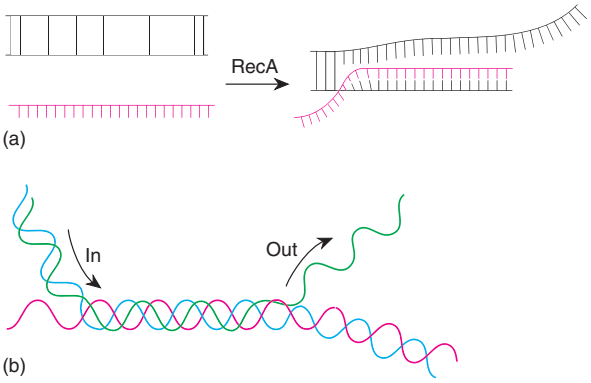


Figure 12.34 One property of the RecA protein causes single-stranded DNA to invade double-stranded DNA and to move along it until a region of complementarity is found. (*a*) Diagrammatic representation of the invasion of the RecA-coated single-stranded DNA. (*b*) A more realistic diagram of the same event. (Reproduced, with permission, from the *Annual Review of Biochemistry,* Volume 61, © 1992 by Annual Reviews, Inc.)

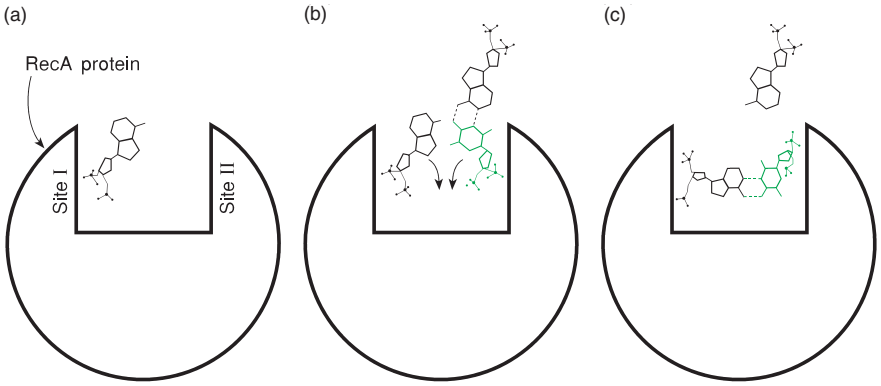


Figure 12.35 A model of how the RecA protein can cause single-stranded DNA to invade a double-stranded molecule. (*a*) Axial view of one nucleotide (with two phosphate groups) of single-stranded DNA attached at site I in this cross-sectional diagram of the RecA protein. The protein is about 60% larger than actually shown. (*b*) Duplex DNA is bound at site II of RecA. (*c*) RecA protein rotates the bases so that the single-stranded DNA forms a complementary base pair with one strand of the duplex, leaving the other strand of the duplex unpaired (see fig. 12.34). (Reprinted with permission from P. Howard-Flanders, et al., "Role of RecA protein spiral filaments in genetic recombination," *Nature,* 309:215–20. Copyright © 1984 Macmillan Magazines, Limited.)

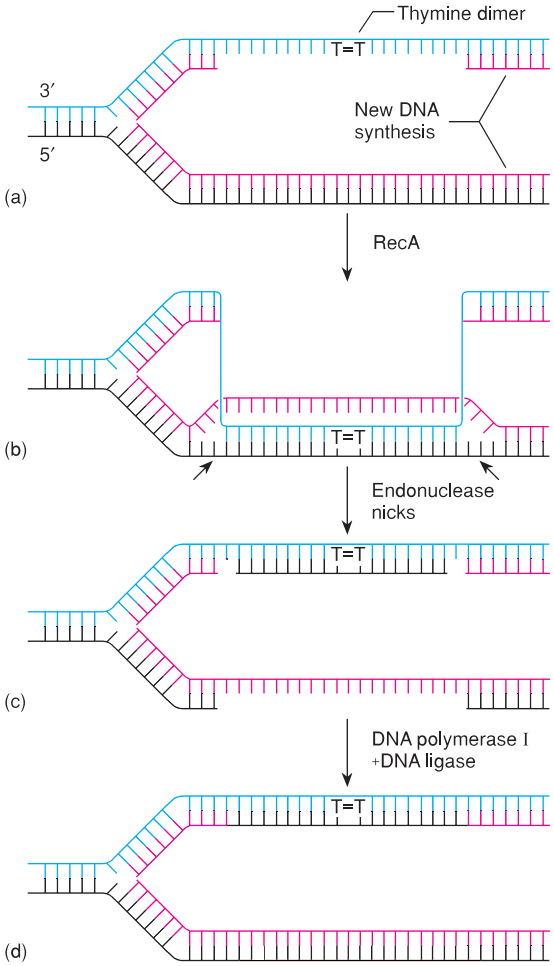


Figure 12.36 RecA-dependent postreplicative DNA repair. DNA polymerase III skips past a thymine dimer during DNA replication (*a*). With the help of RecA, the single strand with the thymine dimer invades the normal sister duplex (*b*). An endonuclease nicks the new duplex at either side of the thymine dimer site, freeing the new duplex with the thymine dimer and leaving the sister duplex single-stranded (*c*). Repair enzymes then create two intact daughter duplexes (*d*).

processes then complete both strands. In figure 12.36*a,* we see a replication fork with a gap in the progeny strand in the region of a thymine dimer. The RecA protein is responsible for the damaged single strand invading the sister duplex (fig. 12.36*b*). Endonuclease activity then frees the double helix containing the thymine dimer (fig. 12.36*c*). DNA polymerase I and DNA ligase return both daughter helices to the intact state (fig. 12.36*d*). The thymine dimer still exists, but now its duplex is intact, and another cell cycle is available for photoreactivation or excision repair to remove the dimer.

