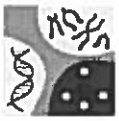
Molecular Genetics



A. History of DNA

Until now, we have looked at genes as abstract entities that somehow control hereditary traits. Through purely genetic analysis, we have studied the inheritance of different genes. But what about the physical nature of the gene? This question puzzled scientists for many years until it was realized that genes are composed of **deoxyribonucleic acid** (DNA) and that DNA has a fascinating structure.

By the 1940's, scientists knew that chromosomes carry hereditary material and consist of DNA and protein. Most researchers thought protein was the genetic material because:

- Proteins are macromolecules with great genetic variation and functional specificity.
- Little was known about nucleic acids.
- The physical and chemical properties of DNA seemed too uniform to account for the multitude of inherited traits.

The interpretation of the **structure** of DNA in **1953** by **James Watson** and **Francis Crick** was one of the most exciting discoveries in the history of genetics. It paved the way for the understanding of gene action and heredity in molecular terms. Before we look at how the solution of DNA structure was achieved, let's review what was known about genes and DNA at the time that Watson and Crick began their historic collaboration:

- Genes – the hereditary "factors" described by Mendel – were known to be associated with specific character traits, but their physical nature was not understood.
- The **one-gene – one-enzyme** theory (described more fully later) postulated that genes control the structure of proteins.
- Genes were known to be carried on chromosomes.
- The chromosomes were found to consist of DNA and protein.
- Research by **Fredrick Griffith** in **1928** and subsequently, by **Oswald Avery** and his coworkers in **1944**, pointed to DNA as the genetic material. These experiments showed bacterial cells that express one phenotype can be **transformed** into cells that express a different phenotype and that the transforming agent is DNA.

B. Evidence that DNA can transform bacteria

In 1928, Frederick Griffith performed experiments which provided evidence that genetic material is a specific molecule.

Griffith was trying to find a vaccine against *Streptococcus pneumoniae*, a bacterium that causes pneumonia in mammals. He knew that:

- There are two distinguishable strains of the *pneumococcus*: one produces smooth colonies (S) and the other rough colonies (R).
- Cells of the smooth strain are encapsulated with a polysaccharide coat and cells of the rough strain are not.
- These alternative phenotypes (S and R) are inherited.

Griffith performed four sets of experiments:

Experiment: Griffith injected live S strain of *Streptococcus pneumoniae* into mice.
Results: Mice died of pneumonia.
Conclusions: Encapsulated strain is pathogenic.

Experiment: Mice were injected with live R strain.
Results: Mice survived and were healthy.
Conclusions: The bacterial strain lacking the polysaccharide coat was non-pathogenic.

Experiment: Mice were injected with heat-killed S strain of *pneumococcus*.
Results: Mice survived and were healthy.
Conclusions: Polysaccharide coat does not cause pneumonia because it is still present in heat-killed bacteria which proved to be non-pathogenic.

Experiment: Heat-killed S cells mixed with live R cells were injected into mice.
Results: Mice developed pneumonia and died. Blood samples from dead mice contained live S cells.
Conclusions: R cells had acquired from the dead S cells the ability to make polysaccharide coats. Griffith cultured S cells from the dead mice. Since the dividing bacteria produced encapsulated daughter cells, he concluded that this newly acquired trait was inheritable. This phenomenon is now called **transformation.**

