CHAPTER 11

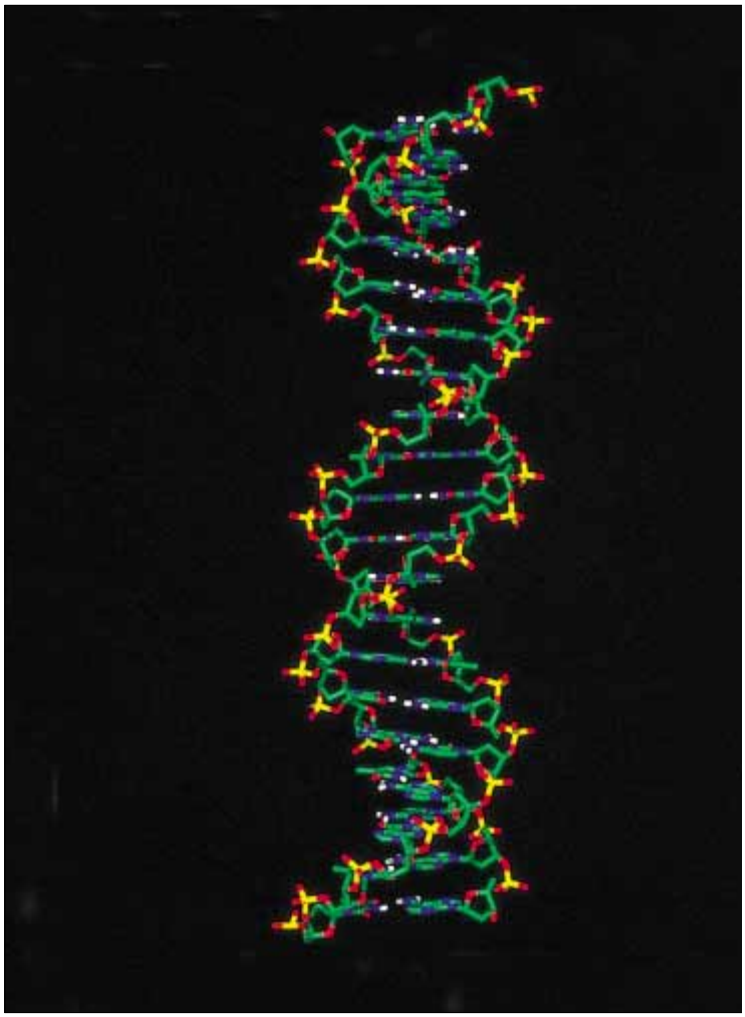
DNA: The Carrier of Genetic Information

Following the rediscovery of Mendel's principles, geneticists conducted elegant experiments to learn how genes are arranged in chromosomes and how they are transmitted from generation to generation. However, two very basic questions remained unanswered: What are genes made of? How do genes work? Although studies of inheritance patterns did not answer these questions, they provided a foundation that allowed scientists to make predictions about the chemical nature of genes and how they might function.

It was obvious that the chemical of which genes are made should have the ability to store information in a form that could be retrieved and used by the cell. But genes had other properties that had to be accounted for as well. Countless genetic experiments on a wide variety of organisms had demonstrated that genes are usually quite stable, being passed unchanged from generation to generation. However, occasionally a gene was observed to convert to a different form; such genetic changes, called **mutations,** were then transmitted unchanged to future generations.

As the science of genetics was developing, the science of biochemistry was flourishing as well. Not surprisingly, there was an increased effort to correlate the known properties of genes with the nature of the various biological molecules. What kind of molecule could store information? How could that information be retrieved and used to direct cellular functions? What kind of molecule could be relatively stable, but have the capacity to change under some circumstances?

Some scientists thought that the problem could never be solved. They thought the information required by a cell to be so complex that no one type of molecule could function as the genetic material. However, as more was learned about the central role that proteins play in virtually every aspect of cellular structure and metabolism, other scientists considered them the prime candidates for the genetic material. In this chapter we discuss how the nucleic acid shown in the photograph, **deoxyribonucleic acid (DNA),** not protein, was found to be the molecule responsible for inheritance, and we examine the unique features of DNA that allow it to carry out this role.



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LEARNING OBJECTIVES

AFTER YOU HAVE STUDIED THIS CHAPTER YOU SHOULD BE ABLE TO

1. Summarize the evidence that accumulated during the 1940s and early 1950s demonstrating that DNA is the genetic material.
2. Relate the chemical and physical features of DNA to the structure proposed by Watson and Crick.
3. Sketch how nucleotide subunits are linked together to form a single DNA strand.
4. Illustrate how the two strands of DNA are oriented with respect to each other.
5. State the base-pairing rules for DNA and describe how complementary bases bind to each other.
6. Cite experimental evidence that allowed scientists to differentiate between semiconservative replication of DNA and alternative models (conservative and dispersive replication).
7. Summarize how DNA replicates and identify some of the unique features of the process.
8. Explain the special constraints on DNA replication that cause it to be (1) bidirectional and (2) discontinuous in one strand and continuous in the other.
9. Compare the organization of DNA in prokaryotic and eukaryotic cells.

MOST GENES CARRY INFORMATION FOR MAKING PROTEINS

The idea that enzymes (which we now know are proteins) and genes are related in some way was first clearly stated by an English physician, Archibald Garrod, who proposed that certain inherited human diseases are caused by a block in a sequence of chemical reactions within the body.

In the first edition of his book, *Inborn Errors of Metabolism* (1908), Garrod discussed a genetic disease called **alkaptonuria,** which has a simple autosomal recessive inheritance pattern. The condition involves the metabolic pathway that breaks down the amino acids phenylalanine and tyrosine, ultimately converting them to carbon dioxide and water. The urine of affected individuals contains an intermediate in this pathway, homogentisic acid, which turns black when exposed to air (Fig. 11–1).

Garrod hypothesized that persons with alkaptonuria lack the enzyme that normally oxidizes homogentisic acid and that this metabolic block causes homogentisic acid to accumulate in their tissues and blood, and to be excreted in their urine. Before the second edition of his book had been published in 1923, it was found that affected persons do indeed lack the enzyme that oxidizes homogentisic acid. Garrod's hypothesis was correct: a mutation in a specific gene could be associated with the absence of a specific enzyme. Shortly thereafter, in 1926, James Sumner purified a different enzyme, urease, and showed it to be a protein; this finding was the first clear evidence that enzymes are proteins.

Despite the implications of these findings, little work was done in this area, primarily because genetically transmitted errors in metabolism appeared to be rare. The lack of experimental subjects made genetic testing and statistical analysis very difficult.

A major advance in understanding the relationship between genes and enzymes came in the early 1940s, when George Beadle and Edward Tatum developed a new approach to the problem. Most efforts until that time had centered on

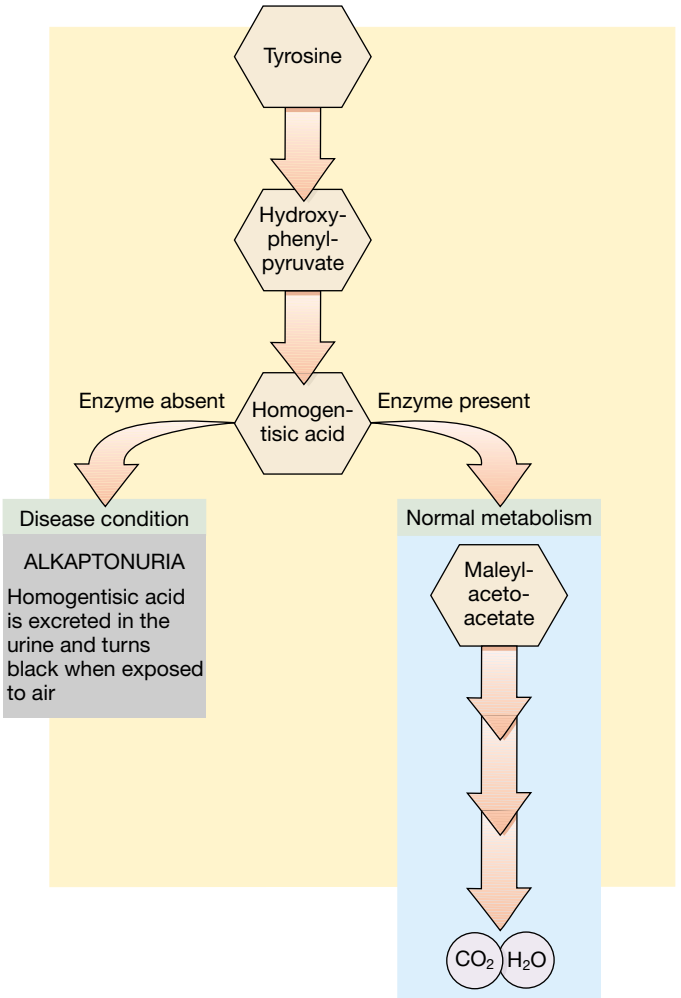


Figure 11–1 An "inborn error of metabolism." Garrod proposed that the alkaptonuria allele causes the absence of a specific enzyme, one that is part of the pathway by which the amino acid tyrosine is catabolized. That enzyme normally converts homogentisic acid to maleylacetoacetate. Homogentisic acid thus accumulates in the blood and is excreted through the urine. When the homogentisic acid in the urine comes in contact with air, it oxidizes and turns black.

studying known loci and attempting to determine what biochemical reactions they affected. Experimenters examined previously identified loci, such as those controlling eye color in *Drosophila* or pigments in plants. They found that specific phenotypes are controlled by a series of biosynthetic reactions, but it was not clear to the investigators whether the genes themselves were acting as enzymes or if they determined the workings of the enzymes in more complex ways.

Beadle and Tatum decided to take the opposite approach. Rather than try to identify the enzymes affected by single genes, they decided to look for mutations interfering with the known metabolic reactions that produce essential molecules such as amino acids and vitamins. They studied the bread mold *Neurospora* (a fungus) for several reasons. First, wild-type[1] *Neurospora* is easy to grow in culture. It can make all of its essential biological molecules when it is grown on a simple minimal medium containing only sugar, salts, and the vitamin biotin. A mutant that cannot make a substance such as an amino acid can still grow if it is simply added to the growth medium.

Second, *Neurospora* grows primarily as a haploid organism. Thus, a recessive mutant allele can be immediately identified because there is no homologous chromosome that could carry a dominant allele that would mask its expression.

Third, the life cycle of *Neurospora* facilitates certain types of manipulations and genetic analysis. *Neurospora* produces large numbers of asexual haploid spores; these can grow and divide mitotically to produce more haploid cells. Two haploid cells can fuse to produce a zygote, which undergoes meiosis to form haploid sexual spores. Thus, researchers can use sexual crosses to perform genetic analyses of isolated mutants. (For an illustration of the generalized life cycles of simple organisms, see Figure 9–12; a more detailed life cycle of organisms similar to *Neurospora* is given in Figure 25–6.)

Beadle and Tatum began by exposing large numbers of wild-type *Neurospora* asexual spores to x rays or ultraviolet radiation to induce mutations. Because each spore contains multiple haploid nuclei, the irradiated cells were mated with another strain, forming zygotes that underwent meiosis to produce haploid sexual spores, each with a single haploid nucleus. (Why did the experimenters not simply irradiate uninucleate sexual spores? The answer is that it is easy to obtain large numbers of asexual spores and they are far more sensitive to the effects of radiation than are sexual spores.) The isolated sexual spores were allowed to grow on a complete medium containing all the amino acids and vitamins normally made by *Neurospora.* Each strain was also tested on a minimal medium. If an isolated strain grew on the complete medium, but failed to grow after transfer to the minimal medium, Beadle and Tatum reasoned that it carried a mutation that made it unable to produce one of the compounds essential for growth. Further testing of the mutant on media containing different combinations of amino acids, purines, vitamins, and so on enabled the investigators to determine the exact compound that was required (Fig. 11–2).

Their findings can be illustrated with a class of mutants that require the amino acid arginine. Beadle and Tatum found that some of the arginine-requiring mutants could grow on ornithine or citrulline, as well as arginine; others could grow on citrulline or arginine; and still others could grow only on arginine (Fig. 11–3*a*). This information was then used to deduce the order of these intermediates in the biochemical pathway leading to arginine (Fig. 11–3*b*).

Using this approach, Beadle and Tatum analyzed large numbers of mutants affecting several metabolic pathways. Each mutant strain was verified by special genetic crossing experiments to have a mutation in only one gene locus. They found that for each individual gene locus identified, only one enzyme was affected. This one-to-one correspondence between genes and enzymes was succinctly stated as the *one gene, one enzyme hypothesis.*

Through the discoveries of Beadle and Tatum and others, the sciences of genetics and biochemistry became ever more closely allied, leading to an evolution of the definition of the gene and additional predictions regarding its chemical nature. The idea that a gene encodes the information required to produce a single enzyme held for almost a decade, until additional findings required a modification of this definition.

It became evident that genes control not only enzymes, but other proteins as well. Linus Pauling and his coworkers were able to demonstrate that the structure of hemoglobin can be altered by a mutation of a single locus. This particular mutant form of hemoglobin is associated with the genetic disease sickle cell anemia (Chapter 15). In addition, various studies showed that many proteins are constructed from two or more polypeptide chains, each of which may be controlled by a different locus. For example, hemoglobin was shown to contain two types of polypeptide chains, the α and β subunits (see Chapter 3). Sickle cell anemia results from a mutation affecting the β subunits.