The SOS Response

Postreplicative repair is part of a cell reaction called the **SOS response.** When an *E. coli* cell is exposed to excessive quantities of UV light, other mutagens, or agents that damage DNA (such as alkylating or cross-linking agents), or when DNA replication is inhibited, gaps are created in the DNA. In the presence of this single-stranded DNA, the RecA protein interacts with the LexA protein, the product of the *lexA* gene. The LexA protein normally represses about eighteen genes, including itself. The other genes include *recA, uvrA, uvrB, and uvrD;* two genes that inhibit cell division, *sulA* and *sulB;* and several others. Each of these genes has a consensus sequence in its promoter called the **SOS box:** 5′-CTGX_{10}CAG-3′ (where X_{10} refers to any ten bases). The LexA protein normally binds at the SOS box, limiting the transcription of these genes. When single-stranded DNA activates RecA, RecA interacts with the LexA protein to trigger the autocatalytic properties of LexA (fig. 12.37). Transcription then follows from all the genes having an SOS box. The two inhibitors of cell division, the products of the *sulA* and *sulB* genes, presumably increase the amount of time the cell has to repair the damage before the next round of DNA replication.

Eventually, the DNA damage is repaired. There is no single-stranded DNA to activate RecA, and, therefore, LexA is no longer destroyed. LexA again represses the suite of proteins involved in the SOS response, and the SOS response is over. Table 12.5 summarizes some of the enzymes and proteins involved in DNA repair.

As we will mention in chapter 14, λ prophage can be induced into vegetative growth by UV light. This is another effect of the SOS response. RecA not only causes the LexA protein to be inactivated, but also directly inactivates the λ repressor, the product of the λ *cI* gene. From an evolutionary point of view, it makes sense for phage λ to have evolved a repressor protein that the RecA protein inactivates. As a prophage, λ is dependent on the survival of the host cell. When that survival might be in jeopardy, the prophage would be at an advantage if it could sense the danger and make copies of itself that could leave the host. One of these times might be when the host has suffered a lot of DNA damage. The SOS response is a signal to a prophage that the cell has received that damage. Hence, the prophage is induced when RecA acts as a protease; the λ repressor is destroyed, the cro protein becomes dominant, and vegetative growth follows. From an evolutionary perspective, the *E. coli* cell has not created an enzyme (RecA) that seeks out the λ repressor for the benefit of λ. Rather, the λ repressor has evolved for its own advantage to be sensitive to RecA.

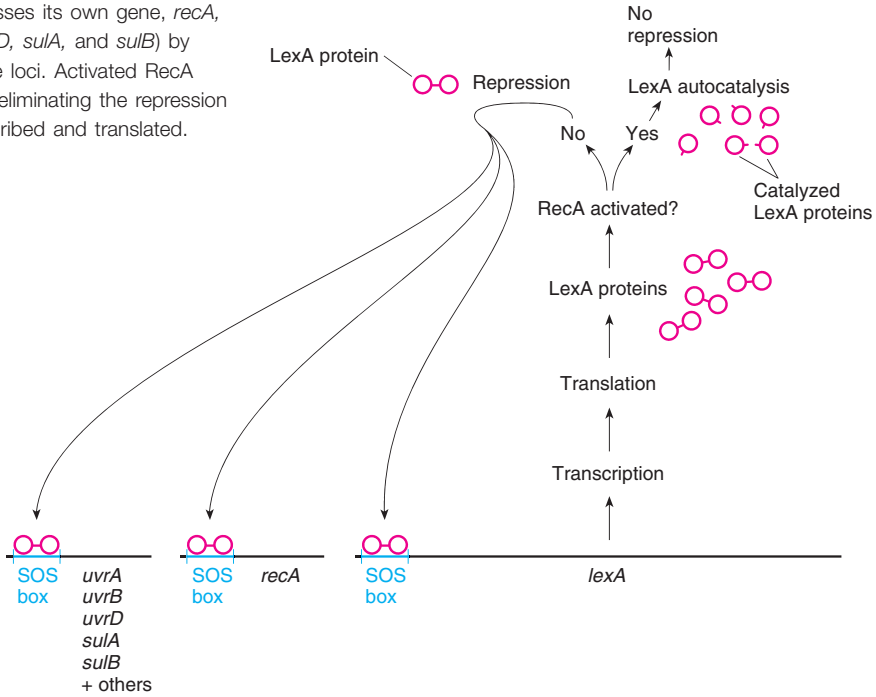


Figure 12.37 The LexA protein represses its own gene, *recA,* and several other loci (*uvrA, uvrB, uvrD, sulA,* and *sulB*) by binding at the SOS box in each of the loci. Activated RecA protein causes autocatalysis of LexA, eliminating the repression of all these loci, which are then transcribed and translated.

RECOMBINATION

Although recombination, the nonparental arrangement of alleles in progeny, can come about both by independent assortment and crossing over, we are concerned here with recombination due to crossing over between homologous pieces of DNA **(homologous recombination).** We briefly discuss transpositional recombination in chapter 14 and site-specific recombination (e.g., λ integration) in chapters 7, 14, and 16.

Recombination is a **breakage-and-reunion** process. Homologous parts of chromosomes come into apposition and are then reconnected in a crosswise fashion (see fig. 6.4). This general model fits what we know about the concordance of recombination and repair: Both involve breakage of the DNA and a small amount of repair synthesis, and both involve some of the same enzymes.

Double-Strand Break Model of Recombination

In 1964, R. Holliday suggested a model of homologous recombination that involved simultaneous breaks in one strand each of the two double helices that were to cross over. In 1983, J. Szostak and colleagues put forth a different model, initiated by a double-strand break in one of the double helices. At first, this model was not considered seriously because a double-strand break was thought too dangerous a DNA lesion for cellular enzymes to create. However, we now know that the double-strand break model is generally correct, and we refer to the **Holliday junction** for an intermediate stage in the process. The model depends on DNA complementarity between the recombining molecules and is thus a model of great precision.

