GLOBAL EDITION

Genetic Analysis

An Integrated Approach

SECOND EDITION

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Brief Table of Contents

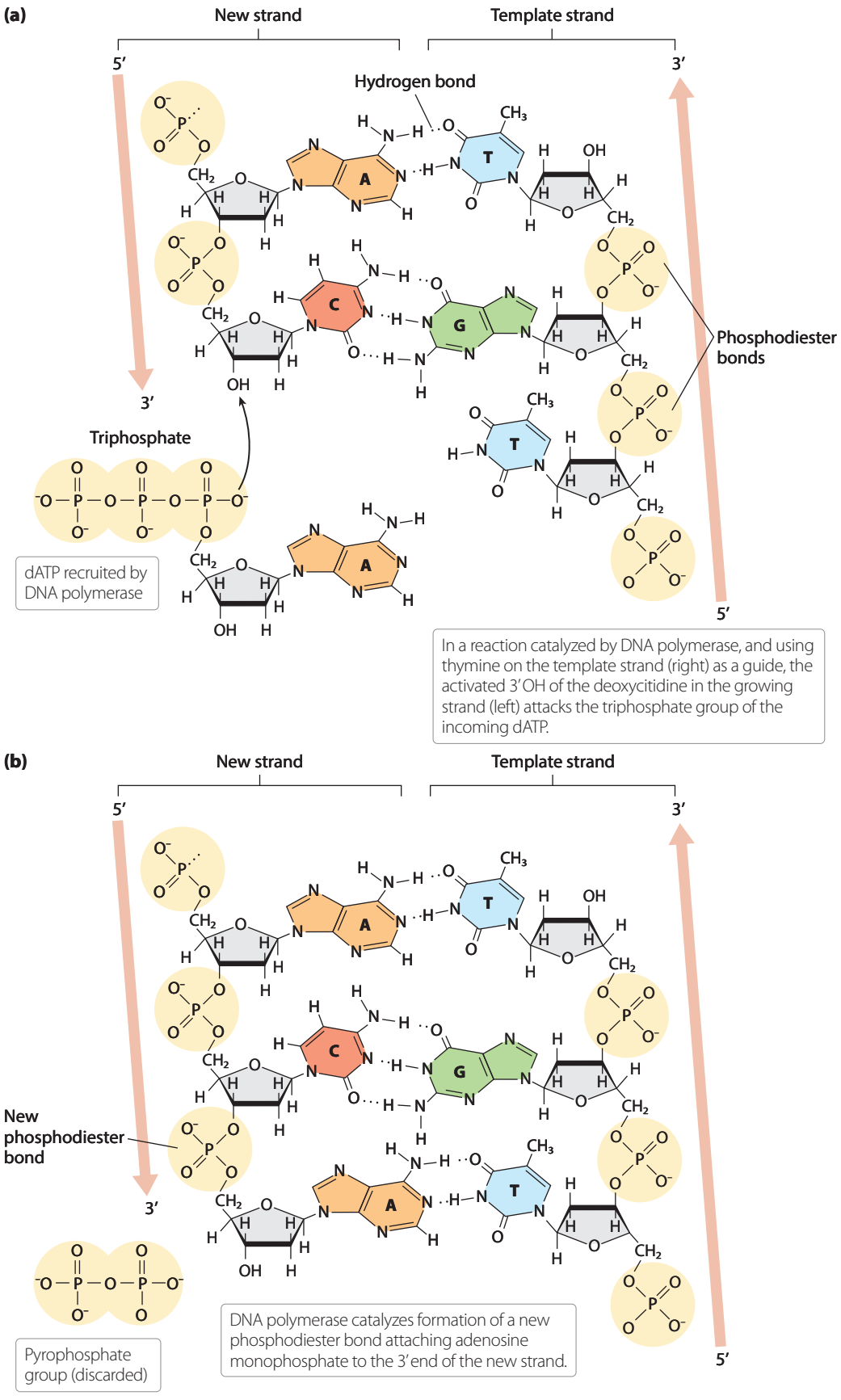

Figure 7.6 **DNA strand elongation.** **(a)** Nucleotides complementary to the template strand are added to the 3′ end of the new strand by DNA polymerase. **(b)** DNA nucleotide triphosphates are recruited by DNA polymerase, which uses catalytic action to remove two phosphates (the pyrophosphate group) and form a new phosphodiester bond.