The definition of a gene was therefore extended to state that one gene is responsible for one polypeptide chain. Even this definition has proved to be only partially correct, although we still define a gene in terms of its product (Chapter 12).

EVIDENCE THAT DNA IS THE HEREDITARY MATERIAL WAS FIRST FOUND IN MICROORGANISMS

During the 1940s most geneticists and biochemists were convinced that the genetic material must be protein. Proteins were known to contain more than 20 different kinds of amino acids in many different combinations, allowing each type of protein

[1] *Wild type* refers to the genotypes and phenotypes most commonly found in natural populations of a particular species. Wild-type alleles are generally thought of as "normal" or nonmutant alleles.

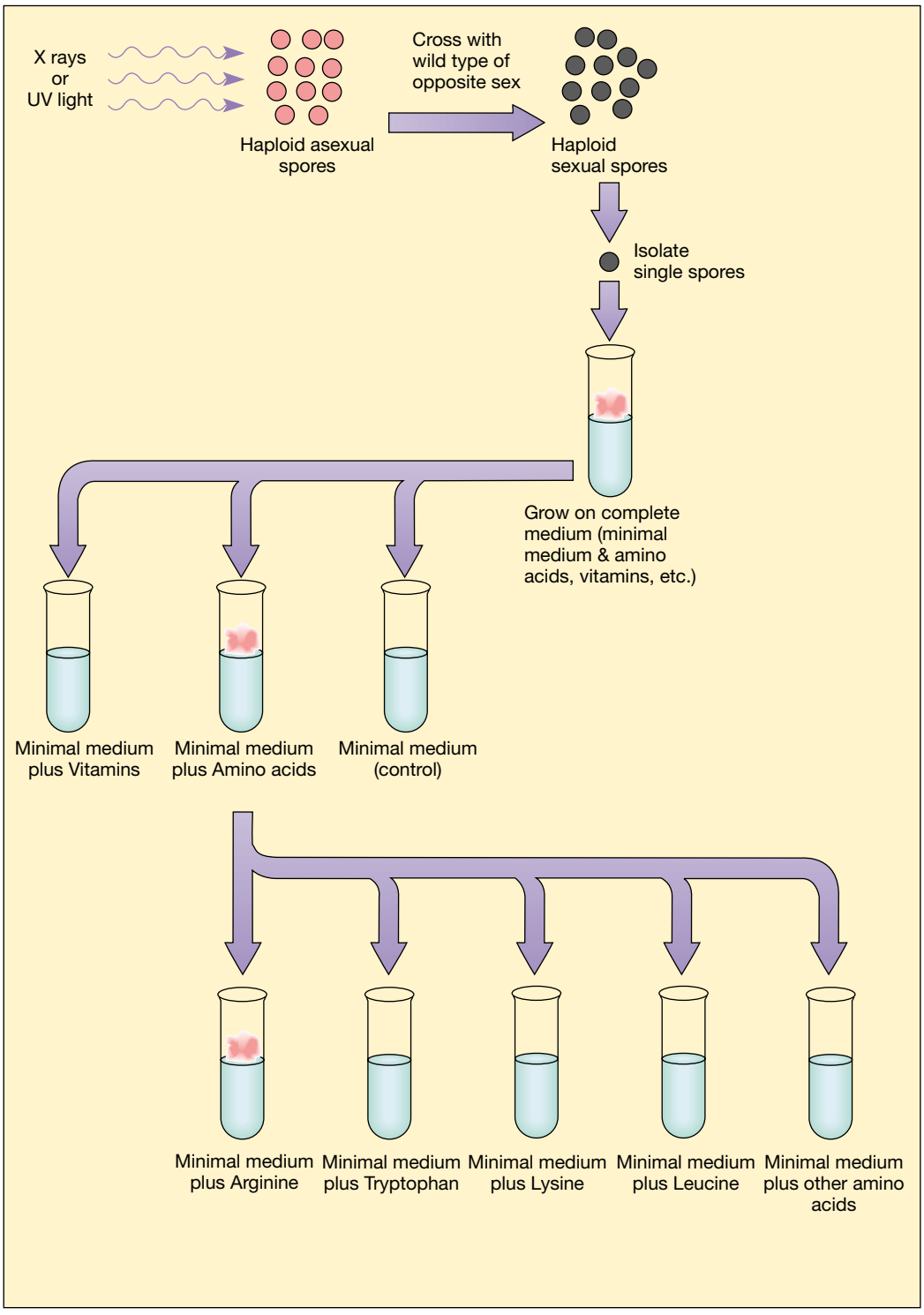


Figure 11–2 **Mutations affecting biochemical pathways.** Beadle and Tatum irradiated multinucleate haploid asexual spores of *Neurospora* to produce random mutations; cultures derived from these spores were then mated with another strain to produce uninucleate haploid sexual spores. Cultures were first established on complete media; subsequent failure to grow on a minimal media indicated a blocked step in a biochemical pathway. The specific nutritional requirement was determined by testing for growth on minimal media supplemented with individual vitamins or amino acids. In this example, the medium containing the amino acid arginine supports growth, indicating that the mutation affects some part of the arginine biosynthetic pathway.

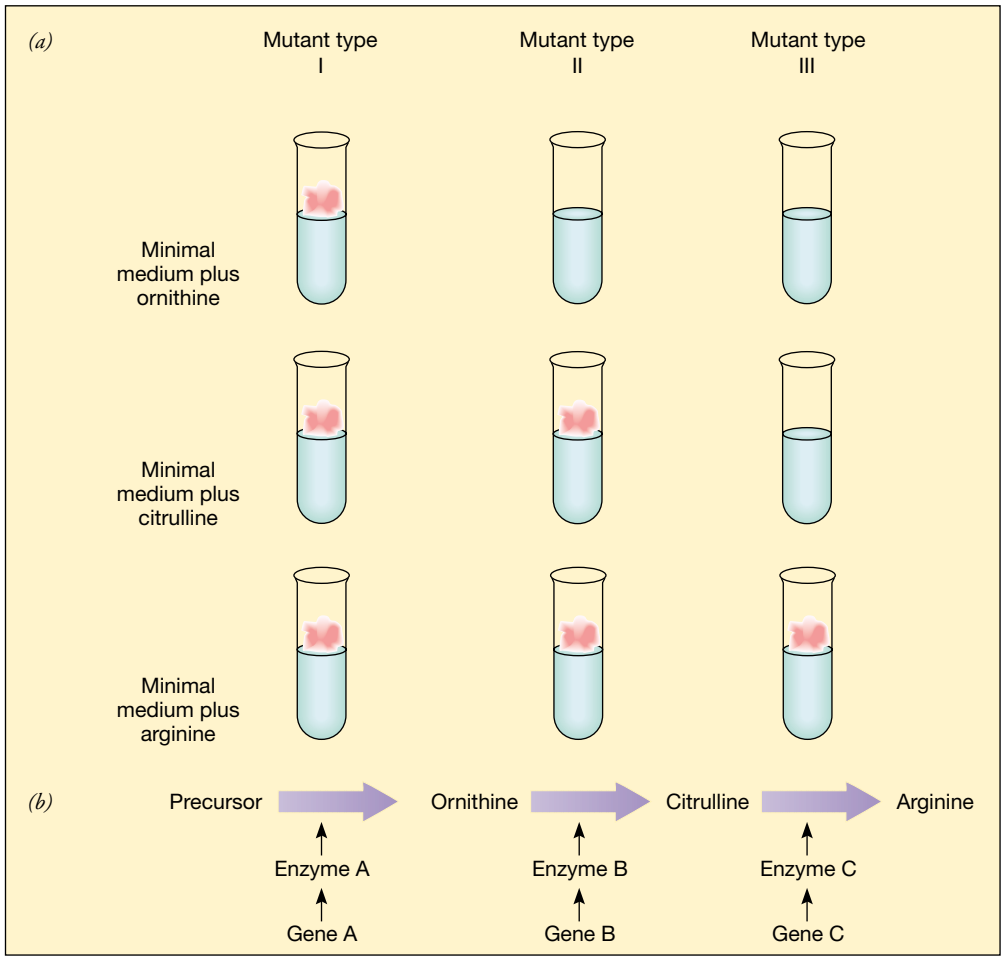


Figure 11–3 **Genes and enzymes.** ***(a)*** In this example, Beadle and Tatum tested a number of different mutant strains that require the amino acid arginine. These were grouped (I, II or III) by their response to various intermediates in the metabolic pathway leading to arginine, which were provided as supplements to the minimal growth medium. ***(b)*** Analysis of the experimental results led to this model for a portion of the arginine biosynthetic pathway (which is actually a cycle). Because Group I strains grew on minimal medium supplemented with ornithine, citrulline, or arginine, they were thought to be missing enzyme A, required for formation of all three compounds. Group II strains were thought to be missing enzyme B because they were unable to grow on minimal medium supplemented with ornithine but allowed the conversion of citrulline to arginine. Group III strains were thought to be missing enzyme C because they grew on minimal medium to which only arginine had been added. A one-to-one correspondence between each specific enzyme and a specific gene locus was verified by special genetic crosses (not shown).

to have unique properties. It had been shown that genes control the production of proteins. Given their obvious complexity and diversity compared with other molecules, proteins seemed to be the stuff of which genes are made.

In contrast, DNA and other nucleic acids were known to be made of only four nucleotides, and what was known about their arrangement made them seem relatively uninteresting to most scientists. For this reason, several early clues to the role of DNA were not widely recognized.

In 1928 Frederick Griffith made a curious observation concerning two strains of pneumococcus bacteria. A smooth (S) strain, named for its formation of smooth colonies on a solid growth medium, was known to be **virulent,** or lethal. When it was injected into mice, the animals contracted pneumonia and died. A related rough (R) strain, which forms colonies with a rough surface, was known to be **avirulent,** or nonlethal. Griffith found that when a mixture of *heat-killed,* virulent S-strain cells and live avirulent R-strain cells was in-

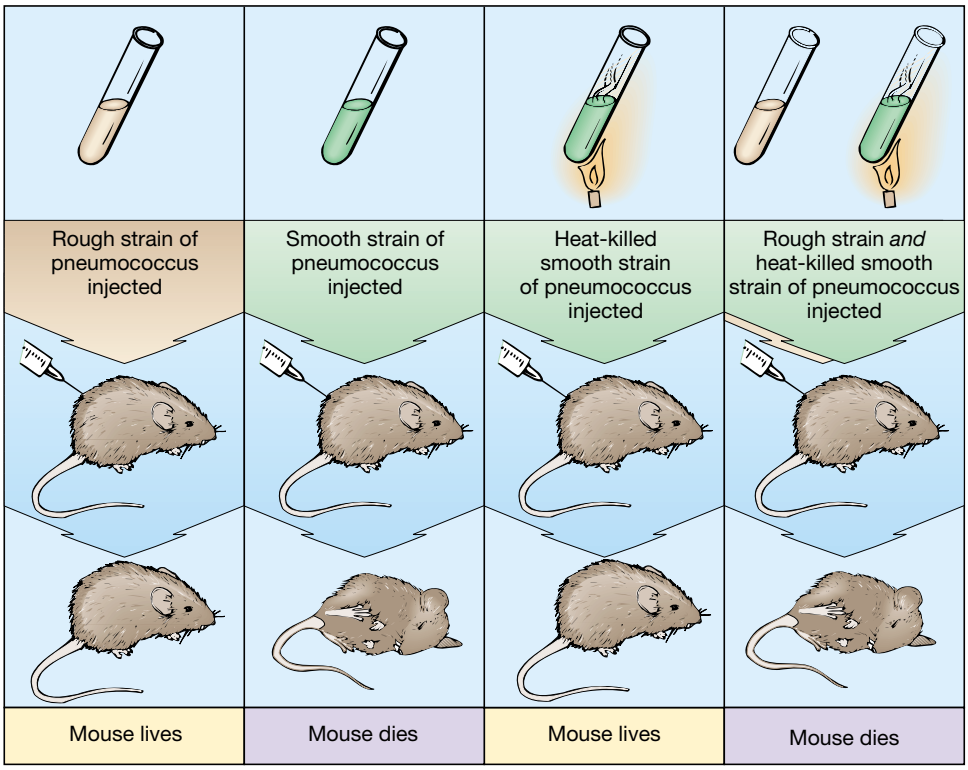


Figure 11–4 Griffith's transformation experiments. Although neither the rough (R) strain nor the heat-killed smooth (S) strain could kill a mouse, a combination of the two did. Autopsy of the dead mouse showed the presence of living, S strain pneumococci. These results indicated that some substance in the heat-killed S strain was responsible for the transformation of the living R strain to a virulent form. Avery and his colleagues later showed that purified DNA isolated from the S-strain confers virulence on the R-strain bacteria, establishing that the DNA carries the necessary information for bacterial transformation.

jected into mice, a high proportion of the mice died. Griffith was then able to isolate living S-strain cells from the dead mice.

Because neither the heat-killed S strain nor the living R strain could be converted to the living virulent form when injected by itself, something in the heat-killed cells appeared to convert the avirulent cells to the lethal form. This phenomenon, called **transformation,** was thought to be caused by some chemical substance (called the "transforming principle") in the dead bacteria that "transformed" a related strain to a genetically stable new form (Fig. 11–4).