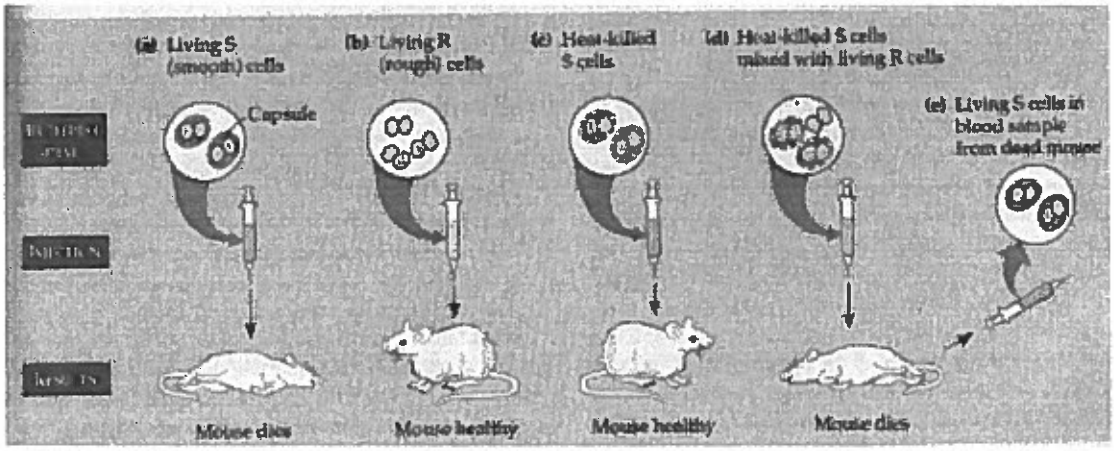


FIGURE 18.1 • Transformation of bacteria. Griffith discovered that (a) the S strain of the bacterium *Streptococcus pneumoniae*, which was protected from a mouse's defensive system by a capsule, was pathogenic; (b) the R strain, a mutant lacking the capsule, was nonpathogenic; (c) heat-killed S cells were harmless; but (d) a mixture of heat-killed S cells and live R cells caused pneumonia and death. (e) Live S bacteria could be retrieved from the dead mice that had been injected with the mixture. Griffith concluded that molecules from the dead S cells had genetically transformed some of the living R bacteria into S bacteria.

Campbell, 5th Edition, pg. 279.

What was the chemical nature of the transforming agent?

- Griffith was unable to answer this question, but other scientists continued the search.
- Griffith's experiments hinted that protein is not the genetic material. Heat denatures protein, yet it did not destroy the transforming ability of the genetic material in the heat-killed S cells.
- In 1944, after a decade of research, Oswald **Avery**, Maclyn **McCarty** and Colin **MacLeod** discovered that the transforming agent had to be DNA.

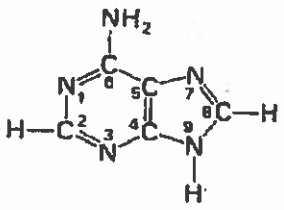
Once most biologists were convinced that DNA was the genetic material, a race was underway to determine how the structure of DNA could account for its role in inheritance.

C. The Structure of DNA

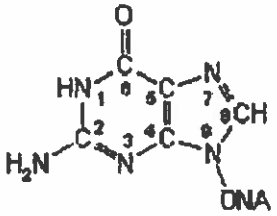
Although the DNA structure was not known, the basic building blocks of DNA had been known for many years. The basic elements of DNA had been isolated and determined by partly breaking up purified DNA.

These studies showed that:

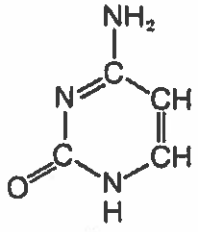
- DNA is composed of only four basic molecules called **nucleotides**, which are identical except that each contains a different nitrogen base.
- Each nucleotide contains **phosphate**, **sugar** (of the **deoxyribose** type) and one of the four bases.
- The four bases are **adenine** (A), **guanine** (G), **cytosine** (C) and **thymine** (T).



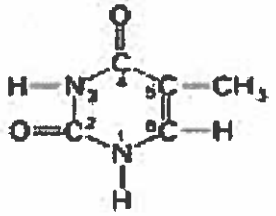
Adenine (A)



Guanine (G)



Cytosine (C)



Thymine (T)