GENETIC ANALYSIS 7.1

PROBLEM A portion of one strand of a DNA duplex has the sequence 5'-ACGACGCTA-3'.

a. Identify the sequence and polarity of the other DNA strand.

b. Identify the *second* nucleotide added if the sequence given is used as a template for DNA replication.

BREAK IT DOWN: DNA nucleotides in one strand of a duplex are complementary to those in the other, and the strands are antiparallel (p. 264).

BREAK IT DOWN: New DNA synthesis progresses 5′-to-3′ to elongate the newly synthesized strand (p. 264).

Solution Strategies	Solution Steps
Evaluate	
1. Identify the topic this problem addresses, and the nature of the required answer.	1. The question concerns a DNA sequence and requests an answer giving the sequence and polarity of the complementary strand.
2. Identify the critical information given in the problem.	2. The sequence and polarity are given for a portion of one DNA strand.
Deduce	
3. Review the general structure of a DNA duplex and the complementarity of specific nucleotides.	3. DNA is a double helix composed of single strands that contain complementary base pairs (A pairs with T, and G with C). The complementary strands are antiparallel (i.e., one strand is 5′ to 3′, and its complement is 3′ to 5′).
Solve	
4. Identify the sequence of the complementary strand.	4. The complementary sequence is TGCTGCGAT.
5. Give the polarity of the complementary strand.	5. The polarity of the complementary strand is 3′-TGCTGCGAT-5′.
6. Identify the second nucleotide added during DNA replication of the given sequence.	6. The second nucleotide added to the newly synthesized strand is adenine, which is complementary to thymine on the template strand.

TIP: DNA polymerase catalyzes the addition of a new nucleotide to the 3′ end of a growing strand.

For more practice, see Problems 5, 8, 9, 16, and 17.

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Two of the three phosphates of a dNTP are removed (as a pyrophosphate group) during phosphodiester bond formation, leaving the nucleotides of a polynucleotide chain in their monophosphate form. Each polynucleotide chain has a **sugar-phosphate backbone** consisting of alternating sugar and phosphate groups throughout its length.

Complementary DNA Nucleotide Pairing

DNA is most stable as a double helix, and the two polynucleotide strands that make up the duplex have a specific relationship that follows two rules: (1) the arrangement of the nucleotides is such that the nucleotide bases of one strand are *complementary* to the corresponding nucleotide bases on the second strand (A pairs with T and G pairs with C), and (2) the two strands are *antiparallel* in orientation (if one strand is, for example 5′-ATCG-3′, then the complementary strand is 3′-TAGC-5′).

Complementary base pairing joins a purine nucleotide on one strand to a pyrimidine nucleotide on the other. The chemical basis of such pairing is the formation of a stable number of hydrogen (H) bonds between the bases of the different strands. Hydrogen bonds are noncovalent bonds that form between the partial charges that are associated with the hydrogen, oxygen, and nitrogen atoms of nucleotide bases. As Figure 7.6 shows, two stable hydrogen bonds form for each A-T base pair, and three hydrogen bonds are formed by each G-C base pair (see also Figure 1.6, p. 40).

Antiparallel strand orientation is essential to the formation of stable hydrogen bonds. In Figure 7.6, notice that the nucleotides in one strand are oriented with their 5′ carbon toward the top and their 3′ carbon toward the bottom. The complementary nucleotides in the other strand are antiparallel; that is, their 5′-to-3′ orientations run in the *opposite* direction. Antiparallel orientation of complementary strands brings the partial charges of complementary nucleotides into alignment to form hydrogen bonds. If complementary strands were to align in parallel (i.e., with their 5′ and 3′ carbons facing in the same direction), the charges of complementary nucleotides would repel, and no hydrogen bonds would form. **Genetic Analysis 7.1** explores relationships between complementary DNA strands.

The Twisting Double Helix

The DNA double helix has an axis of helical symmetry, an imaginary line that passes lengthwise through the core of the double helix and marks the center of the molecule.