In 1944, O. T. Avery, C. M. MacLeod, and M. McCarty of the Rockefeller Institute chemically identified Griffith's transforming principle as DNA. Although today we consider their findings to be the first demonstration that DNA is the genetic material, not all scientists of the time were convinced. One argument given was that the DNA preparations used might have been contaminated with a tiny amount of protein, which might have been responsible for the results. This was not a trivial objection, because it was well known that a very small amount of an enzyme could have significant biological effects.

During the next few years, new evidence accumulated that the haploid nuclei of pollen grains and gametes such as sperm contain only half the amount of DNA found in diploid somatic cells of the same species. Because the idea that genes are on chromosomes was generally accepted, these findings correlating DNA content with chromosome number provided strong circumstantial evidence of DNA's importance in inheritance.

In 1952 Alfred Hershey and Martha Chase performed a series of elegant experiments on the reproduction of **bacteriophages** (viruses that infect bacteria; see Chapter 23) (Fig. 11–5). Their demonstration that bacteriophages inject their DNA into bacterial cells, leaving most of their protein on the outside, emphasized the significance of DNA in viral reproduction and was seen by many as another important indication of the role of DNA as the hereditary material.

THE STRUCTURE OF DNA ALLOWS IT TO CARRY INFORMATION AND TO BE FAITHFULLY DUPLICATED

DNA was not widely accepted as the genetic material until James Watson and Francis Crick proposed a model for its structure that had extraordinary explanatory power. The story of how the structure of DNA came to be determined is one of the most remarkable chapters in the history of modern biology.

A great deal was known about the physical and chemical properties of DNA when Watson and Crick became interested in the problem. Their all-important contribution was to integrate all this information into a model that demonstrated how the molecule can both carry information and serve as its own template (pattern) for duplication.

Nucleotides can be covalently linked in any order to form long polymers

As discussed in Chapter 3, each DNA building block is a **nucleotide** consisting of a pentose sugar **(deoxyribose),** a phosphate, and one of four nitrogenous bases (Fig. 11–6*a*). The

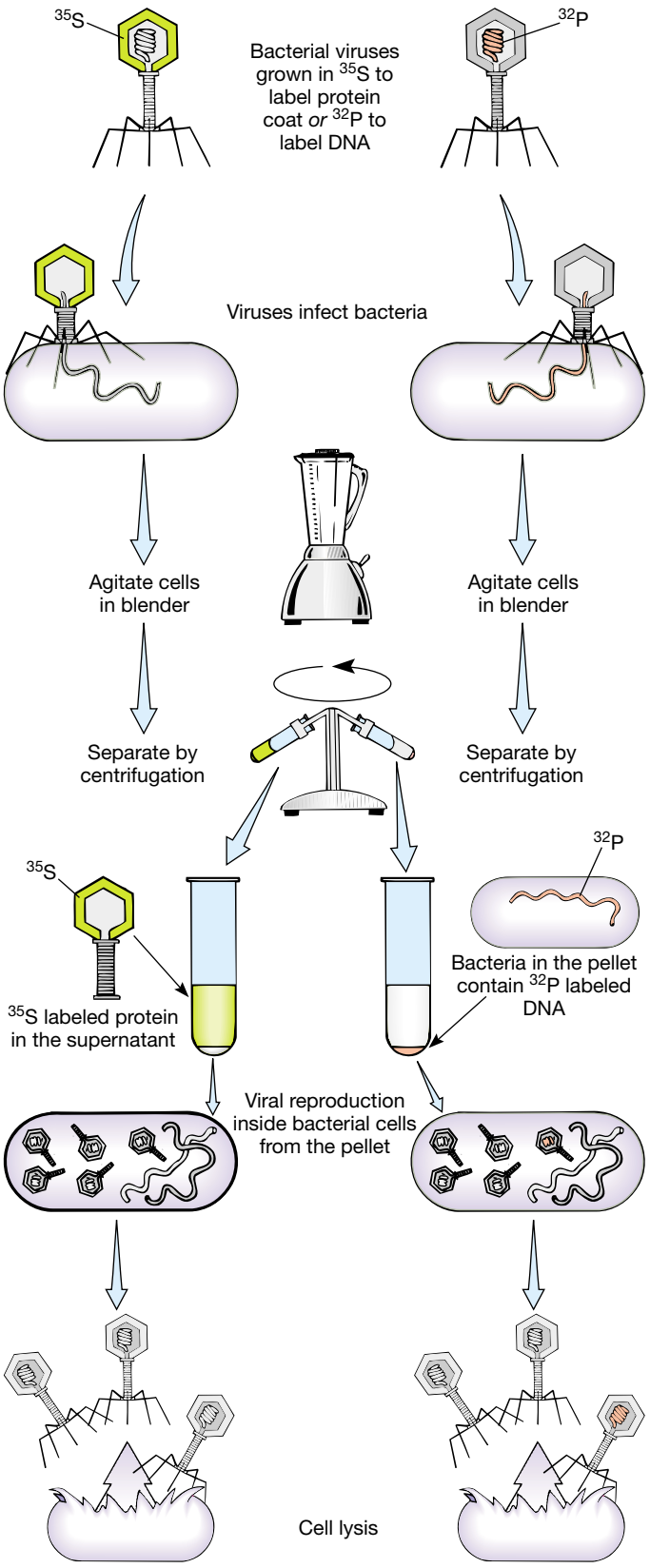


Figure 11–5 **The Hershey-Chase experiments.** Although bacteriophage protein coats labeled with the radioactive isotope ^{35}S *(left)* could be separated from infected bacterial cells without interfering with viral reproduction, viral DNA labeled with the radioactive isotope ^{32}P *(right)* could not, thus demonstrating that the DNA enters the cells and is required for synthesis of new protein coats and DNA.

nitrogenous base is attached to the 1′ carbon of the sugar, and the phosphate is attached to the 5′ carbon.[2] The bases include two **purines—adenine (A)** and **guanine (G)**—and two **pyrimidines—thymine (T)** and **cytosine (C).**

The nucleotides are linked by covalent bonds to form an alternating sugar-phosphate backbone. The 3′ carbon of one sugar is bonded to the 5′ phosphate of the adjacent sugar to form a 3′, 5′ **phosphodiester linkage** (Fig. 11–6*b*). It is therefore possible to form a polymer of indefinite length, with the nucleotides linked in any order. We now know that most DNA molecules found in cells are millions of bases long. Figure 11–6*a* also illustrates that a single polynucleotide chain is directional. No matter how long the chain may be, one end (the **5′ end**) has a 5′ carbon and the other (the **3′ end**) has a 3′ carbon that is not linked to another nucleotide.

DNA is made of two polynucleotide chains intertwined to form a double helix

Important information about the structure of DNA came from **x-ray diffraction** studies on crystals of purified DNA, carried out by Rosalind Franklin in the laboratory of M. H. F. Wilkins. X-ray diffraction is a powerful method for determining distances between atoms of molecules arranged in a regular, repeating crystalline structure (Fig. 11–7). X rays have such extremely short wavelengths that they can be scattered by the electrons surrounding the atoms in a molecule. Atoms with dense electron clouds (e.g., phosphorus, oxygen) tend to deflect electrons more strongly than do atoms with lower atomic numbers.

When a crystal is exposed to an intense beam of x rays, the regular arrangement of the atoms in the crystal causes the x rays to be diffracted, or bent, in specific ways. The pattern of diffracted x rays is seen on film as dark spots. Mathematical analysis of the arrangement and distances between the spots can then be used to determine precise distances between atoms and their orientation within the molecules.

Franklin had already produced x-ray crystallographic films of DNA patterns when Watson and Crick began to pursue the problem of DNA structure. The pictures clearly showed that DNA has a type of helical structure, and three major types of regular, repeating patterns in the molecule with the dimensions 0.34 nanometer, 3.4 nanometers, and 2.0 nanometers were evident. Franklin and Wilkins had inferred from these patterns that the nucleotide bases (which are flat molecules) are stacked like rungs of a ladder. Using this information, Watson and Crick began to build scale models of the DNA components and then fit them together to agree with the experimental data.

After a number of trials, the two worked out a model that fit the existing data (Fig. 11–8). The nucleotide chains conformed to the dimensions of the x-ray data only if each DNA molecule consisted of *two* polynucleotide chains arranged in a

[2]It is conventional to number the atoms in a molecule, using a system devised by organic chemists. In nucleic acid chemistry the "prime" designations, such as 2′, designate individual carbon atoms in the sugar ring, to distinguish them from carbon atoms in the base.

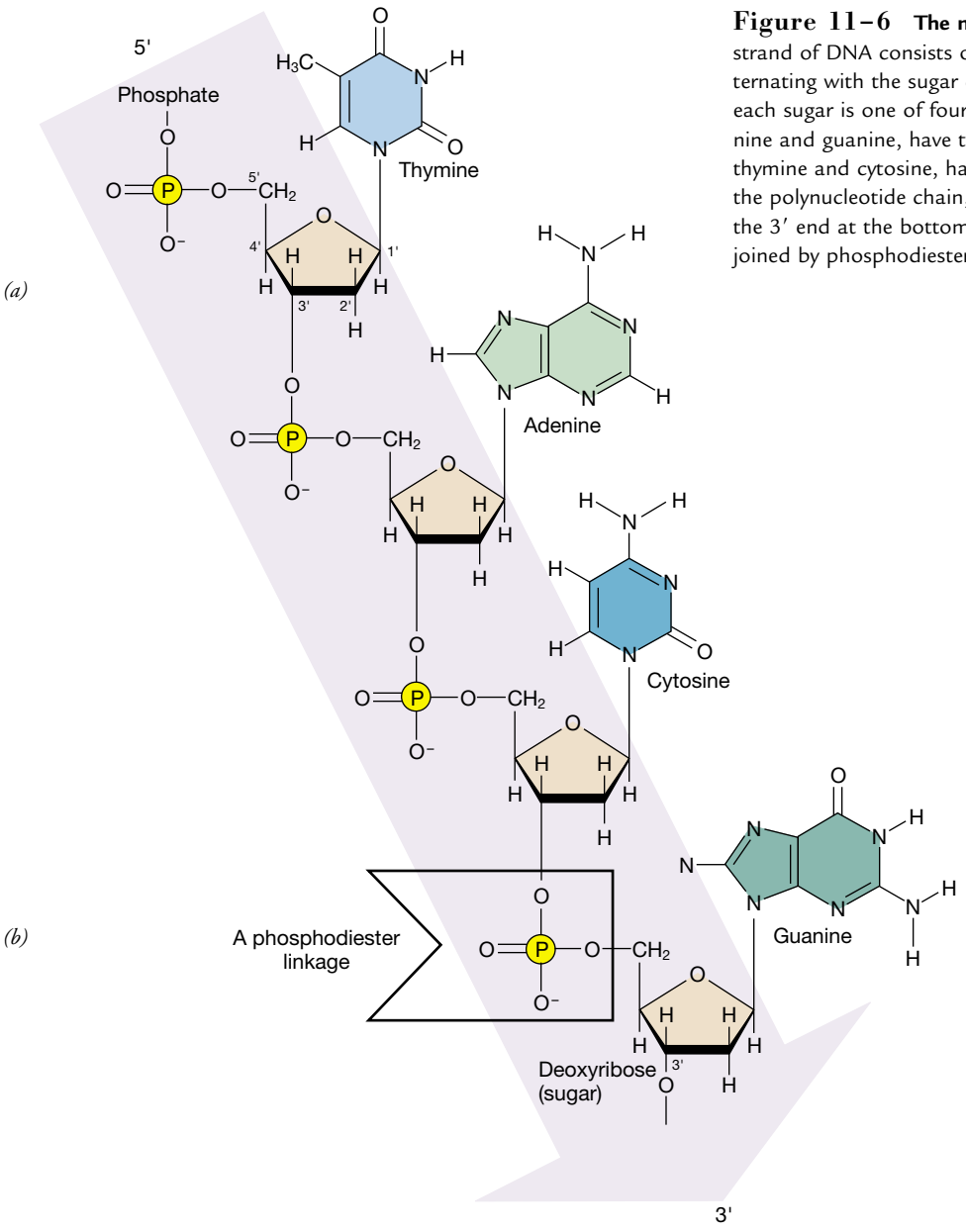


Figure 11–6 **The nucleotide subunits of DNA.** *(a)* A single strand of DNA consists of a backbone made of phosphate groups alternating with the sugar deoxyribose *(tan)*. Linked to the 1′ carbon of each sugar is one of four nitrogenous bases. The purine bases, adenine and guanine, have two-ring structures; the pyrimidine bases, thymine and cytosine, have one-ring structures. Note the polarity of the polynucleotide chain, with the 5′ end at the top of the figure and the 3′ end at the bottom. ***(b)*** Sugars of adjacent nucleotides are joined by phosphodiester linkages.