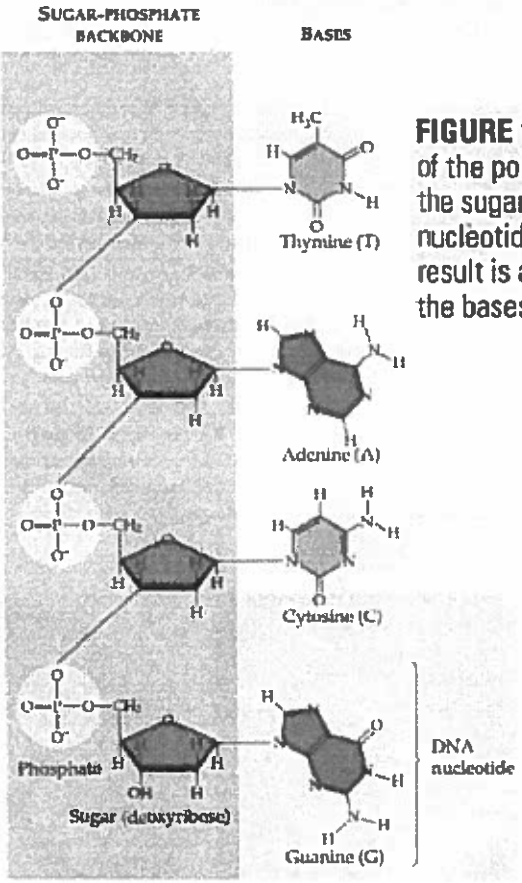


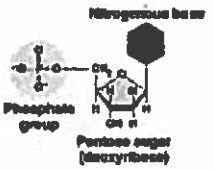
FIGURE 16.3 • The structure of a DNA strand. Each nucleotide unit of the polynucleotide chain consists of a nitrogenous base (T, A, C, or G), the sugar deoxyribose, and a phosphate group. The phosphate of one nucleotide is attached to the sugar of the next nucleotide in line. The result is a "backbone" of alternating phosphates and sugars, from which the bases project.

Campbell, 5th Edition, pg. 281.

Terms to Review

Nucleic Acid: A polymer consisting of many nucleotide monomers; serves as a blueprint for proteins, and, through the actions of proteins, for all cellular activities. The two types are DNA and RNA.

Nucleotide: The building block of a nucleic acid, consisting of a five carbon sugar covalently bonded to a nitrogenous base and a phosphate group.



Covalent Bond: A type of strong chemical bond in which two atoms share one pair of electrons.

Purines: nitrogenous bases with two organic rings.

Example. adenine and guanine

Pyrimidines: nitrogenous bases with a single organic ring.

Example. cytosine and thymine

NOTE: Purines are about twice as wide as pyrimidines.

D. Discovery of the Double Helix

By the beginning of the 1950s, since the arrangement of covalent bonds in a nucleic acid polymer was well established, the competition focused on discovering the **three dimensional structure of DNA.**

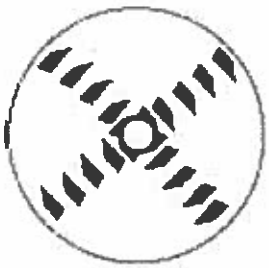
Among scientists working on the problem were:

Linus Pauling – California Institute of Technology
Maurice Wilkins and **Rosalind Franklin** – King's College in London
James D. Watson (American) and **Francis Crick** – Cambridge University

How did Watson and Crick deduce the structure of DNA?

James Watson went to Cambridge to work with Francis Crick who was studying protein structure with X-ray crystallography.

- Watson saw a photo of DNA produced by **Rosalind Franklin**. Watson and Crick deduced from Franklin's X-ray data that:



a. DNA is a **helix** with a uniform width of **2 nm**. This width suggested that it had two strands.
b. Purine and pyrimidine bases are stacked 0.34 nm apart.
c. The helix makes **one full turn** every **3.4 nm** along its length.
d. There are ten layers of nitrogenous base pairs in each turn of the helix.

Watson and Crick built scale models of a double helix that would conform to the X-ray data and the known chemistry of DNA.

- One of their unsuccessful attempts placed the sugar-phosphate chain inside the molecule.
- Watson next put the sugar-phosphate chains on the outside which allowed the more hydrophobic nitrogenous bases to swivel to the interior away from the aqueous medium.
- Their proposed structure is a **ladder-like molecule** twisted into a spiral, with sugar-phosphate backbones as uprights and pairs of nitrogenous bases as rungs.
- The two sugar-phosphate backbones of the helix are **antiparallel** – they run in opposite directions.

Watson and Crick finally solved the problem of DNA structure be proposing that there is a specific pairing between nitrogenous bases. After considering several arrangements, they concluded:

- To be consistent with a 2 nm width, a purine on one strand must pair with a pyrimidine on the other.
- Base structure dictates which pairs of bases can hydrogen bond. The **base pairing rule** is that **adenine** can only pair with **thymine**, and **guanine** with **cytosine**.