We begin with two double helices lined up as they would be, for example, in a meiotic tetrad, ready to undergo recombination (fig. 12.38*a*). The first step of the process is a double-stranded break in one of the double helices. In eukaryotes, the protein Spo11 accomplishes

R. Holliday (1932–). (Courtesy of James L. German, III, M.D.)

Table 12.5 **Some of the Enzymes and Proteins Involved in DNA Repair in *E. coli,* Not Including DNA Polymerase I and III, DNA Ligase, and Single-Strand Binding Proteins**

Enzyme	Gene	Action
Damage Reversal		
DNA photolyase	*phr*	Undimerizes thymine dimers
DNA methyltransferase	*ada*	Demethylates guanines in DNA
Base Excision Repair		
Uracil-DNA glycosylase	*ung*	Removes uracils from DNA
Endonuclease IV	*nfo*	Nicks AP sites on the 5′ side
Exonuclease, lyase, or phosphodiesterase	*several*	Removes base-free nucleotide
Nucleotide Excision Repair		
UvrA	*uvrA*	With UvrB, locates thymine dimers and other distortions
UvrB	*uvrB*	Nicks DNA on the 3′ side of the lesion
UvrC	*uvrC*	Nicks DNA on the 5′ side of the lesion
UvrD (helicase II)	*uvrD*	Unwinds oligonucleotide
Mismatch Repair		
MutH	*mutH*	Nicks DNA at recognition sequence
MutL	*mutL*	Recognizes mismatch
MutS	*mutS*	Binds at mismatch
MutU (UvrD)	*mutU*	Unwinds oligonucleotide
Exonucleases	*recJ, xseA, sbcB*	Degrades unwound oligonucleotide
DNA methylase	*dam*	Methylates 5′-GATC-3′ DNA sequences
Double-Strand Break Repair		
Ku	*Ku70, Ku80*	Binds to broken chromosomal ends
PK_{CS}	PK_{CS}	Protein kinase
DNA ligase IV	*LIG4*	Ligates broken ends of DNA
XRCC4	*XRCC4*	Stabilization protein
Postreplicative Repair		
Polymerase IV	*DinB*	DNA polymerase
Polymerase V	*UmuC, UmuD*	DNA polymerase
Polymerase η	*RAD30*	DNA polymerase
Polymerase ζ	*REV3, REV7*	DNA polymerase
RecA	*recA*	Single-stranded DNA invades double-stranded DNA; causes LexA to autocatalyze; protease
LexA	*lexA*	Represses SOS proteins
SulA, SulB	*sulA, sulB*	Inhibit cell division

this. The break is followed by 5′ → 3′ exonuclease activity to widen the gaps formed in the double helix and create 3′ single-stranded tails (fig. 12.38*b, c*). These tails are coated with RecA protein that then catalyzes the invasion of one of the single strands into the intact double helix in direct apposition (fig. 12.38*d*). Repair of single-stranded DNA by DNA polymerase I and DNA ligase then replaces sections of previously digested DNA (fig. 12.38*e*). At this point, there is no "lost" genetic material; however the two double helices are interlocked and need to be freed of each other. Before that happens, however, **branch migration** can take place, a process in which the crossover point can slide down the duplexes (fig. 12.38*f*). In *E. coli,* the RuvAB complex, the product of the *ruvA* and *ruvB* genes that together form an ATP-dependent motor, moves the junction point

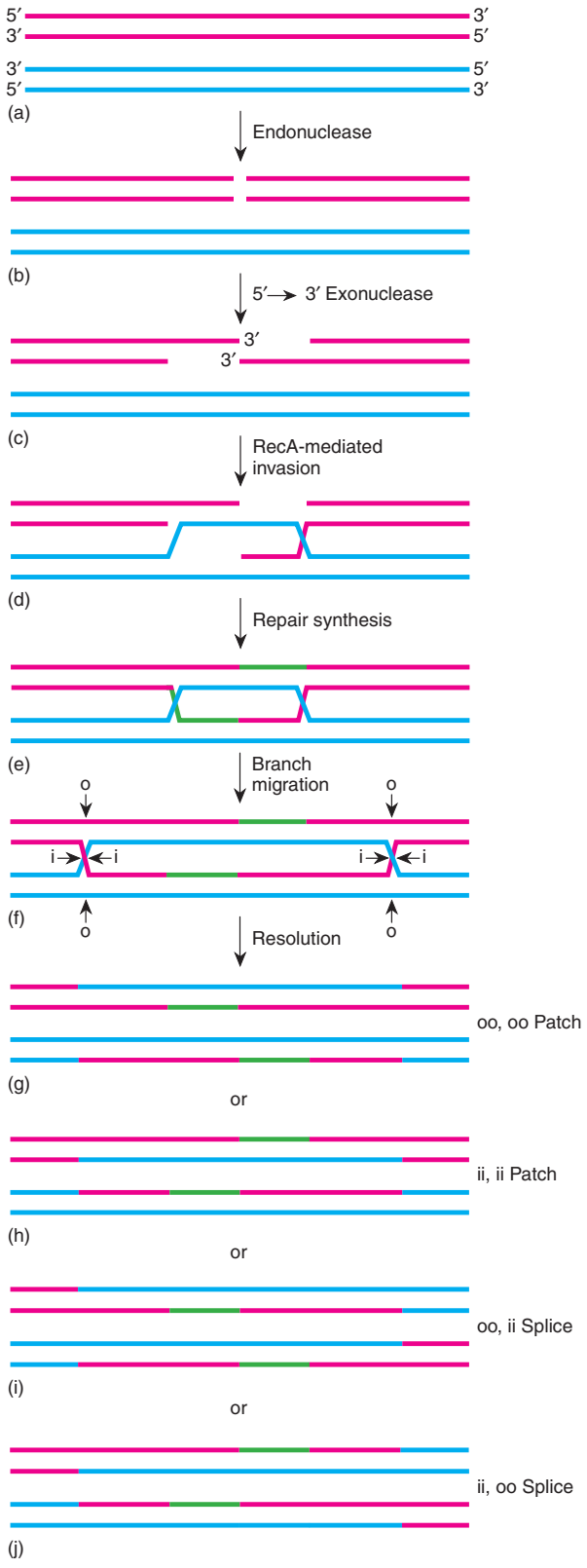


Figure 12.38 The double-strand break model of genetic recombination. Two homologous duplexes (*a: red, blue*) of the four present in a meiotic tetrad are shown. An endonuclease creates a double-stranded break in one of the duplexes (*b*). A 5′→ 3′ exonuclease then digests away from the break in both directions, creating 3′ tails (*c*). RecA-mediated invasion occurs in the second duplex (*d*), followed by repair synthesis to close all gaps (*e*). Branch migration then takes place (*f*). Each of the Holliday junctions is then resolved independently, either by nicks in the two outer strands (*o*) or the two inner strands (*i*). Therefore, four resolution structures are possible (*g–j*). In patches, the ends of each duplex are the same as the original, indicating that there may not be recombination for loci flanking the point of crossover. In splices, the ends of each duplex have recombined, indicating that flanking loci may have crossed over. (Reprinted from *Cell*, Vol. 87, Frank Stahl, "Meiotic Recombination in Yeast: Coronation of the Double-Stranded-Break Repair Model," pp. 965–968, Copyright © 1996, with permission from Elsevier Science.)