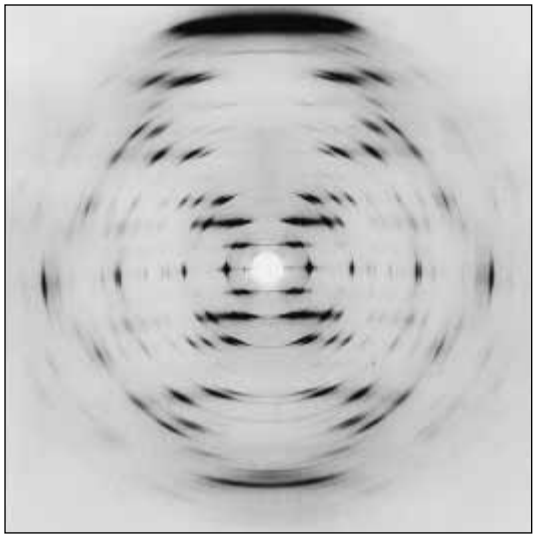


Figure 11–7 **X-ray diffraction image of DNA.** Important clues about DNA structure are provided by detailed mathematical analysis of measurements of x-ray diffraction images of the lithium salt of DNA. The diagonal pattern of spots stretching from 11 o'clock to 5 o'clock and from 1 o'clock to 7 o'clock provides evidence for the helical structure of DNA. The elongated horizontal patterns at the top and bottom indicate that the purine and pyrimidine bases are stacked 0.34 nanometers apart and are perpendicular to the axis of the DNA molecule. *(Dr. S.D. Dover, Division of Biomolecular Sciences, Kings College, London)*

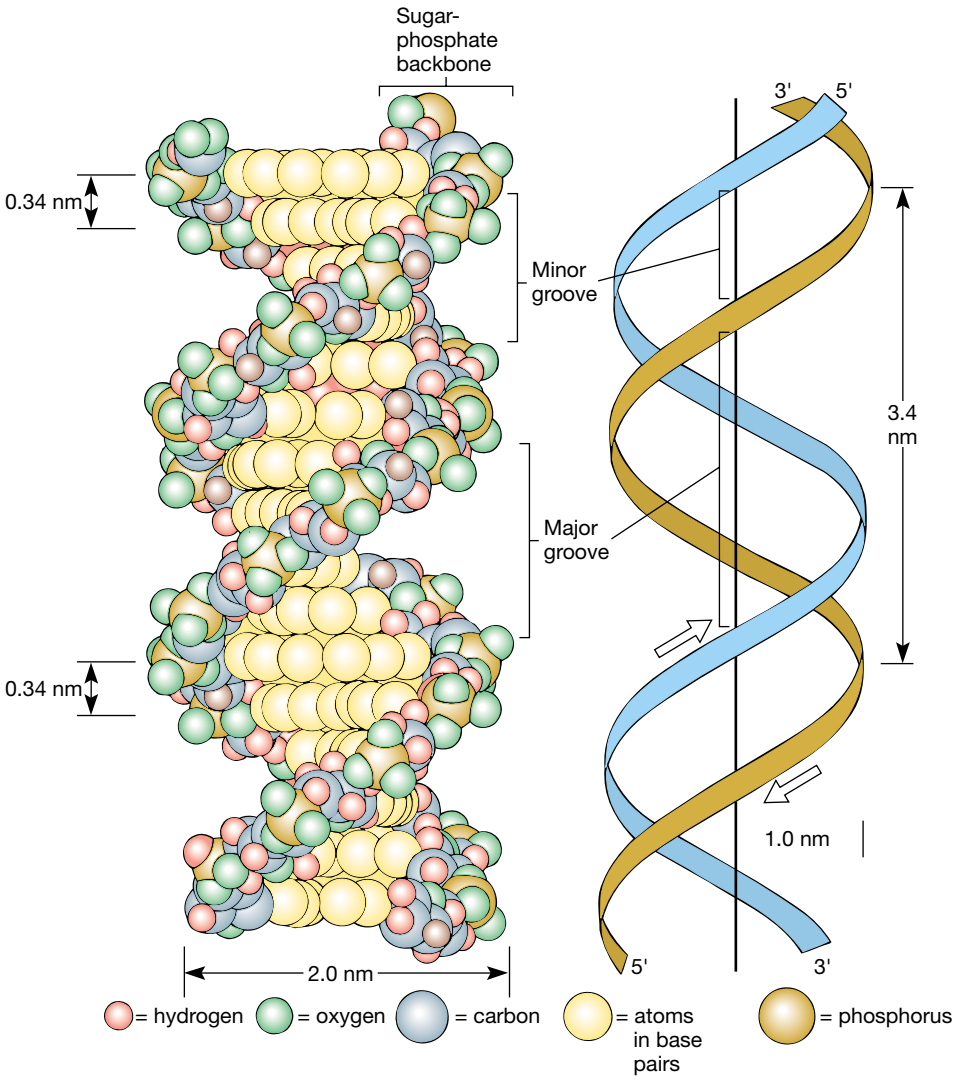


Figure 11–8 **Structure of the DNA molecule.** On the left is a space-filling model of the DNA double helix. The measurements on the diagrammatic model on the right match those derived from x-ray diffraction images. The ribbons represent the sugar-phosphate backbone of each strand; the thick arrows indicate that the two strands extend in opposite directions.

coiled **double helix.** In their model, the sugar-phosphate backbones of the two chains form the outside of the helix. The bases belonging to the two chains associate as pairs in the center. The reasons for the repeating patterns of 0.34-nanometer and 3.4-nanometer measurements are readily apparent from the model: each pair of bases is exactly 0.34 nanometer from the adjacent pairs above and below. Because exactly ten base pairs are present in each full turn of the helix, each turn is 3.4 nanometers high. To fit the data, the two chains must run in opposite directions; therefore, each end of the double helix must have an exposed 5′ phosphate on one strand and an exposed 3′ hydroxyl group on the other. Because the two strands run in opposite directions, they are said to be **antiparallel** to each other.

In double-stranded DNA, hydrogen bonds form between adenine and thymine and between guanine and cytosine

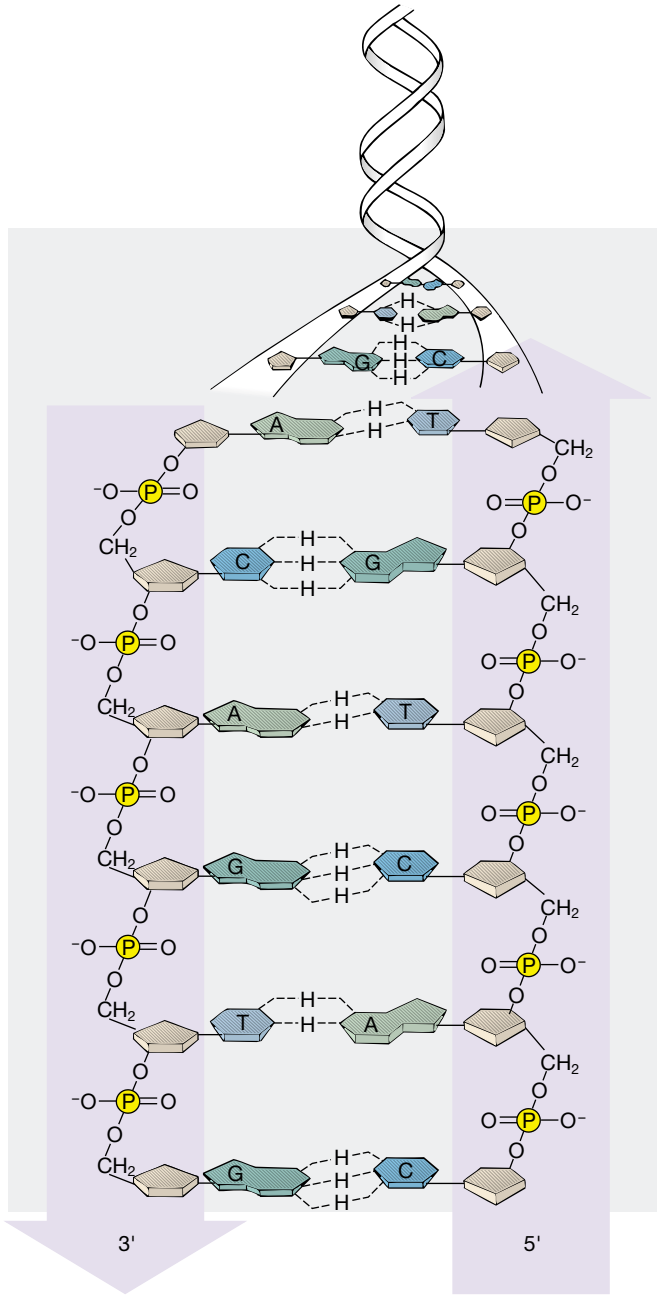
Other features of the model integrated important information about the chemical composition of DNA with the x-ray diffraction data. By 1950, the base composition of DNA from a number of organisms and tissues had been determined by Erwin Chargaff and his coworkers at Columbia University. They found a simple relationship among the bases that turned out to be an important clue to the structure of DNA. Regardless of the source of the DNA, in Chargaff's words, the "ratios of purines to pyrimidines and also of adenine to thymine and of guanine to cytosine were not far from 1." In other words, in DNA molecules, A=T and G=C.

The x-ray diffraction studies indicated that the double helix has a precise and constant width, as shown by the 2.0-nanometer reflections. This finding is actually connected to Chargaff's rules. Notice in Figure 11–6 that the pyrimidines (cytosine and thymine) contain only one ring of atoms, whereas the larger purines (guanine and adenine), contain two rings. Study of the models made it clear to Watson and Crick that if each cross-rung of the ladder were to contain one purine and one pyrimidine, the width of the helix at that point would be exactly 2.0 nanometers; the combination of two purines (each of which is 1.2 nanometers wide) would be wider and that of two pyrimidines would be narrower.

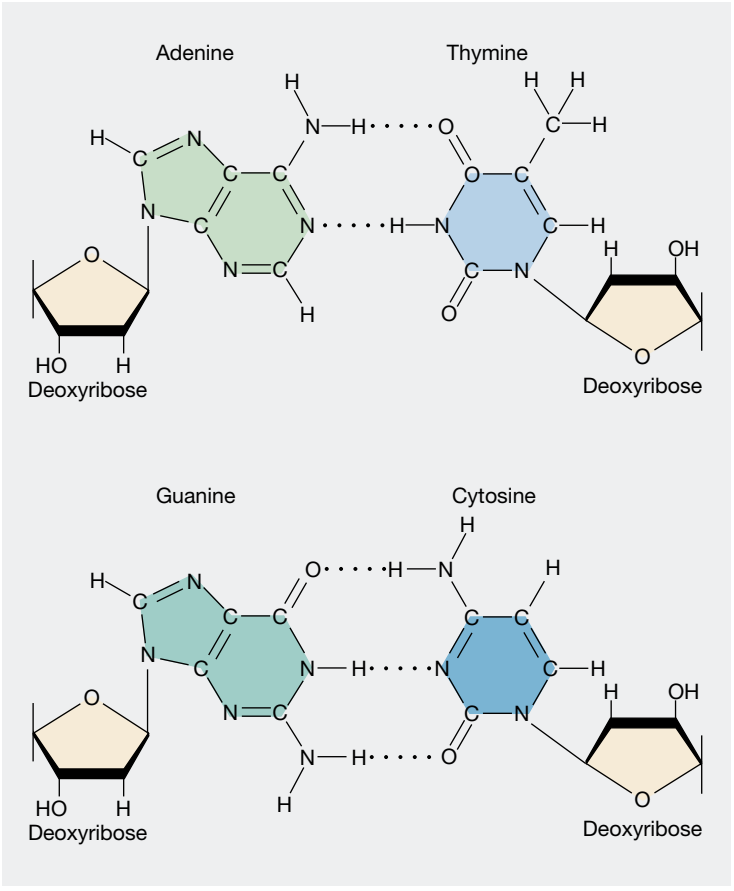
Further examination of the model showed that adenine can pair with thymine (and guanine with cytosine) in such a way that hydrogen bonds form between them; the opposite combinations, cytosine with adenine and guanine with thymine, do not lead to favorable hydrogen bonding.

The nature of the hydrogen bonding between adenine and thymine and between guanine and cytosine is shown in Figure 11–9. Two hydrogen bonds can form between adenine and thymine, and three between guanine and cytosine. This concept of *specific base-pairing* neatly explains Chargaff's rules. The amount of cytosine has to equal the amount of guanine, because every cytosine in one chain must have a paired guanine in the other chain. Similarly, every adenine in the first chain must have a thymine in the second chain. Thus, the sequences of bases in the two chains are **complementary,** but not identical, to each other. In other words, the sequence of nucleotides in one chain dictates the complementary sequence of nucleotides in the other. For example, if one strand has the sequence:

3′—AGCTAC—5′



(*a*)



(*b*)

Figure 11–9 **Hydrogen bonding between bases.** The two strands of a DNA double helix are associated by hydrogen bonding between the bases. ***(a)*** The two sugar-phosphate chains run in opposite directions. This orientation permits the complementary bases to pair. ***(b)*** Diagram of the hydrogen bonding between base pairs adenine (A) and thymine (T) *(top)* and guanine (G) and cytosine (C) *(bottom)*. The AT pair has two hydrogen bonds; the GC pair has three.

then the other strand has the complementary sequence:

5′—TCGATG—3′

The double-helix model strongly suggested that the sequence of bases in DNA can provide for the storage of genetic information. Although there are restrictions on how the bases pair with each other, the number of possible sequences of bases in a strand is virtually unlimited. Because a DNA molecule in a cell can be millions of nucleotides long, it can store enormous amounts of information, usually comprising a large number of genes.