The molecular dimensions of DNA are measured using the unit called an angstrom (Å) or in nanometers (nm). One angstrom is equal to 10^{-10} meters, or 1 ten-billionth of a meter, and 1 nm equals one-billionth of a meter, or 10^{-9} meters. In DNA, the distance from the axis of symmetry to the outer edge of the sugar-phosphate backbone is 10 Å (1 nm), and the molecular diameter is 20 Å (2 nm) at any point along the length of the helix (**Figure 7.7a**). The 20-Å molecular diameter results from complementary pairing of each purine with the complementary pyrimidine (A with T, G with C) and gives each base pair the same dimension.

Nucleotide base pairs are spaced at intervals of 3.4 Å along DNA duplexes. This tight packing of DNA bases in the duplex leads to **base stacking,** the offsetting of adjacent base pairs so that their planes are parallel, and imparts a twist to the double helix. Figure 7.7a shows that one complete helical turn spans 34 Å. This span is occupied by approximately 10.5 base pairs. **Figure 7.7b** is a space-filling model that illustrates base-pair stacking and the twisting of the sugar-phosphate backbones. **Figure 7.7c** is a ball-and-stick model illustrating how base pairs twist around the axis of symmetry to create the helical spiral.

Base-pair stacking creates two grooves in the double helix, gaps between the spiraling sugar-phosphate backbones that partially expose the nucleotides. The alternating grooves, known as the **major groove** and **minor groove,** are highlighted in Figures 7.7b and 7.7c. The major groove is approximately 12 Å wide, and the minor groove is approximately 6 Å wide. The major and minor grooves are regions where DNA-binding proteins can most easily make direct contact with nucleotides along one or both strands of the double helix. In this chapter and in later chapters, we discuss many of the important functions DNA-binding proteins perform, such as regulating the initiation of transcription and controlling the onset and progression of DNA replication. Most of these functions depend on the presence of characteristic sequences of DNA nucleotides. DNA-binding proteins gain access to DNA nucleotides in major and minor grooves of the molecule.

The models of the DNA double helix presented in Figure 7.7 illustrate the most common and most stable form of DNA, known as B-form DNA, which has a right-handed twisting of the sugar-phosphate backbone. B-form DNA is overwhelmingly the most common DNA structure in organisms. Two other rarer and less stable forms of the DNA double helix have also been identified. A-form DNA is more compact than B-form DNA, with about 11 base pairs per complete helical twist and a higher degree of tilt of the base pairs relative to the