(fig. 12.39). As the junction points move, they create heteroduplex DNA, places where the two strands of each double helix come from different original helices. These stretches have the potential to produce mismatches where the two chromatids differed originally. To resolve the cross-linked duplexes, a second cut at each junction is required.

Each of the two crossover points is a Holliday junction. If we open these junctions, we can see that each can be resolved in two different ways. (RuvC endonuclease, the protein product of the *ruvC* gene, resolves the Holliday junctions in *E. coli;* see fig. 12.39. RuvC cuts the Holliday junction at the consensus sequence 5′[A or T]TT[G or C]-3′. The cut is on the 3′ side of the two thymines.) Since there are two Holliday junctions per crossover, there are four potential combinations, as shown in figure 12.38*g–j.* Some of these combinations produce patches, where no recombination takes place among loci to the sides of the hybrid piece. Other combinations produce splices, where reciprocal recombination of loci takes place at the ends. The Holliday junctions can be seen in the electron microscope (fig. 12.40). Note that homology-directed recombination to repair double-strand breaks is similar to the process shown in figure 12.38.

Bacterial Recombination

In bacterial recombination, a linear molecule recombines with a circular molecule (see fig. 7.15). Usually, invading DNA originates in the linear molecule. The RecBCD protein, whose subunits are the products of the *recB, recC,* and *recD* loci, initiates the first steps in forming an invading linear DNA molecule. RecBCD is a helicase, an exonuclease, and an endonuclease.

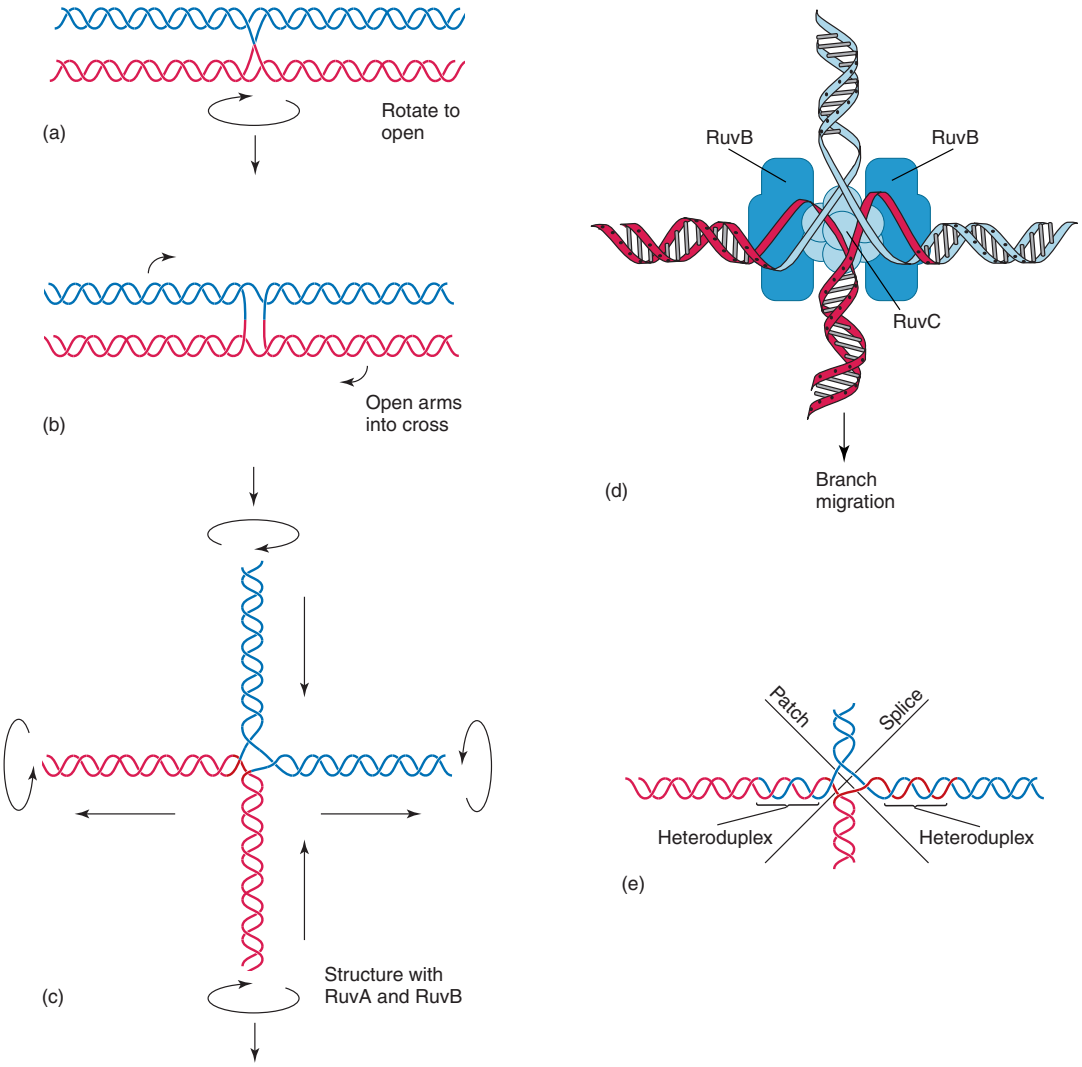


Figure 12.39 Branch migration at a Holliday junction. (*a*) Two double helices (*red, blue*) are connected by a crossover. (*b*) The structure opens when one of the double helices rotates. (*c*) We further clarify the structure by separating the arms into an open cross, showing the direction in which the arms move during branch migration (*arrows:* the equivalent of pulling out on the left and right arms, drawing in the top and bottom arms). (*d*) A more realistic drawing with the RuvA and RuvB proteins, indicating one of the RuvA tetramers behind the center of the cross. A second tetramer (not shown) is located above the cross center, forming a RuvA sandwich of the cross center. The RuvB hexamers are shown on either side of the cross. (*e*) RuvC can resolve the cross to form either a splice or a patch, depending on which cut is made. (Reprinted with permission from *Nature,* Vol. 374, C. Parsons, et al., "Structure of a Multisubunit Complex that Promotes DNA Branch Migration." Copyright © 1995 Macmillan Magazines Limited.)