DNA REPLICATION IS SEMICONSERVATIVE

Two immediately apparent and distinctive features of the Watson-Crick model made it seem more plausible that DNA is the genetic material. We have already mentioned that DNA can carry coded information in its sequence of bases. The model also suggested a way in which information in DNA could be precisely copied, a process known as **DNA replication.** The importance of the replication mechanism was known to Watson and Crick, who noted in a classic and now famous understatement at the end of their first brief paper, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

The model suggested that, because the nucleotides pair with each other in a complementary fashion, each strand of the DNA molecule could serve as a template, or pattern, for the synthesis of the opposite strand. It would simply be necessary for the hydrogen bonds between the two strands to break and the two chains to separate. Each half-helix could then pair with complementary nucleotides to replace its missing partner. The result would be two DNA double helices, each identical to the original one and consisting of one original strand from the parent molecule and one newly synthesized complementary strand. This type of information copying is known as a **semiconservative replication** mechanism. The recognition that DNA could be copied in this way suggested how DNA could provide a third essential characteristic of genetic material—the ability to mutate (see *Making the Connection: Mutations and the Structure of DNA*).

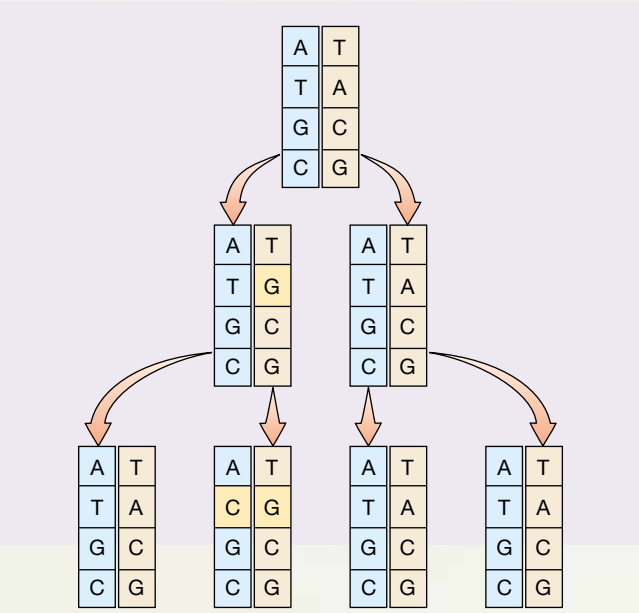
Although the semiconservative replication mechanism suggested by Watson and Crick was (and is) a simple and compelling model, experimental proof was needed to establish that DNA in fact duplicates in that manner. First it was necessary

MAKING THE CONNECTION

MUTATIONS AND THE STRUCTURE OF DNA

How do new genetic variants arise? This question was of great interest to geneticists, who had long known that mutations, or genetic changes, could arise in genes and then be transmitted faithfully to succeeding generations. When the double-helix model was proposed, it seemed plausible that mutations could represent a change in the sequence of bases in the DNA. One could predict that if DNA is copied by a mechanism involving complementary base pairing, any change in the sequence of bases on one strand would result in a new sequence of complementary bases during the next replication cycle. The new base sequence would then be passed on to daughter molecules by the same mechanism used to copy the original genetic material, as if no change had occurred.

In the example shown in the figure, an adenine base in one of the DNA strands has been changed to guanine. This could occur by a rare error in DNA replication or by one of several other known mechanisms. (There are systems of enzymes that repair errors when they occur, but not all mutations are corrected properly.) When the DNA molecule is replicated again, one of the strands gives rise to a molecule exactly like the parent strand; the other (mutated) strand gives rise to a molecule with a new combination of bases that will be stably transmitted generation after generation.



Perpetuation of a mutation. The process of DNA replication can stabilize a mutation so that it will be passed to future generations.

to rule out several other possibilities. For example, with a *conservative replication* mechanism, both parent (or old) strands would remain together, and the two newly synthesized strands would form a second double helix. As a third alternative, the parental and newly synthesized strands might become randomly mixed during the replication process; this possibility was known as *dispersive replication.* To discriminate among the semiconservative replication mechanism and the other possibilities, it was necessary to distinguish between old and newly synthesized strands of DNA.

One way to accomplish this is to use a heavy-nitrogen isotope, nitrogen-15 (ordinary nitrogen is nitrogen-14), to label DNA strands by making them more dense. Large molecules such as DNA can be separated on the basis of differences in their density, using a technique known as **density gradient centrifugation.** When DNA is mixed with a solution containing cesium chloride (CsCl) and centrifuged at high speed, the solution forms a density gradient in the centrifuge tube, ranging from a low density region at the top to a region of highest density at the bottom. During centrifugation the DNA molecules migrate to the region of the gradient identical to their own density.

In 1957, Matthew Meselson and Franklin Stahl grew cells of the bacterium *Escherichia coli* on a medium that contained nitrogen-15 in the form of ammonium chloride (NH_4Cl). The cells used the nitrogen-15 to synthesize bases, which then became incorporated into DNA (Fig. 11–10). The resulting heavy nitrogen-containing DNA molecules were extracted from some of the cells. When they were subjected to density gradient centrifugation, they accumulated in the high-density region of the gradient. The rest of the bacteria (which also contained nitrogen-15-labeled DNA) were transferred to a different growth medium in which the NH_4Cl contained the naturally abundant, lighter nitrogen-14 isotope; they were then allowed to undergo additional cell divisions.

The newly synthesized DNA strands were expected to be less dense because they incorporated bases containing the lighter nitrogen-14 isotope. Indeed, double-stranded DNA from cells isolated after one generation had an intermediate density, indicating that they contained half as many nitrogen-15 atoms as the "parent" DNA. This finding supported the semiconservative model, which predicted that each double helix should contain a previously synthesized strand (heavy in this case) and a newly synthesized strand (light in this case). It was inconsistent with the conservative model, which predicted that there should be two classes of double stranded molecules, those with two heavy strands and those with two light strands.

After another cycle of cell division in the medium with the lighter nitrogen-14 isotope, two types of DNA appeared in the density gradient. One consisted of "hybrid" DNA helices (with one nitrogen-15 strand and one nitrogen-14 strand), whereas the other contained only DNA with the naturally occurring light isotope. This finding refuted the dispersive model, which predicted that all strands should have intermediate density. Instead, each strand of the parental double-helix molecule was conserved, but in a *different* daughter molecule, exactly as predicted by the semiconservative replication model.

DNA replication is complex and has a number of unique features

Although the general principles of DNA replication are simple and straightforward predictions from the Watson-Crick model, the process actually requires a complex "replication machine" containing a large number of proteins and enzymes. Many of the essential features of DNA replication are universal, although some differences exist between prokaryotes and eukaryotes because their DNA is organized differently. In bacterial cells such as *E. coli,* most or all of the DNA is in the form of a single, *circular,* double-stranded molecule. Each unreplicated eukaryotic chromosome contains a single, *linear,* double-stranded molecule associated with a great deal of protein.

DNA strands must be unwound during replication

Watson and Crick recognized that in their double-helix model the two DNA strands are wrapped around one another like the strands of a rope. If we try to pull the strands apart, the rope must either rotate or twist into tighter coils. We would expect similar things to happen when complementary DNA strands are separated for replication. Separating the two strands of DNA is accomplished by **DNA helicase enzymes** that travel along the helix, opening the double helix as they move. Once the strands are separated, **helix-destabilizing proteins** bind to single DNA strands, preventing re-formation of the double helix until the strands are copied. Enzymes called **topoisomerases** produce breaks in the DNA molecules and then rejoin the strands, relieving strain and effectively preventing the formation of knots during replication.

DNA synthesis always proceeds in a 5′ → 3′ direction

The enzymes that catalyze the linking together of the nucleotide subunits are called **DNA polymerases.** They have several limitations that contribute to the complexity of the replication process. They are able to add nucleotides only to the 3′ end of a growing polynucleotide strand, and this strand must be paired with the strand being copied (Fig. 11–11). Nucleotides with three phosphate groups are used as substrates for the polymerization reaction. As the nucleotides are linked together, two of the phosphates are removed. Like the hydrolysis of ATP, these reactions are strongly exergonic (see Chapter 6) and do not require additional energy. Because the new polynucleotide chain is elongated by the linkage of the 5′

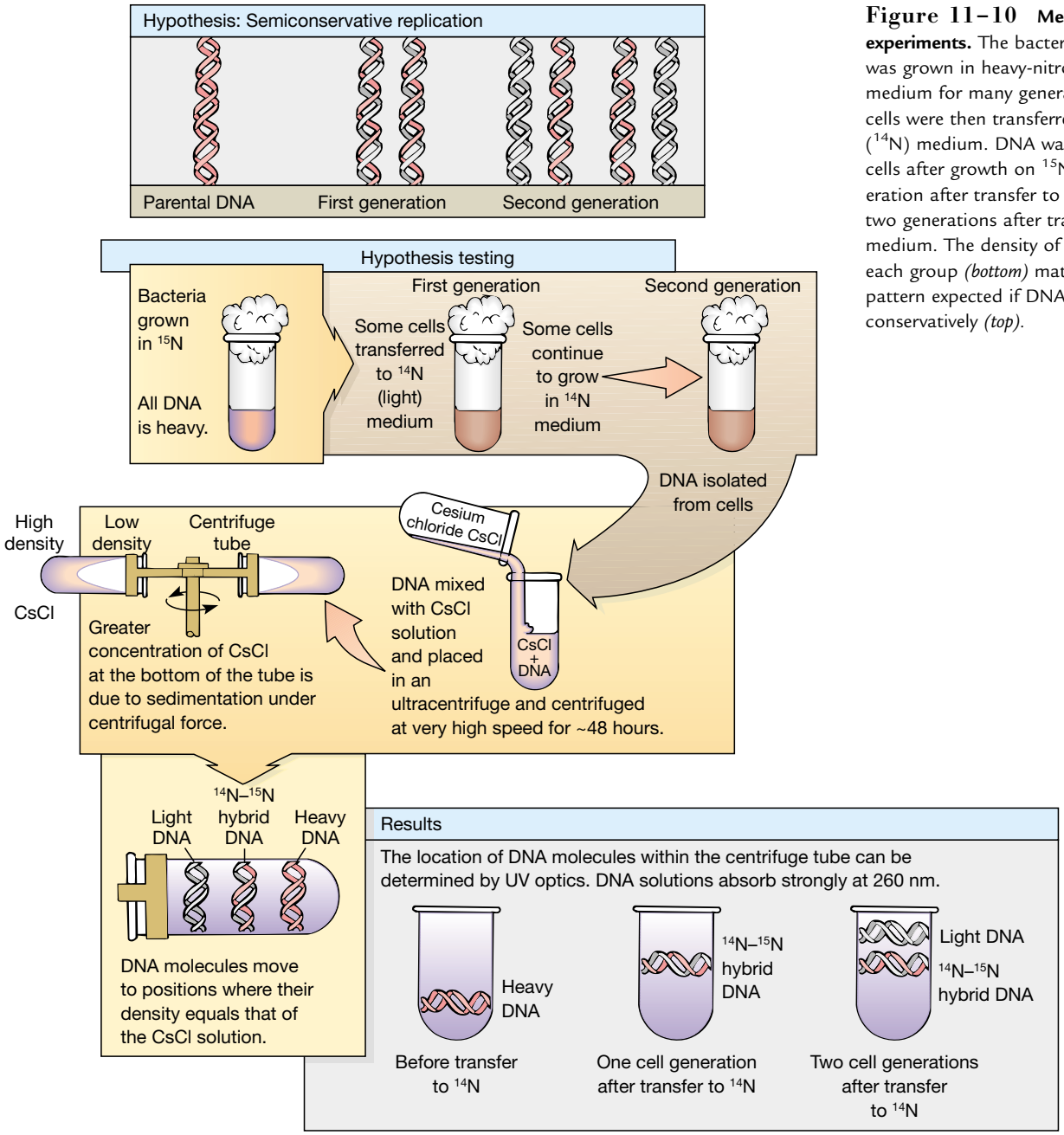


Figure 11–10 Meselson and Stahl's experiments. The bacterium *Escherichia coli* was grown in heavy-nitrogen (^{15}N) growth medium for many generations. Some of the cells were then transferred to light-nitrogen (^{14}N) medium. DNA was isolated from cells after growth on ^{15}N medium one generation after transfer to ^{14}N medium, and two generations after transfer to ^{14}N medium. The density of the molecules in each group *(bottom)* matches the labeling pattern expected if DNA is replicated semiconservatively *(top)*.

phosphate group of the next nucleotide subunit to the 3′ hydroxyl group of the sugar at the end of the preexisting strand, the new strand of DNA always grows in the 5′ → 3′ direction.

DNA synthesis requires an RNA primer

A second limitation of the DNA polymerases is that they can add nucleotides only to the 3′ end of an *existing* polynucleotide strand. So how can DNA synthesis be initiated once the two strands are separated? The answer is that a short piece (usually about five nucleotides) of an **RNA primer** is first synthesized at the point of initiation of replication (Fig. 11–12).