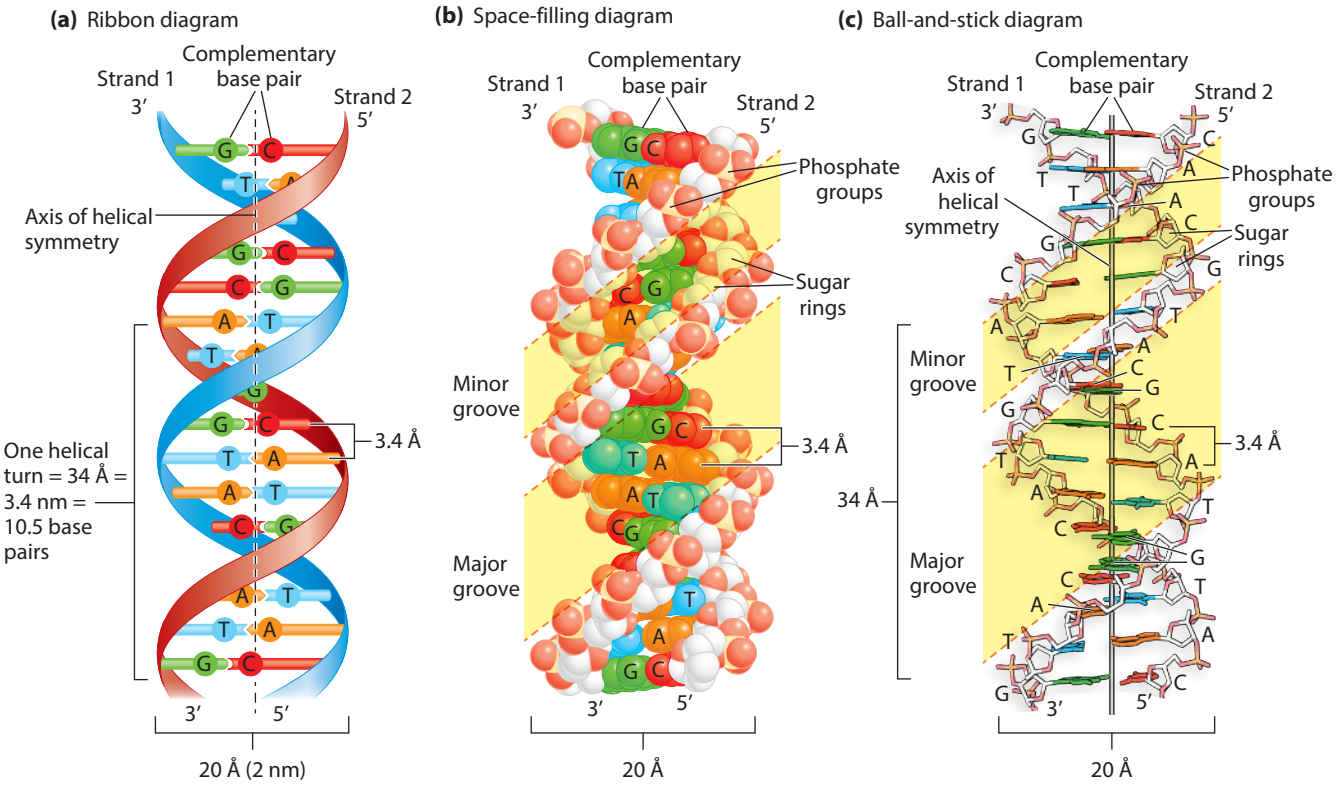

Figure 7.7 **The DNA double helix.** **(a)** Ribbon diagram, **(b)** space-filling diagram, and **(c)** ball-and-stick diagram show the sugar-phosphate backbones, base pairs, major and minor grooves, and dimensions of the DNA duplex.

backbone. A-form DNA is occasionally detected in cells. The third form of DNA, Z-form DNA, is quite different from A-form and B-form DNA. Z-form DNA has a left-handed twist that gives the sugar-phosphate backbone a zigzag appearance—hence the name Z-form. Z-form DNA occurs in the presence of a high concentration of positively charged ions. Only a tiny portion of total cellular DNA is ever in the Z form, and its physiological significance in cells is not known.

7.3 DNA Replication Is Semiconservative and Bidirectional

Given the role of DNA as an information repository and an information transmitter, the integrity of the nucleotide sequence of DNA is of paramount importance. Each time DNA is copied, the new version must be a precise duplicate of the original version. The high fidelity of DNA replication is essential to reproduction and to the normal development of biological structures and functions. Without faithful DNA replication, the information of life would become hopelessly garbled by rapidly accumulating mutations that would threaten survival.

Considering the importance of DNA throughout the biological world, it was no surprise to discover that the general mechanism of DNA replication is the same in all organisms. This universal process evolved in the earliest life-forms and has been retained for billions of years. As organisms diverged and became more complex, however, an array of differences did develop among DNA replication proteins and enzymes. Despite the diversification of these specific components of DNA replication, three attributes of DNA replication are shared by all organisms:

1. Each strand of the parental DNA molecule remains intact during replication.
2. Each parental strand serves as a template directing the synthesis of a complementary, antiparallel daughter strand.
3. Completion of DNA replication results in the formation of two identical daughter duplexes, each composed of one parental strand and one daughter strand.

As we describe DNA replication in bacteria, archaea, and eukaryotes in following sections, we will point out similarities and differences among the domains. The shared features of DNA replication are present because all life evolved from a common origin. At the same time, the differences in DNA replication between the domains are also the result of evolution, which favored specific adaptations.

Three Competing Models of Replication

In their famous 1953 paper describing the structure of DNA, Watson and Crick concluded with the observation

> It has not escaped our notice that the specific base-pairing we have proposed immediately suggests a possible copying mechanism for the genetic material.

Specifically, Watson and Crick recognized that a consequence of complementary base pairing was that nucleotides on one strand of the duplex could be used to identify the nucleotides of the other strand. Watson and Crick presumed that DNA replication used the nucleotide sequence of each strand to form a new pair of DNA duplexes, hypothesizing that each DNA strand of the original duplex would act as a template for the synthesis of a new daughter strand. Watson and Crick did not know the precise mechanism by which template-based replication took place, however, raising the crucial question of what the exact mechanism of replication might be.