The RecBCD protein enters a DNA double helix from one end and travels along it in an ATP-dependent process. As it travels along the DNA, it acts as a 3′ → 5′ exonuclease, degrading one strand of the linear double helix (fig. 12.41). This process continues until RecBCD comes to a **chi site,** the sequence 5′-GCTGGTGG-3′, which appears about a thousand times on the *E. coli* chromosome. RecBCD's recognition of that sequence attenuates its 3′ → 5′ exonuclease activity and enhances its 5′ → 3′ exonuclease activity, begun after an endonucleolytic cleavage. From that point on, RecBCD creates a 3′ overhang or tail. That tail is coated by RecA and then

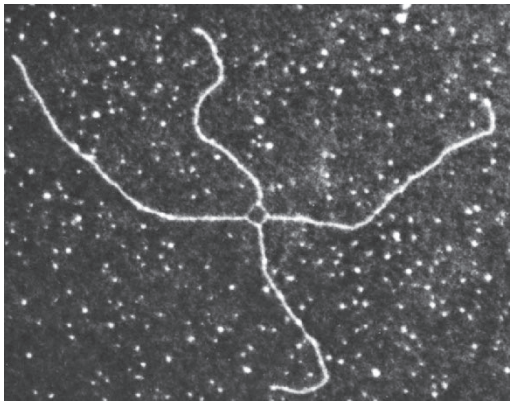


Figure 12.40 A Holliday intermediate structure, equivalent to the structure seen in figure 12.39*c*. Each arm is about 1 micron long. (H. Potter and D. Dressler. "DNA recombination: In vivo and in vitro studies," *Cold Spring Harbor Symposium on Quantitative Biology,* Volume XLIII, 1979, pp. 969–85.)

invades the circular bacterial chromosome to initiate a crossover event (fig. 12.41). After this pairing, the unpaired segments of the double helix of the bacteria and the exogenote are both degraded. Finally, DNA ligase seals the circular double helix. The resulting hybrid DNA will then be open to mismatch repair that can restore either original base pairs or base pairs from the invading DNA (see below).

Hybrid DNA

The result of bacterial recombination or meiotic recombination with branch migration is a length of hybrid DNA. This **hybrid DNA,** also called **heterozygous DNA** or **heteroduplex DNA,** has one of two fates, if we assume a difference in base sequences in the two strands. Either the heteroduplex can separate unchanged at the next cell division, or the cell's mismatch repair system can repair it (fig. 12.42). Without appropriate methylation cues, the mismatch repair system can convert the CA base pair to either a CG or a TA base pair. If TA were the original bacterial base pair, conversion to CG would be a successful recombination, whereas return of the CA to TA would be restoration rather than recombination.

Recombination in yeast, or any other eukaryote, generates two heteroduplexes. The repair process can cause **gene conversion** (fig. 12.43), the alteration of progeny ratios indicating that one allele was converted to another, a phenomenon seen in up to 10% of yeast asci. The mismatched AC will be changed to an AT or a GC base pair; the mismatched TG base pair will be changed to TA or CG. The result of the repair, as shown at the bottom of figure 12.43, can be gene conversion in which an expected ratio of 2:2 ($a^-\ a^-\ a^+\ a^+$) is converted to a 3:1 ratio ($a^-\ a^-\ a^-\ a^+$) or a 1:3 ratio ($a^-\ a^+\ a^+\ a^+$). If the heteroduplexes are not repaired, then a single cell generates both kinds of offspring after one round of DNA replication. Thus, the colony from the cell will be half wild-type (a^+) and half mutant (a^-). We see this phenomenon only in an Ascomycete fungus, such as yeast, in which all the products of a single meiosis remain together.

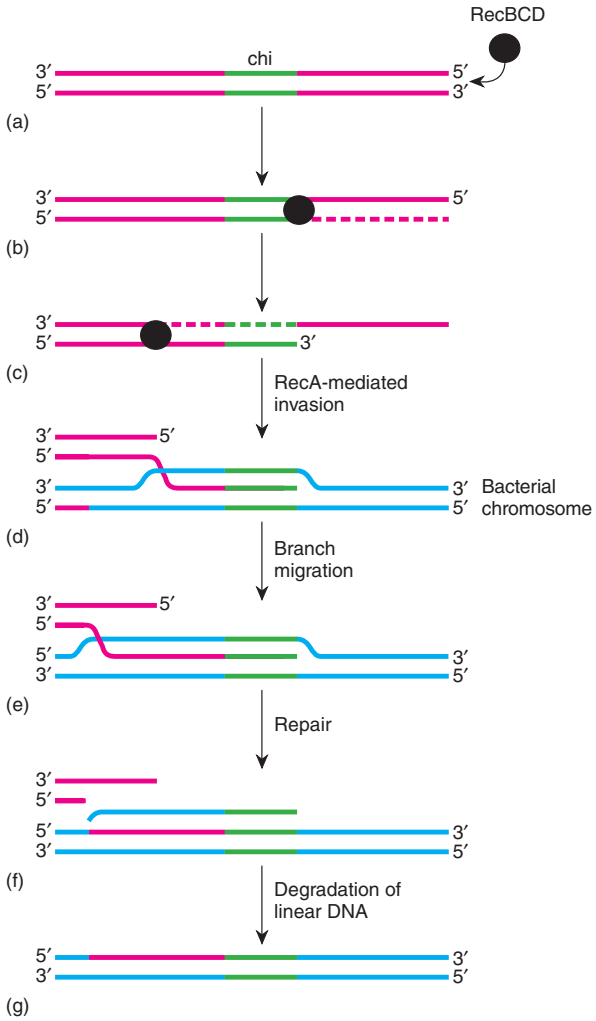


Figure 12.41 RecBCD enters a linear DNA double helix (*red*) at one end and travels along it, digesting the 3′ strand. When the protein encounters a chi site (*green*), it cuts the other strand and begins acting as a 3′ → 5′ exonuclease, creating a 3′ overhang (*b, c*). The 3′ overhang can then invade a double helix mediated by RecA. Repair and degradation of the linear DNA results in hybrid DNA in the bacterial chromosome, which can be fixed by the mismatch repair system.