RNA, or **ribonucleic acid** (see Chapters 3 and 12), is a nucleic acid polymer consisting of nucleotide subunits that can associate by complementary base-pairing with the single-stranded DNA template. The RNA primer is synthesized by a protein complex known as a **primosome,** which includes an enzyme that is able to start a new strand of RNA opposite a

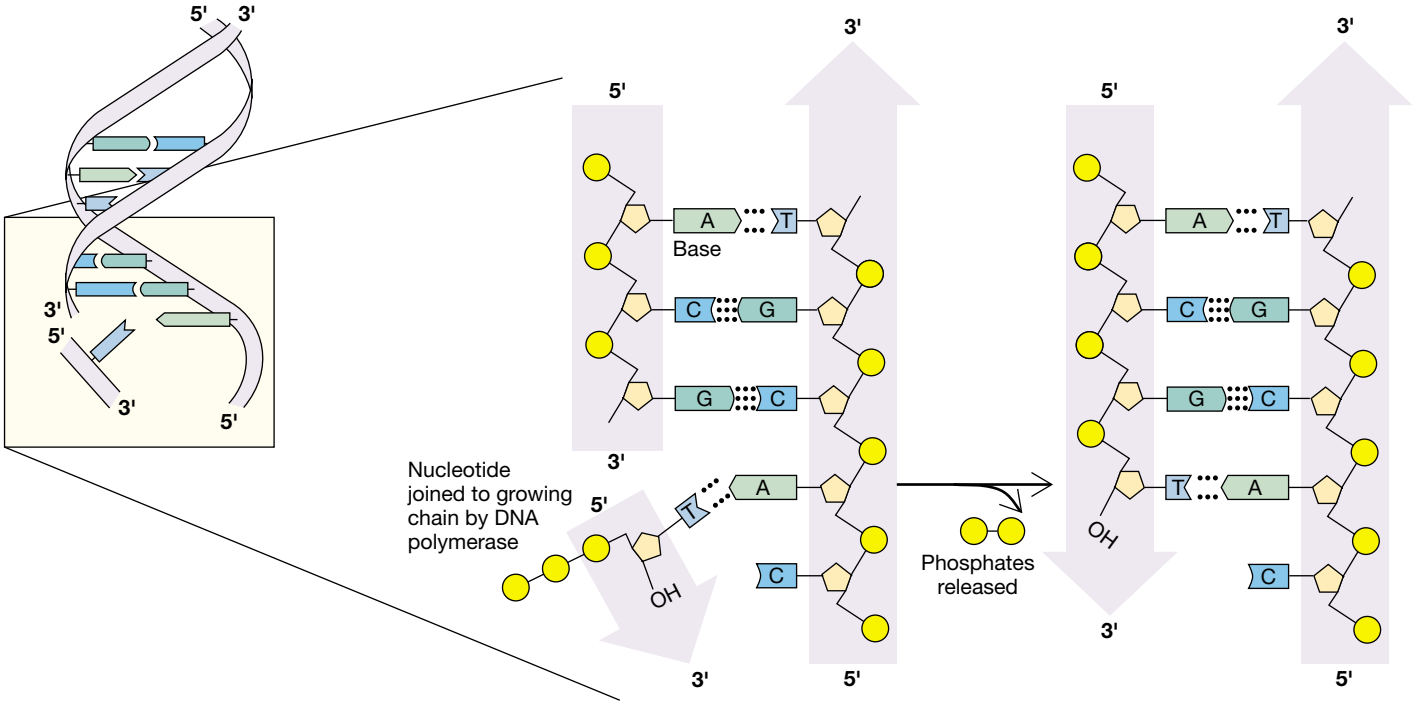


Figure 11–11 **DNA replication.** The polymerase enzymes that catalyze the polymerization reactions add one nucleotide at a time to the 3′ end of a growing chain.

DNA strand. After a few nucleotides have been added, the primosome is displaced by DNA polymerase, which can then add subunits to the 3′ end of the short RNA primer. The primer is later degraded by specific enzymes, and the space is filled in with DNA.

DNA replication is discontinuous in one strand and continuous in the other

A major obstacle in understanding DNA replication was the fact that the complementary DNA strands are antiparallel. Because DNA synthesis proceeds only in the direction of 5′ → 3′ (which means that the strand being copied is being read in a 3′ → 5′ direction), it would seem necessary to copy one of the strands starting at one end of the double helix and the other strand starting at the opposite end. We know, however, that this is not the case.

DNA replication begins at specific sites on the DNA molecule, termed **origins of replication,** and both strands are replicated at the same time at a Y-shaped structure called the **replication fork** (Fig. 11–13). The position of the replication fork is constantly moving as replication proceeds. Two identical DNA polymerase molecules are responsible for replication. One of these adds nucleotides to the 3′ end of the new strand that is always growing *toward* the replication fork. Because this strand can be formed smoothly and continuously, it is called the **leading strand.**

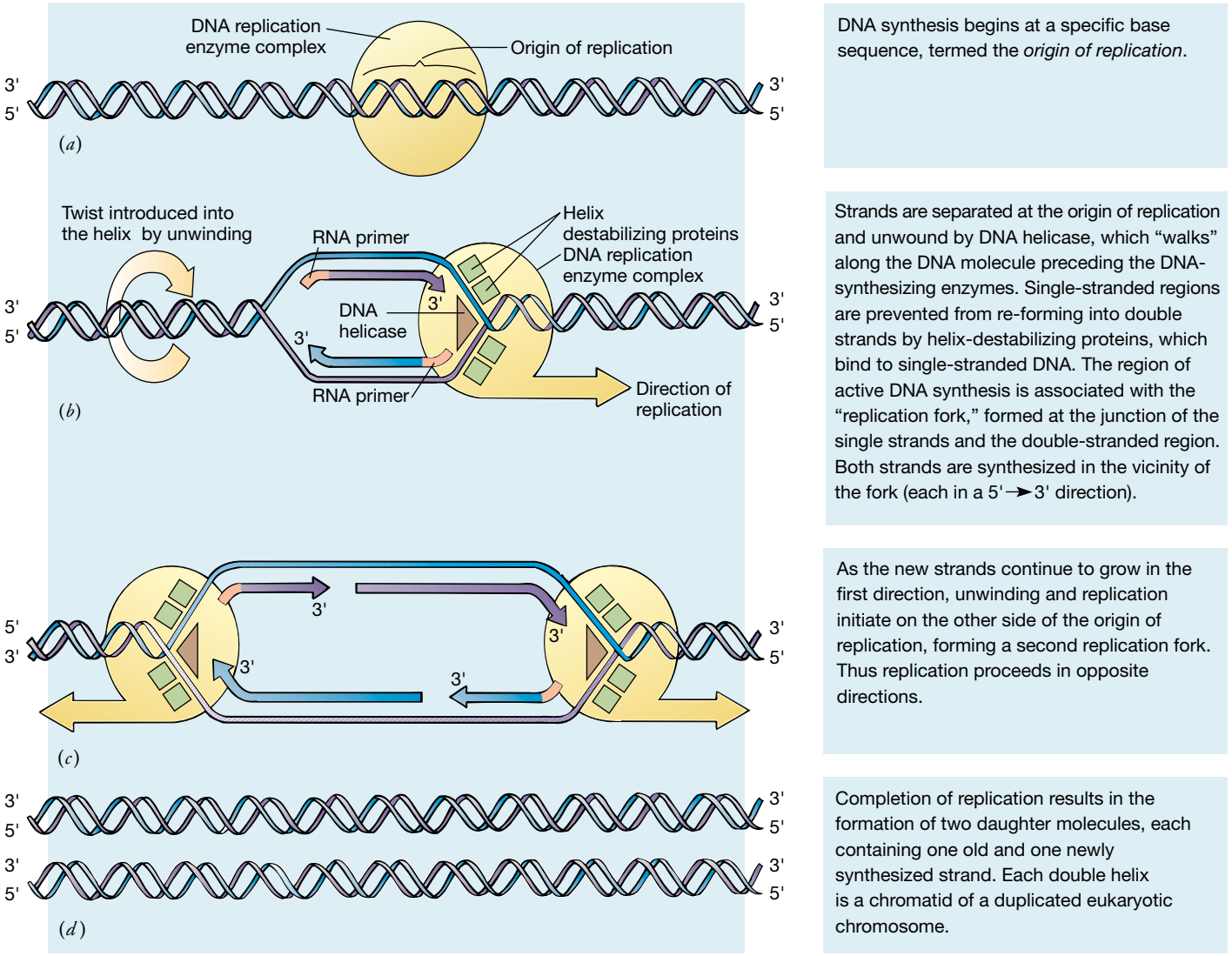
A separate (but identical) DNA polymerase molecule adds nucleotides to the 3′ end of the other new strand, termed the **lagging strand.** The lagging strand is always growing *away* from the replication fork. Only short pieces can be synthesized because the DNA polymerase enzyme would need to move far away from the fork if it were to add continuously to the 3′ end of that strand. These 100- to 1000-nucleotide pieces are called **Okazaki fragments** after their discoverer, Reijii Okazaki.

Each Okazaki fragment is initiated by a separate RNA primer and is then extended toward the 5′ end of the previously synthesized fragment by DNA polymerase. When the RNA primer of the previously synthesized fragment is reached, the primer is degraded and the resulting gap is filled in with DNA. The fragments are then joined together by **DNA ligase,** an enzyme that links the 3′ end of one DNA fragment to the 5′ end of another.

It has been suggested that simultaneous synthesis of both the leading and lagging strands is possible because the lagging strand (and the DNA strand from which it is copied) forms a loop. This loop allows DNA polymerase to synthesize the lagging strand while remaining close the replication fork as it works.

Most DNA synthesis is bidirectional

When double-stranded DNA is separated, two replication forks are formed, allowing the molecule to be replicated in



DNA synthesis begins at a specific base sequence, termed the *origin of replication*.

Strands are separated at the origin of replication and unwound by DNA helicase, which "walks" along the DNA molecule preceding the DNA-synthesizing enzymes. Single-stranded regions are prevented from re-forming into double strands by helix-destabilizing proteins, which bind to single-stranded DNA. The region of active DNA synthesis is associated with the "replication fork," formed at the junction of the single strands and the double-stranded region. Both strands are synthesized in the vicinity of the fork (each in a 5'→3' direction).

As the new strands continue to grow in the first direction, unwinding and replication initiate on the other side of the origin of replication, forming a second replication fork. Thus replication proceeds in opposite directions.

Completion of replication results in the formation of two daughter molecules, each containing one old and one newly synthesized strand. Each double helix is a chromatid of a duplicated eukaryotic chromosome.

Figure 11–12 **Overview of DNA replication.** This process requires a number of steps involving several enzymes and RNA primers.

both directions from the origin of replication. Prokaryotic cells usually have only one origin of replication on each circular DNA molecule (Fig. 11–14*a*), so the two replication forks proceed around the circle and eventually meet at the other side to complete the formation of two new DNA molecules.

A eukaryotic chromosome is composed of one, extremely long, linear DNA molecule, so the process is speeded up by having multiple origins of replication (Fig. 11–14*b–d*). Synthesis continues at each replication fork until it meets one coming from the opposite direction, resulting in the formation of a chromosome containing two DNA double helices (each of which corresponds to a chromatid). The ends of eukaryotic chromosomes; known as **telomeres,** present special problems in replication (see *On the Cutting Edge: Telomeres, Cellular Aging, and Cancer on page 263*).

DNA IN CHROMOSOMES IS PACKAGED IN A HIGHLY ORGANIZED WAY

Prokaryotic and eukaryotic cells differ markedly in their DNA content as well as in the organization of DNA molecules. An *E. coli* cell normally contains about 4×10^6 base pairs (almost 1.35 millimeters) of DNA in its single circular DNA molecule. In fact, the total length of the DNA is about 1000 times greater than the length of the cell itself. Therefore the DNA molecule must, with the help of special proteins, be twisted and folded compactly to fit inside the bacterial cell.

A typical eukaryotic cell contains much more DNA than a bacterium does, and it is organized in the nucleus as multi-

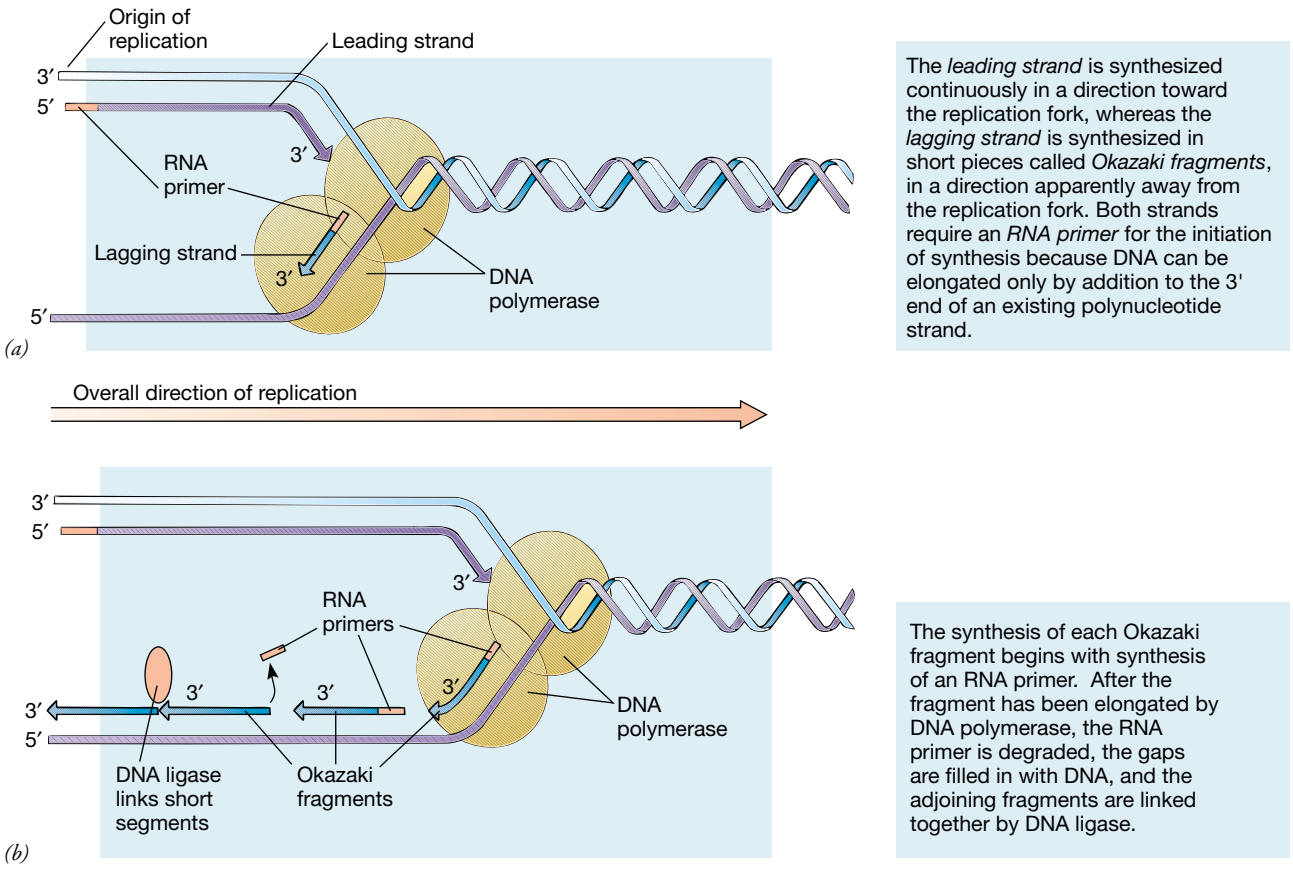


Figure 11–13 **Leading and lagging DNA strands.** Because elongation can proceed only in a 5′ → 3′ direction, the two strands at the replication fork are copied in different ways, each by a separate DNA polymerase molecule.

ple chromosomes; these vary widely in size and number among different species. Although a human cell nucleus is about the size of a large bacterial cell, it contains almost 1000 times the amount of DNA found in *E. coli*. The haploid DNA content of a human cell is about 3×10^9 base pairs; if stretched end to end, it would be almost 1 meter long.