Almost immediately after the DNA structure was identified, three competing models of DNA replication emerged (**Figure 7.8**). The models shared the idea that the two original strands (the parental strands) of the duplex act as templates to direct the assembly of newly synthesized DNA by complementary base pairing. The models also predicted that the completion of DNA replication produced two identical DNA duplexes (daughter duplexes). The models differed, however, in describing the makeup of the daughter duplexes. The ❶ **semiconservative DNA replication** model—which proved to be correct—proposed that each daughter duplex contains one original parental strand of DNA and one complementary, newly synthesized daughter strand. The ❷ **conservative DNA replication** model predicts that one daughter duplex contains the two strands of the parental molecule and the other contains two newly synthesized daughter strands. Lastly, the ❸ **dispersive DNA replication** model predicts that each daughter duplex is a composite of interspersed parental duplex segments and daughter duplex segments.

The Meselson-Stahl Experiment

In 1958, Matthew Meselson and Franklin Stahl took advantage of the newly developed method of high-speed cesium chloride (CsCl) density gradient ultracentrifugation to decipher the mechanism of DNA replication in an experiment of beautiful simplicity. In this analytical method, a tube filled with a CsCl mixture is subjected to high ultracentrifuge speeds that exert thousands of gravities of separating force, creating a graded variation in density—a density gradient—throughout the CsCl mixture. When substances are placed in the CsCl gradient and ultracentrifugation

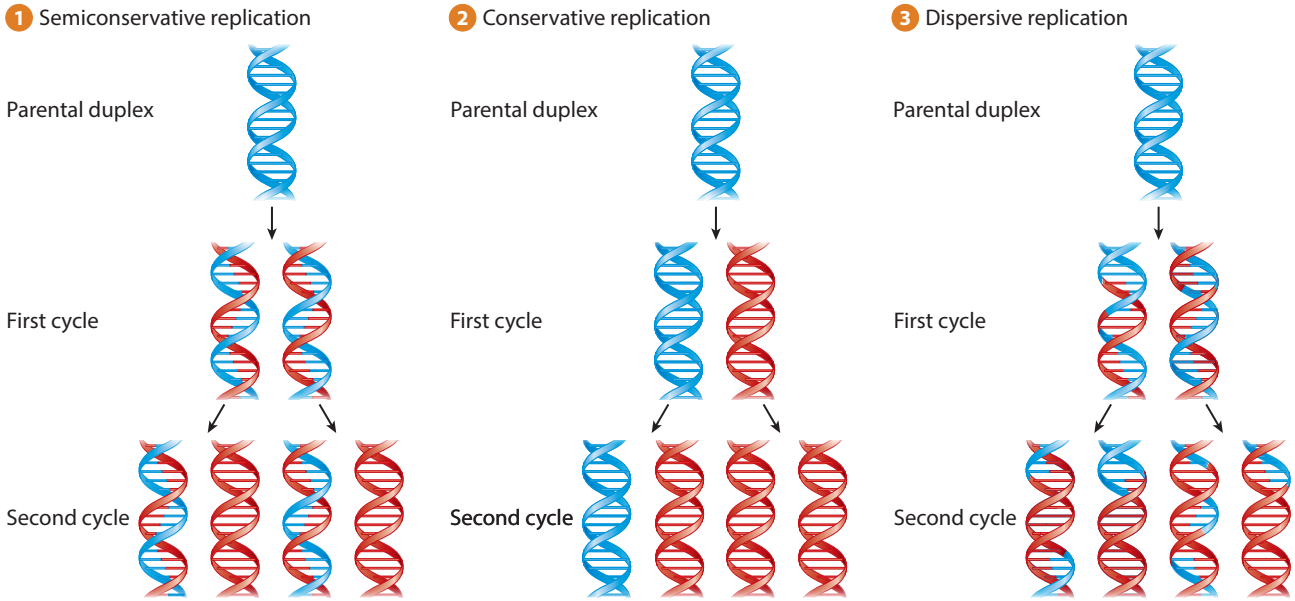

Figure 7.8 Three proposed mechanisms of DNA replication tested by Meselson and Stahl. The results expected for two cycles of DNA replication are shown for each model.

takes place, the substances migrate until they reach the point in the density gradient where their molecular density is matched by that of the gradient. Migration stops at that point. This technique is capable of separating molecules that have only slightly different molecular weights.