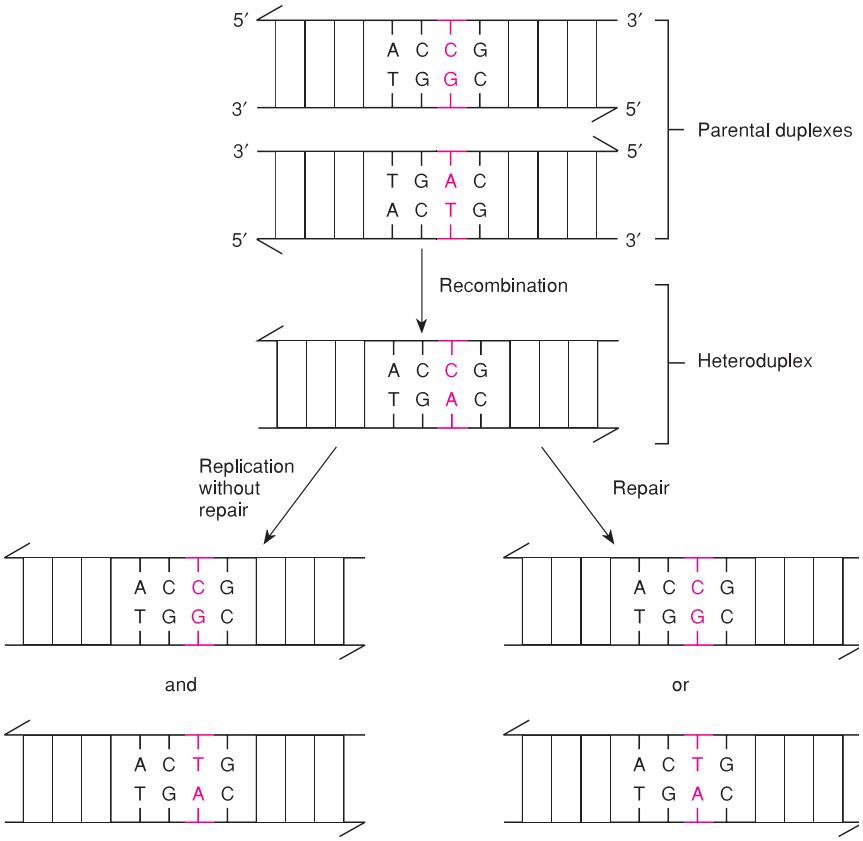


Figure 12.42 Fate of a heteroduplex DNA. *Recombination* results in heteroduplex DNA with mismatched bases. *Replication without repair* produces two different daughter molecules. *Repair* converts the mismatched base pair to one or the other normal base pair.

SUMMARY

STUDY OBJECTIVE 1: To look at the nature of mutation in prokaryotes 316–317

In 1943, Luria and Delbrück demonstrated that bacterial changes are true mutations similar to mutations in higher organisms. They showed that a high variability occurs in the number of mutants in small cultures as compared with the number of mutants in repeated subsamples of a large culture. Mutations thus occur spontaneously; they are caused by mutagens, which include chemicals and radiation. This chapter is concerned primarily with point mutations rather than changes in whole chromosomes or chromosomal parts.

STUDY OBJECTIVE 2: To analyze functional and structural allelism and examine the mapping of mutant sites within a gene 317–324

Allelism is defined by the *cis-trans* complementation test. Complementation implies independent loci, or nonallelic genes. Lack of complementation implies allelism. Functional alleles that differ from each other at the same nucleotides are also called structural alleles. Benzer used T4 phages to do fine-structure studies using complementation testing and deletion mapping.

STUDY OBJECTIVE 3: To verify the colinearity of gene and protein 324–325

Yanofsky demonstrated colinearity of the gene and protein. He had the advantage of working with a gene whose protein product was known.

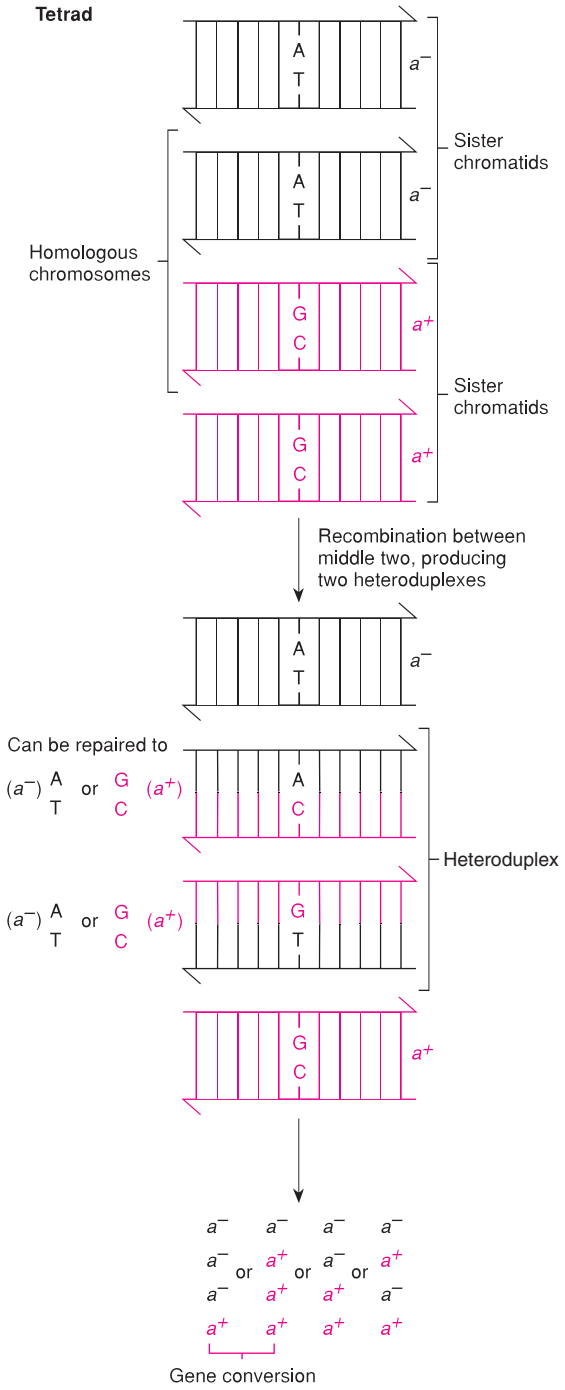


Figure 12.43 Recombination and repair can cause gene conversion. During recombination, heteroduplex DNA is formed, containing mismatched base pairs. Without methylation cues, repair enzymes convert the mismatch to a complementary base pair, in a random fashion—that is, an AC base pair can be converted to either an AT (a^- allele) or a GC (a^+ allele) base pair. Two of the four possible repair choices create 3:1 ratios of alleles rather than the expected 2:2 ratios in the offspring. The 3:1 ratio represents gene conversion.