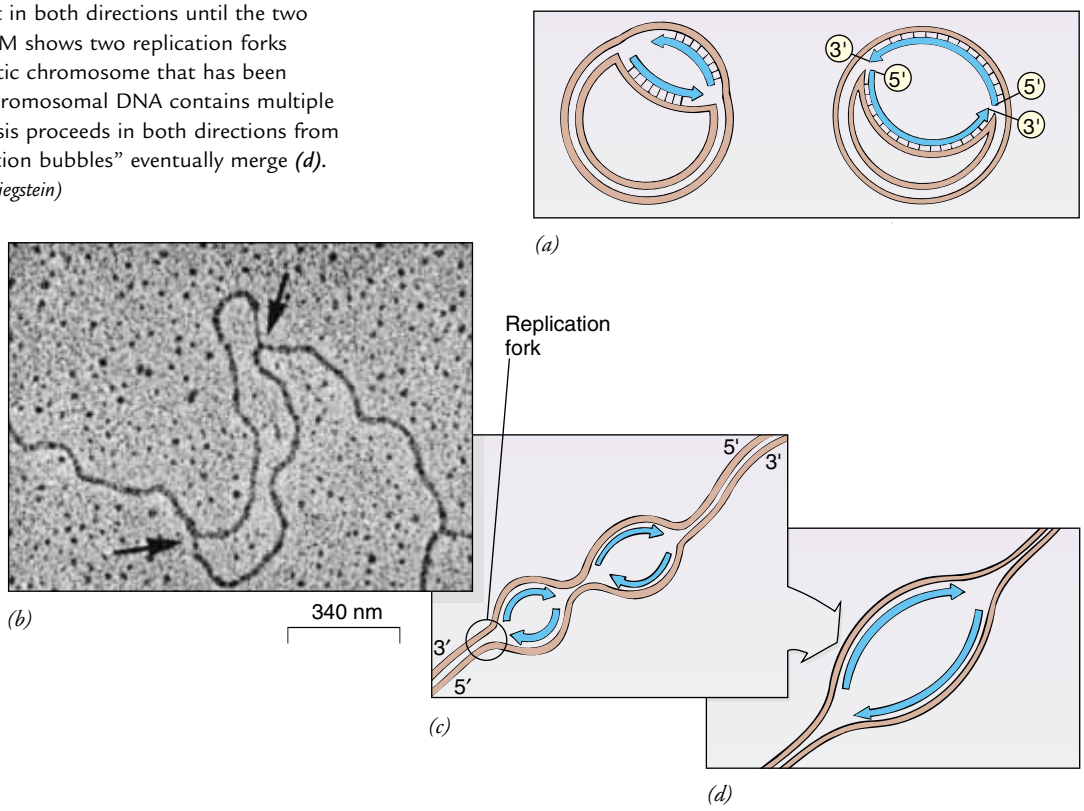
In eukaryotes, DNA, which is negatively charged, is associated with positively charged basic proteins known as **histones**[3] to form structures called **nucleosomes.** The fundamental unit of the nucleosome complex consists of a beadlike structure with 146 base pairs of DNA wrapped around a disc-shaped core of eight histone molecules (two each of four different histone types) (Fig. 11–15). Although the nucleosome was originally defined as a bead plus a DNA segment that links it to an adjacent bead, today the term more commonly refers only to the bead itself (i.e., the eight histones and the DNA wrapped around them).

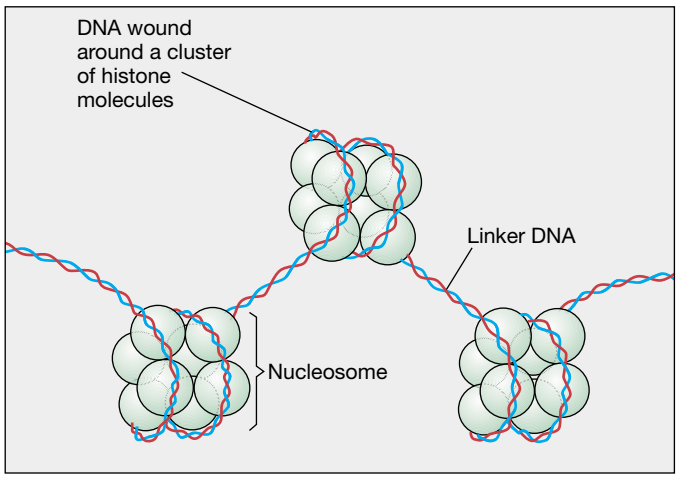
The nucleosomes are part of the **chromatin,** the complex of nucleic acids and protein that makes up the chromosomes. The higher order structures of chromatin are illustrated in Figure 11–16. For example, a fifth type of histone, known as histone H1, is associated with the linker DNA and is responsible for packing adjacent nucleosomes (each of which is 11 nm in diameter) together to form a 30-nm-diameter thread. These 30-nm-diameter threads form large coiled loops held together by a set of nonhistone **scaffolding proteins.** The loops then interact in complex ways to form the chromatin found in a condensed metaphase chromosome.

Nucleosomes function like tiny spools, thereby preventing DNA strands from becoming tangled. The importance of this role is underscored by Figure 11–17, which illustrates the dense packing of the DNA fibers of a mouse chromosome after the histones have been removed. However, their role is more than structural, for their arrangement also affects the activity of the DNA with which they are associated (Chapter 13).

[3]A few types of eukaryotic cells lack histones. Conversely, histones do occur in one group of prokaryotes, the Archaebacteria (Chapter 23).

Figure 11–14 **Bidirectional DNA replication.** The leading strands and lagging strands are not represented in the illustrations. ***(a)*** The circular DNA in *E. coli* has only one origin of replication. DNA synthesis proceeds from that point in both directions until the two replication forks meet. ***(b)*** This TEM shows two replication forks *(arrows)* in a segment of a eukaryotic chromosome that has been partly replicated. ***(c)*** Eukaryotic chromosomal DNA contains multiple origins of replication. DNA synthesis proceeds in both directions from each origin until adjacent "replication bubbles" eventually merge ***(d)***. *(b, Courtesy of D.S. Hogness and H.J. Kriegstein)*





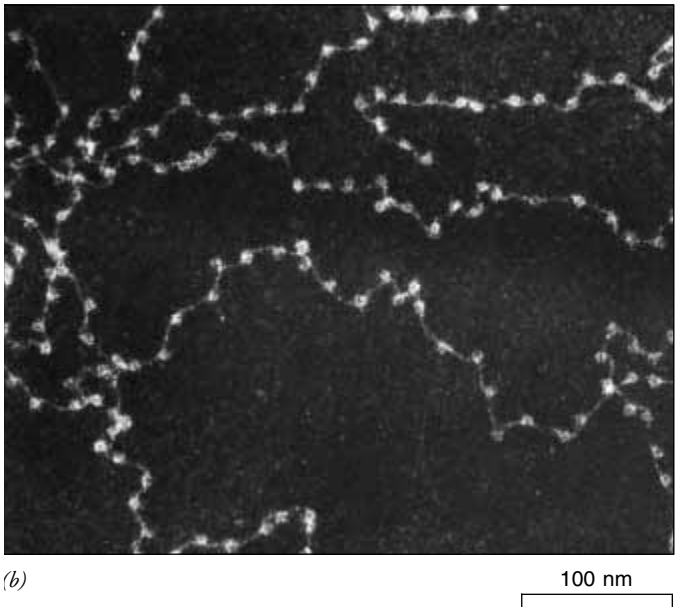


Figure 11–15 **Nucleosomes.** ***(a)*** A model for the structure of a nucleosome. Each nucleosome bead contains a set of eight histone molecules; these form a protein core around which the double-stranded DNA is wound. The DNA surrounding the histone consists of 146 nucleotide pairs; another segment of DNA, about 60 nucleotide pairs long, links nucleosome beads. ***(b)*** TEM of nucleosomes from the nucleus of a chicken red blood cell. Normally nucleosomes are packed more closely together, but the preparation procedure has spread them apart, revealing the DNA linkers. *(b, courtesy of D.E. Olins and A.L. Olins)*

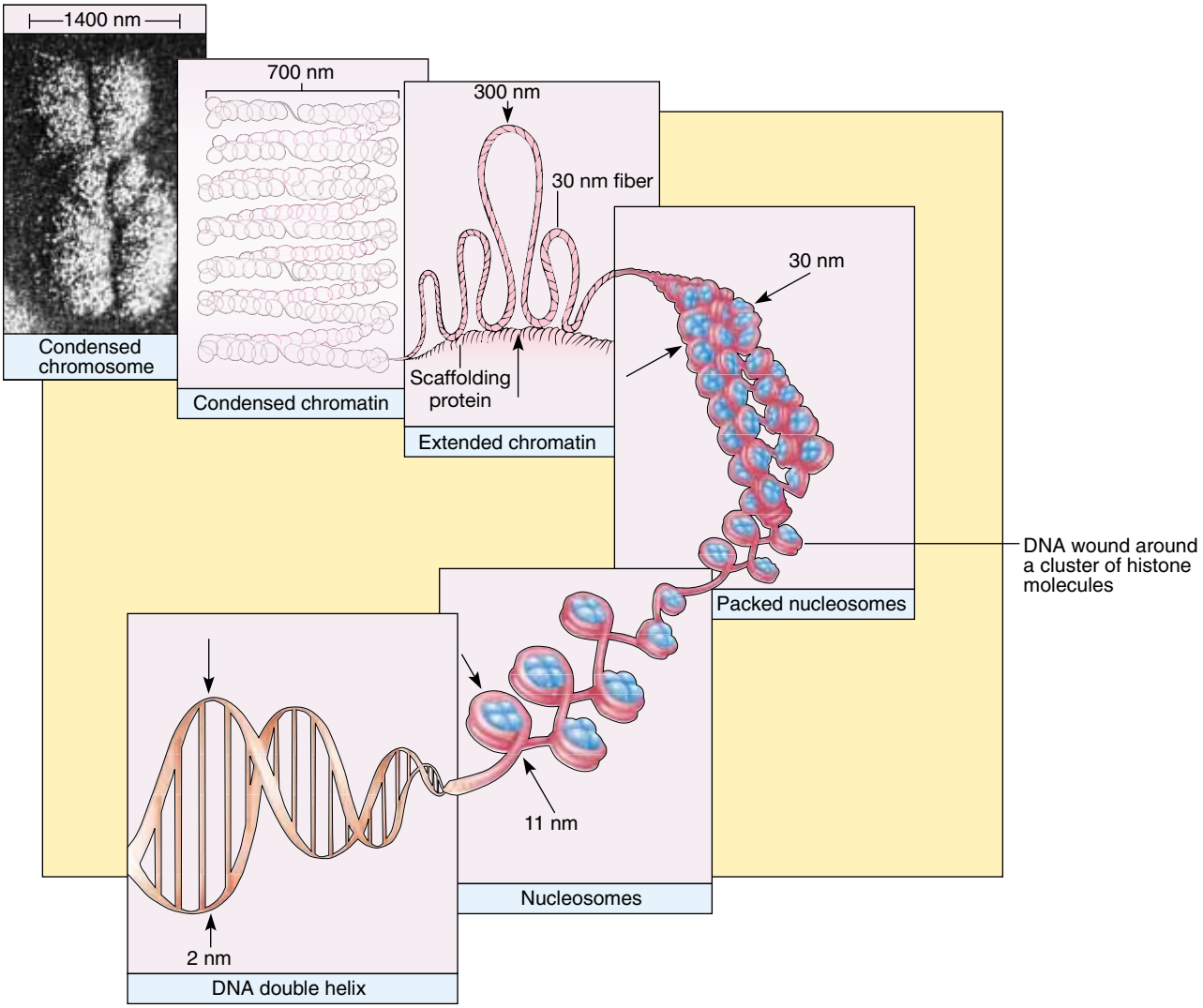


Figure 11–16 **Organization of a eukaryotic chromosome.**
(Visuals Unlimited/K.G. Murti)