Meselson and Stahl began their experiment by growing *Escherichia coli* in a growth medium containing the rare heavy isotope of nitrogen, ^{15}N, for many generations. Under these growth conditions, parental DNA is fully saturated with heavy-isotope-containing nitrogen. All the DNA duplexes contain only the heavy nitrogen isotope, and they are designated $^{15}N/^{15}N$ to signify the incorporation of ^{15}N in both strands of the duplex. (By the same token, a DNA duplex composed of two strands containing only ^{14}N, the normal isotope of nitrogen, is designated $^{14}N/^{14}N$, and a duplex with one strand containing each isotope is designated $^{15}N/^{14}N$.) DNA collected for CsCl gradient analysis from this starting generation, designated generation 0, was exclusively $^{15}N/^{15}N$. Next, some of these ^{15}N-labeled *E. coli* were transferred to a new growth medium containing only the normal light isotope of nitrogen, ^{14}N. At the end of each successive DNA replication cycle, DNA was collected from a few cells on the ^{14}N medium for CsCl analysis. Growth in this medium leads to the incorporation of DNA nucleotides containing the light isotope into newly synthesized strands.

Figure 7.9 shows the results of CsCl gradient analysis of DNA collected from three replication cycles, beginning with generation 0. The experimental results are consistent with the semiconservative model only. The conservative model predicted DNA molecules with two distinct densities after generation 1 ($^{15}N/^{15}N$ and $^{14}N/^{14}N$). The results reject this model. Similarly, the dispersive model predicted a single DNA density in all generations. The generation 2 results reject this replication model. The data are consistent with the predictions of the semiconservative model of DNA replication through generation 3 shown and beyond. Within a few years of Meselson and Stahl's identification of semiconservative replication in bacteria, the mechanism was identified experimentally in eukaryotes as well, solidifying the idea that all life shares the same general process of DNA replication, as a consequence of life's single origin and the evolutionary connections among living things.

Origin and Directionality of Replication in Bacterial DNA

Solving the riddle of the basic mechanism of DNA replication introduced new questions about how replication is initiated and how it progresses. Does replication commence at specific points on each chromosome? If so, how many such points does a chromosome have? Does DNA replication progress in one direction or in both directions from a replication origin? Experimental evidence clearly demonstrates that DNA replication is most often **bidirectional,** progressing in both directions from a single **origin of replication** in bacterial chromosomes and from multiple origins of replication in eukaryotic chromosomes.

In 1963, John Cairns reported the first evidence of a single origin of DNA replication in *E. coli.* Based on Cairn's evidence, it appeared that once replication gets underway in bacteria, there is expansion around the origin of replication, forming a **replication bubble,** as seen in Figure 7.10. The image shown in the figure is similar to the type of result Cairns obtained, but by itself, it did not allow a determination as to whether replication takes place in one direction away from the origin (unidirectional) or