STUDY OBJECTIVE 4: To study mutagenesis 325–338

After a mutation, the normal phenotype, or an approximation of it, can be restored either by back mutation or suppression. Intragenic suppression occurs when a second mutation within the same gene causes a return of normal or nearly normal function. Intergenic suppression occurs when a second mutation happens, usually in a transfer RNA gene, that counteracts the original mutation. Nonsense, missense, and frameshift mutations can all be suppressed.

Spontaneous mutation probably occurs primarily because of tautomerization of the bases of DNA. If a base is in the rare form during DNA replication, it can form unusual or mutant base pairings. We describe the mechanisms of action of the most common mutagens.

STUDY OBJECTIVE 5: To investigate the processes of DNA repair and recombination 339–352

DNA repair processes can be divided into four categories: damage reversal, excision repair, double-strand break repair, and postreplicative repair. Photoreactivation is an example of damage reversal. Thymine dimers are undimerized by a photolyase enzyme in the presence of light energy. Excision repair removes a damaged section of a DNA strand. Repair enzymes fill in the gap. Excision repair can be divided into three types. In base excision repair, bases are removed by environmental causes or by glycosylases that sense damaged bases. AP endonuclease and an exonuclease, phosphodiesterase, or lyase then removes the base-free nucleotide. Some enzymes use base flipping to gain access to nucleotides in the double helix.

In nucleotide excision repair, enzymes in the Uvr system remove a patch containing the lesion, usually a thymine dimer. In mismatch repair, enzymes of the Mut system use methylation cues to remove a progeny patch containing the mismatch.

Double-strand break repair relies on one of two mechanisms. In nonhomologous end joining, the cell simply brings the broken ends back together. In homology-directed recombination, the cell repairs the broken ends using a recombinational mechanism.

Postreplicative repair fills in gaps left by DNA polymerase III. Some polymerase enzymes can use lesions as templates. Otherwise, the RecA protein is central to the process. A single strand from the undamaged duplex is

used to fill the gap in the damaged duplex. Single-stranded DNA induces the SOS response, which temporarily eliminates LexA-mediated repression.

Recombination in eukaryotes begins with a double-stranded break in one double helix, followed by invasion of one of the ends into the other double helix. Repair, ligation, and branch migration follow. The crossover points, called Holliday junctions, need to be resolved, resulting in patches and splices. In *E. coli,* the RecBCD protein invades linear DNA, creating tails for invasion of the circular bacterial chromosome. Recombination results in heteroduplex DNA, which, if repaired, can lead to gene conversion. Thus, a battery of enzymes within the cell can modify DNA. These enzymes serve in DNA replication, repair, and recombination.

SOLVED PROBLEMS

PROBLEM 1: An investigator isolates two recessive wing mutants of *Drosophila melanogaster.* The flies differ in wing vein pattern. Are the mutations that cause these variants allelic?

Answer: To verify allelism, the investigator must create a heterozygote of the two mutations by either mating the flies, if they are of opposite sexes, or breeding each mutant into a separate stock for new matings. If the heterozygotes are of the wild-type, then the mutations are not allelic. If the heterozygote has a mutant phenotype, then we presume the mutations are functional alleles. (Allelism should be verified in females to be sure that the locus is not on the X chromosome, since males have only one.) If the mutations are functional alleles, it is possible to determine whether they are also structural alleles by looking for wild-type offspring of the heterozygote. If they occur at a rate higher than the background mutation rate, the alleles are not structural alleles. If wild-type offspring occur at the mutation rate, the alleles are presumably structural.

PROBLEM 2: What is the difference between mismatch repair and AP repair?

Answer: Both processes are similar in that they entail removal of an incorrect base in a DNA double helix by an excision process followed by a repair process. The processes differ in the event that triggers them. Mismatch repair is triggered by a base pair that does not occupy the correct space in the double helix—that is, by a non-Watson and Crick pairing (not AT or GC). AP repair is triggered by enzymes that recognize a missing base.

PROBLEM 3: What role does the RecBCD protein play in recombination?

Answer: For recombination to take place in *E. coli,* a single strand of DNA from the exogenote must insinuate itself into the chromosomal double helix with the help of the RecA protein. It is the RecBCD protein that creates the single-stranded DNA. It does so by traveling down the double helix, creating a single-strand tail in its wake. At chi sites, it switches the activity of the enzyme from a $3' \rightarrow 5'$ exonuclease to a $5' \rightarrow 3'$ exonuclease, creating a $3'$ tail. RecA can then act on this single-stranded tail to initiate recombination.

EXERCISES AND PROBLEMS*

MUTATION

1. Construct a data set that Luria and Delbrück might have obtained that would prove the mutation theory wrong.
2. What types of enzymatic functions are best studied using temperature-sensitive mutations?
3. Seven arginine-requiring mutants of *E. coli* were independently isolated. All pairwise matings were done (by transduction) to determine the number of loci (complementation groups) involved. If a (+) in the following figure indicates growth and a (−) no growth on minimal medium, how many complementation groups are involved? Why is only "half" a table given? Must the upper left to lower right diagonal be all (−)?

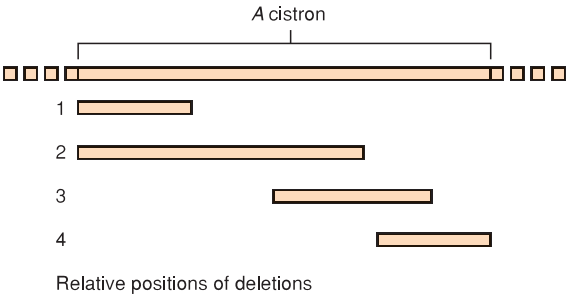
	1	2	3	4	5	6	7
1	–	+	+	+	+	–	–
2		–	+	+	–	+	+
3			–	–	+	+	+
4				–	+	+	+
5					–	+	+
6						–	–
7							–

4. Several *r*II mutations (M to S) have been localized to the *A* cistron because of their failure to complement with a known deletion in that cistron. The phages carrying these mutations are then mated pairwise with the following series of subregion deletions. The mating is done on *E. coli* B and plated out on *E. coli*

*Answers to selected exercises and problems are on page A-13.