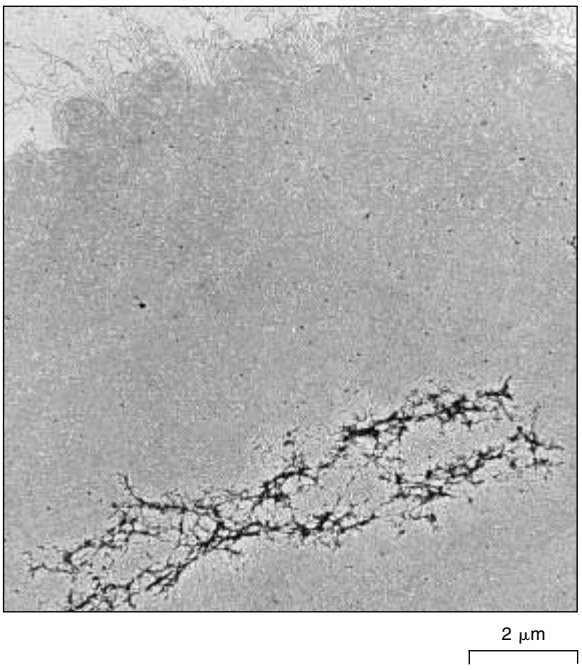


Figure 11–17 **A chromosome depleted of histones.** Note how densely packed the DNA fibrils are in this TEM of a mouse chromosome, even though they have been released from the histone proteins that organize them into tightly coiled structures. The dark structure extending from left to right across the bottom of the photograph is composed of scaffolding proteins. *(Courtesy of U. Laemmli, from* Cell *12:817, 1988. Copyright by Cell Press)*

ON THE CUTTING EDGE

Telomerase, Cellular Aging, and Cancer

HYPOTHESIS: The number of cell divisions that normal human somatic cells can undergo when grown in culture is limited by the length of their telomeric DNA.

METHOD: To test the prediction that cells will be able to undergo more divisions if their telomeric DNA is lengthened, researchers used a virus to introduce the gene coding for the catalytic subunit of telomerase into cultured normal human cells, which normally lack telomerase activity.

RESULTS: The cells produced active telomerase, which elongated the telomeres. The cells continued to actively proliferate for many cell cycles beyond the point at which cell division would normally cease.

CONCLUSION: The life spans of cells can be extended by lengthening the telomeric DNA of their chromosomes.

Unlike prokaryotic DNA, which is circular, eukaryotic chromosomes have free ends. This simple fact has far-reaching consequences. Because of the discontinuous way in which DNA polymerases work, they are unable to complete replication neatly when they reach the end of the DNA, and must therefore leave a small portion unreplicated. This situation is less dangerous than it sounds because chromosomes have end caps (telomeres) that do not contain protein-coding genes, but instead consist of short, simple, noncoding DNA sequences that are repeated many times. Therefore, although a small amount of telomeric DNA fails to replicate each time the cell divides, no essential genetic information is lost.

Telomeric DNA can be lengthened by a special DNA replication enzyme known as **telomerase.** This enzyme, which was discovered in 1984 by Carol W. Greider and Elizabeth H. Blackburn, is typically present in cells that can divide an unlimited number of times, including unicellular organisms and many types of cancer cells.

In animals such as humans, active telomerase is usually present in germ line cells (the cells that give rise to eggs and sperm) but not in the normal cells of the body, known as somatic cells. There has been much speculation that this lack of telomerase activity could contribute to the phenomenon known as **cellular aging.** Cellular aging has been analyzed since the 1960s, following the pioneering studies of Leonard Hayflick, who showed that normal human somatic cells grown in culture lose their ability to divide after a limited number of cell divisions. Furthermore, the number of cell divisions is determined by the age of the individual from whom the cells were taken. Cells from a 70 year old can divide only 20 to 30 times, as compared with those from an infant, which can divide 80 to 90 times.

Although correlations between the ability of cells to undergo unlimited divisions and the presence of telomerase activity have been repeatedly noted, this connection remained controversial. Proof of a causal relationship was lacking until Andrea G. Bodnar and her colleagues at the Geron Corporation teamed up with researchers from the University of Texas Southwestern Medical Center to conduct a direct test.* Using the techniques of recombinant DNA technology (see Chapter 14), they infected cultured normal human cells with a virus that carried the genetic information coding for the catalytic subunit of telomerase. Not only did the cells produce active telomerase, which elongated the telomeres significantly, but the cells continued to divide long past the point at which cell divisions would normally cease. These findings have revived interest in telomeres, particularly with respect to their apparent dual roles in aging and cancer, and are leading to more hypotheses that can be experimentally tested.

*Bodnar, A.G., M. Ouellette, M. Frolkis, S.E. Holt, C.-P. Chiu, G.B. Morin, C.B. Harley, J.W. Shay, S. Lichsteiner, and W.E. Wright. "Extension of Life-Span by Introduction of Telomerase into Normal Human Cells." *Science,* Vol. 279, 16 Jan. 1998.

SUMMARY WITH KEY TERMS

I. Many early geneticists thought that genes were made of proteins. Proteins were known to be complex and variable, whereas nucleic acids were thought of as rather simple molecules with a limited ability to store information.
   A. Garrod's work on inborn errors of metabolism and that of Beadle and Tatum with *Neurospora* mutants suggested that each protein is specified by a single gene.
   B. Several lines of evidence supported the idea that DNA is the genetic material.
      1. In **transformation** experiments, the DNA of one strain of bacteria can endow related bacteria with new genetic characteristics.
      2. When a bacterial cell becomes infected with a virus, only the DNA from the virus enters the cell; this DNA is sufficient for the virus to reproduce and form new virus particles.
   C. Watson and Crick's studies on the structure of DNA demonstrated how information can be stored in the molecule's structure and how DNA molecules can serve as templates for their own duplication.

II. DNA is a very regular polymer of **nucleotides.**
   A. Each nucleotide subunit contains a nitrogenous base, which may be one of the **purines (adenine** or **guanine)** or one of the **pyrimidines (thymine** or **cytosine).** Each base is covalently linked to a five-carbon sugar, **deoxyribose,** which is covalently bonded to a phosphate group.

B. The backbone of each single DNA chain is formed by alternating sugar and phosphate groups, joined by covalent **phosphodiester linkages.** Each phosphate group is attached to the 5′ carbon of one deoxyribose and to the 3′ carbon of the neighboring deoxyribose.
C. Each DNA molecule is composed of two polynucleotide chains that associate as a **double helix.** The two chains are **antiparallel** (meaning they run in opposite directions); at each end of the DNA molecule one chain has an exposed 5′ deoxyribose carbon (the **5′ end**) and the other has an exposed 3′ deoxyribose carbon (the **3′end**).
D. The two chains of the helix are held together by hydrogen bonding between specific base pairs. Adenine (A) forms two hydrogen bonds with thymine (T); guanine (G) forms three hydrogen bonds with cytosine (C).
1. **Complementary base-pairing** between A and T and between G and C is the basis of Chargaff's rules, which state that A=T and G=C.
2. Because the two strands of DNA are held together by complementary base-pairing, it is possible to predict the base sequence of one strand if one knows the base sequence of the other strand.

III. During **DNA replication,** the two strands of the double helix unwind. Each strand serves as a template for the formation of a new complementary strand.
A. DNA replication is **semiconservative;** that is, each daughter double helix contains one strand from the parent molecule and one newly synthesized strand.
B. DNA replication is a complex process requiring a number of different enzymes.
1. The enzyme that adds new deoxyribonucleotides to a growing DNA strand is a **DNA polymerase.**
2. Additional enzymes and other proteins are required to unwind and stabilize the separated DNA helix and to form **RNA primers. Topoisomerases** prevent tangling and knotting, and **DNA ligase** links together fragments of newly synthesized DNA.
C. DNA synthesis always proceeds in a 5′ → 3′ direction. This requires that one DNA strand (the **lagging strand**) be synthesized discontinuously, as short **Okazaki fragments.** The opposite strand (the **leading strand**) is synthesized continuously.
D. DNA replication is bidirectional, starting at the **origin of replication** and proceeding in both directions from that point. A eukaryotic chromosome may have multiple origins of replication and may be replicating at many points along its length at any one time.
E. Eukaryotic chromosome ends, known as **telomeres,** shorten slightly with each cell cycle, but can be extended by the **telomerase** enzyme. The absence of telomerase activity in certain cells may be a cause of **cellular aging.**

IV. DNA is organized in a cell.
A. Prokaryotic cells usually have circular DNA molecules.
B. Eukaryotic chromosomes have several levels of organization.
1. The DNA is associated with **histones** (basic proteins) to form **nucleosomes,** each of which consists of a histone bead with DNA wrapped around it.
2. The nucleosomes are organized into large coiled loops held together by nonhistone **scaffolding proteins.**
3. DNA molecules are much longer than the nuclei or the cells that contain them. The organization of DNA into chromosomes allows the DNA to be accurately replicated and segregated into daughter cells without tangling.

POST-TEST

1. Which of the following inspired Avery and his coworkers to do the experiments that demonstrated that the transforming principle in bacteria is DNA? (a) the fact that A=T and G=C (b) Beadle and Tatum's work on biochemical pathways in *Neurospora* (c) Meselson and Stahl's studies on DNA replication in *E. coli* (d) Griffith's experiments on smooth and rough strains of pneumococcus (e) Hershey and Chase's experiments on the reproduction of bacteriophages
2. Which of the following was commonly thought to be true (by scientists) at the time that Beadle and Tatum began their work on *Neurospora?* (a) enzymes are proteins (b) DNA specifies the structure of proteins (c) DNA replication is semiconservative (d) DNA is a double helix (e) the number of purines in DNA is equal to the number of pyrimidines
3. The statement "DNA replicates by a semiconservative mechanism" means that (a) only one DNA strand is copied (b) first one DNA strand is copied, and then the other strand is copied (c) the two strands of a double helix have identical base sequences (d) some portions of a single DNA strand are old, and other portions are newly synthesized (e) each double helix consists of one old and one newly synthesized strand
4. Multiple origins of replication (a) speed up replication of eukaryotic chromosomes (b) allow the lagging strands and leading strands to be synthesized at different replication forks (c) help to relieve strain as the double helix is unwound (d) prevent mutations (e) are necessary for the replication of a circular DNA molecule in bacteria
5. Topoisomerases (a) synthesize DNA (b) synthesize RNA primers (c) join Okazaki fragments (d) break and rejoin DNA to resolve knots that have formed (e) prevent single DNA strands from joining to form a double helix
6. A phosphate in DNA is (a) hydrogen bonded to a base (b) covalently linked to two bases (c) covalently linked to two deoxyriboses (d) hydrogen-bonded to two additional phosphates (e) covalently linked to a base, a deoxyribose, and another phosphate
7. Which of the following depicts the relative arrangement of the complementary strands of a DNA double helix?
(a) 5′—5′ / 3′—3′ (b) 3′—5′ / 3′—5′ (c) 3′—3′ / 3′—3′ (d) 5′—5′ / 5′—5′ (e) 3′—5′ / 5′—3′
8. A lagging strand is formed by (a) joining primers (b) joining Okazaki fragments (c) joining leading strands (d) breaking up a leading strand (e) joining primers, Okazaki fragments, and leading strands
9. The immediate source of energy for DNA replication is (a) the hydrolysis of nucleoside triphosphates (b) the oxidation of NADPH (c) the hydrolysis of ATP (d) electron transport (e) the breaking of hydrogen bonds
10. A nucleosome consists of (a) DNA and scaffolding proteins (b) scaffolding proteins and histones (c) DNA and histones (d) DNA, histones, and scaffolding proteins (e) histones only

REVIEW QUESTIONS

1. How did the experiments of Avery and coworkers point to DNA as the essential genetic material? Did the Hershey-Chase experiment establish that DNA is the genetic material in all organisms? Did either of these experiments demonstrate how DNA could function as the chemical basis of genes?
2. Sketch the structure of a single strand of DNA. What types of subunits make up the chain? How are they linked?
3. Describe the structure of double-stranded DNA as determined by Watson and Crick.
4. Does a single strand of DNA obey Chargaff's rules? How do Chargaff's rules relate to the structure of DNA?
5. What are some of the mechanical problems encountered in DNA replication? How are they dealt with by the cell?
6. Why is DNA replication continuous for one strand but discontinuous for the other?
7. Compare the structures of a bacterial DNA molecule and a eukaryotic chromosome. What effects do these differences have on replication?
8. Describe how both prokaryotic and eukaryotic cells cope with the large discrepancy between the length of their DNA molecules and the size of the cell or nucleus.

YOU MAKE THE CONNECTION

1. What characteristics must a molecule have if it is to serve as genetic material? What important features of the structure of DNA are consistent with its role as the chemical basis of heredity?
2. In Chapter 10 we discussed the fact that heritable variation is essential for the study of inheritance. What role did mutant strains play in Beadle and Tatum's development of the one gene, one enzyme hypothesis?

RECOMMENDED READINGS

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