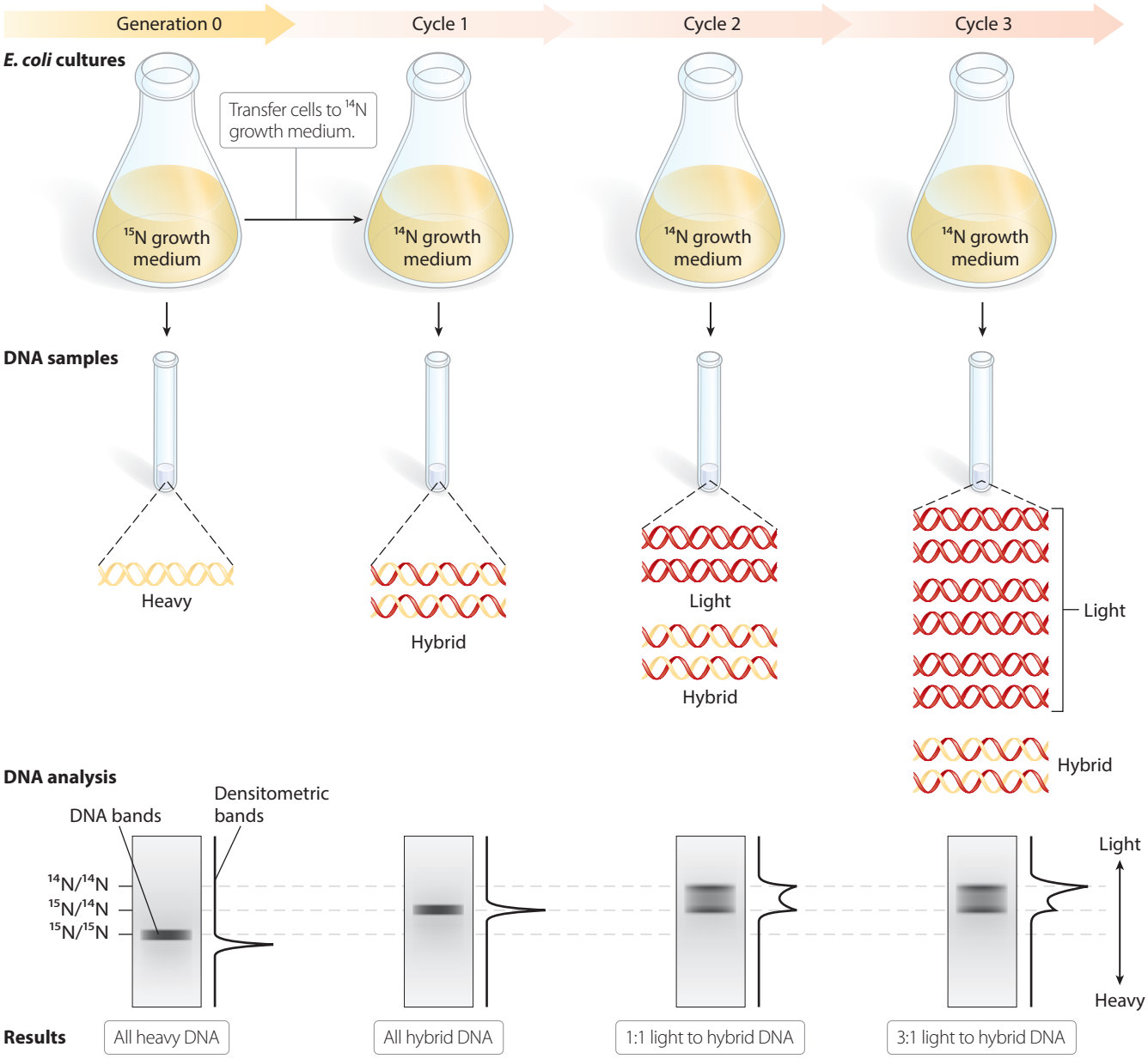

Figure 7.9 **The Meselson-Stahl experimental results.** Photographs of DNA bands in centrifuge tubes and densitometry scans (lower) identify the duplex DNA composition at each stage and are consistent only with semiconservative DNA replication. The semiconservative replication process is interpreted for each replication cycle.

in both directions (bidirectional). The resolution of this uncertainty held important implications. If DNA replicated bidirectionally, the time required to replicate a bacterial chromosome would be, give or take, about half that required if replication were unidirectional.

The replication bubble is where active DNA replication takes place. If replication were unidirectional, the origin of replication would eventually also serve as the terminus of replication, once the process was completed around the circumference of the circular bacterial chromosome. If, on the other hand, replication were bidirectional. Bidirectionality of replication would also mean that each end of the replication bubble would contain a **replication fork** where new DNA nucleotides are added to elongating daughter strands. Furthermore, bidirectional replication would also mean that because the growth of the replication bubble progresses in both directions from the origin of replication, the terminus of replication would be halfway around the chromosome from the origin of replication. In contrast, unidirectional replication would mean that the origin and the terminus were at the same location.

In 1968, Joel Huberman and Arthur Riggs used a technique called pulse-chase labeling to produce the first experimental evidence of bidirectional replication in mammalian chromosomes (Figure 7.11). In pulse-chase labeling experiments, cells are exposed alternately to high levels of a