K12 (see the figure). A (+) shows the presence of plaques on K12, whereas a (−) shows an absence of growth. Look at the deletion map of the area and localize each of the *r*II *A* mutations on this map.

	Deletion			
Mutant	**1**	**2**	**3**	**4**
M	+	+	–	+
N	+	–	–	+
O	+	+	+	–
P	+	+	–	–
Q	+	–	+	+
R	–	–	+	+
S	–	–	–	+



5. A *Drosophila* worker isolates four eye-color forms of the fly: wild-type, white, carmine, and ruby. (The worker does not know that white, carmine, and ruby are three separate loci on the X chromosome.) What crosses could the researcher make to determine allelic relations of the genes? What results would be expected? A new mutant, eosin, is isolated. What crosses should be carried out to determine that eosin is an allele of white?

6. Define structural and functional alleles. What is the *cis* part of a *cis-trans* complementation test?

7. Did Benzer and Yanofsky work with genes that had intervening sequences? What relevance might introns have to their work?

8. How can intra-allelic complementation result in incorrect conclusions about allelism?

9. *E. coli* bacteria of strain K12 are lysogenic for phage λ. Why won't *r*II mutants of phage T4 grow in these bacteria?

10. Diagram the tautomeric base pairings in DNA. What base pair replacements occur because of the shifts?

11. What is the difference between a substrate and a template transition mutation?

12. Describe two mechanisms for transversion mutagenesis.

13. 5-bromouracil, 2-aminopurine, proflavin, ethyl ethane sulfonate, and nitrous acid are chemical mutagens. What does each do?

14. A point mutation occurs in a particular gene. Describe the types of mutational events that can restore a functional protein, including intergenic events. Consider missense, nonsense, and frameshift mutations.

15. Why does misalignment result in addition or deletion of bases?

16. What are the differences and similarities between intergenic and intragenic suppression?

17. Eight independent mutants of *E. coli* requiring tryptophan (*trp*) are isolated. Complementation tests are performed on all pairwise combinations. Based on the results shown, determine how many genes you have identified and which mutants are in which genes (+ = complementation; − = no complementation).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	–	+	–	–	+	+	+	–
2		–	+	+	–	+	+	+
3			–	–	+	–	–	–
4				–	+	+	+	–
5					–	+	+	+
6						–	–	+
7							–	+
8								–

18. Complementation tests are usually done with recessive mutations for if the mutations were dominant, all progeny would be mutant, regardless of whether the genes are allelic. Suppose you have isolated in a diploid species two independent dominant mutations that each confer resistance to the drug cycloheximide. Call these mutations *Chx-1* and *Chx-2*. What crosses can you perform to determine whether the mutations are allelic? Your crosses should allow you to determine whether the mutations are allelic, nonallelic and unlinked, or nonallelic and linked.

19. A series of overlapping deletions in phage T4 are isolated. All pairwise crosses are performed, and the progeny scored for wild-type recombinants. In the following table, + = wild-type progeny recovered; − = no wild-type progeny recovered.

	1	**2**	**3**	**4**	**5**
1	–	+	–	–	–
2		–	+	+	–
3			–	+	+
4				–	–
5					–

a. Draw a deletion map of these mutations.
b. A point mutation, 6, is isolated and crossed with all of the deletion strains. Wild-type recombinants are recovered only with strains 2 and 3. What is the location of the point mutation?

20. Hydroxylamine is a chemical that causes exclusively C → T transition mutations. Can hydroxylamine reverse nonsense mutations? Explain.

21. A nonsense suppressor is isolated and shown to involve a tyrosine transfer RNA. When this mutant transfer RNA is sequenced, the anticodon turns out to be normal, but a mutation is found in the dihydrouridine loop. What does this finding suggest about how a transfer RNA interacts with the messenger RNA?

22. Devise selection-enrichment procedures for isolating the following kinds of mutants:
a. extra-large bacterial cells
b. nonmotile ciliated protozoans

23. Two chemically induced mutants, *x* and *y*, are treated with the following mutagens to see if revertants can be produced: 2-amino purine (2AP), 5-bromouracil (5BU), acridine dye (AC), hydroxylamine (HA), and ethylmethanesulfonate (EMS). In the following table, + = revertants and − = no revertants. For each mutation, determine the probable base change that occurred to change the wild-type to the mutant.

	Chemical				
Mutant	**2AP**	**5BU**	**AC**	**HA**	**EMS**
x	−	+	−	+	+
y	+	−	−	−	−

24. What situation will lead to a false positive in a complementation test—or, in other words, indicate two genes when, in fact, the mutations are in the same gene?

25. What situation will lead to false negatives in a complementation test—or, in other words, indicate mutations are in the same gene when, in fact, they are in different genes?

26. Suppose you repeat the Luria-Delbrück fluctuation test, but this time you look for *lac* colonies. Your "individual cultures" produce the following numbers of lac colonies: 20, 25, 22, 18, 24, 19, 17, 25, 26, and 18. Subsamples from the bulk culture give results identical to these. What can you conclude?

27. You have isolated a new histidine auxotroph, and, despite all efforts, you cannot produce any revertants. What probably happened to produce the original mutant?

DNA REPAIR

28. UV light causes thymine dimerization. Describe the mechanisms, in order of efficiency, that can repair the damage. Name the enzymes involved.

29. What types of damage do excision repair endonucleases recognize?

30. What are the functions of the RecA protein? How is it involved in phage λ induction? (*See also* RECOMBINATION)

RECOMBINATION

31. Diagram, in careful detail, a recombination by way of the double-strand break model. What enzymes are required at each step?

32. What are the different enzymes involved in reciprocal and nonreciprocal recombination?

CRITICAL THINKING QUESTIONS

1. Charles Yanofsky demonstrated colinearity of the gene and its protein product. What are the alternatives to colinearity?

2. Comment on the statement that DNA is a molecule designed for replication and repair.

Suggested Readings for chapter 12 are on page B-10.