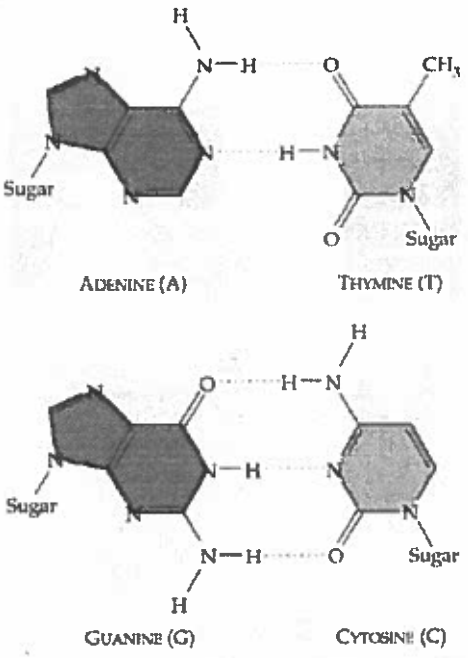


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FIGURE 16.6 • Base pairing in DNA. The pairs of nitrogenous bases in a DNA double helix are held together by hydrogen bonds as shown here.

- The base-pairing rule is significant because:
  - Since adenine (A) must pair with thymine (T), their amounts in a given DNA molecule will be **about the same**. Similarly, the amount of guanine (G) equals the amount of cytosine (C).
  - It suggests the **general mechanism for DNA replication**. If bases form specific pairs, the information on one strand complements that along the other.
  - It dictates the combination of **complementary base pairs**, but places no restriction on the linear sequence of nucleotides along the length of a DNA strand.
    - The sequence of bases can be highly variable which makes it suitable for coding genetic information.
  - Though hydrogen bonds between paired bases are weak bonds, collectively they stabilize the DNA molecule.

FIGURES OF THE DNA MOLECULE.

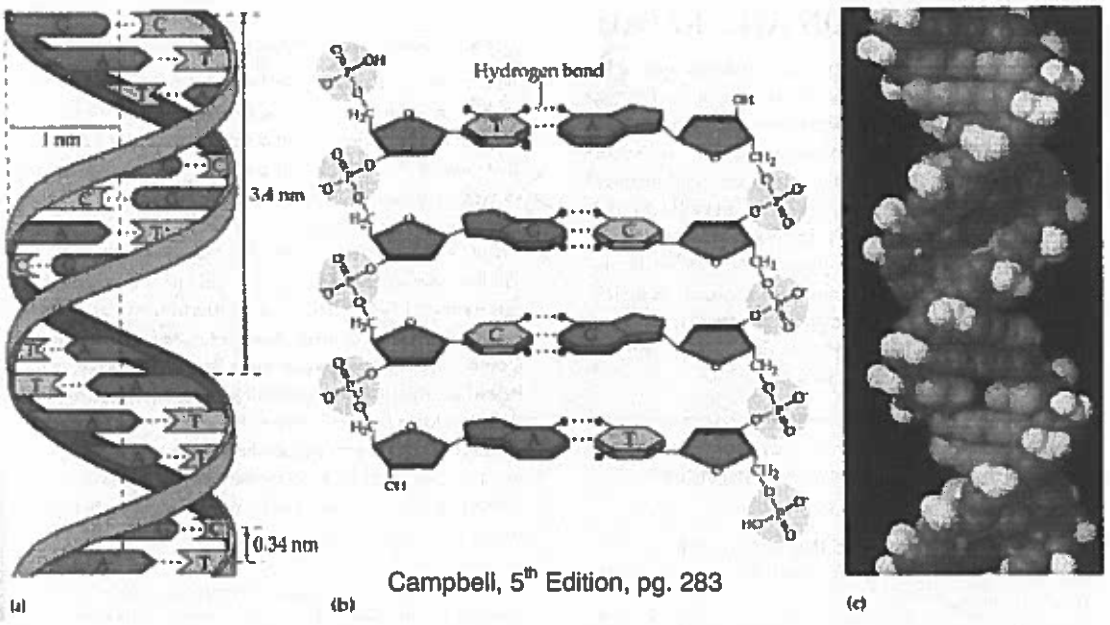

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FIGURE 16.5 • The double helix. **(a)** The "ribbons" in this diagram represent the sugar-phosphate backbones of the two DNA strands. The helix is "right-handed," curving up to the right. The two strands are held together by hydrogen bonds (dotted lines) between the nitrogenous bases, which are paired in the interior of the double helix. **(b)** Partial chemical structure, with the two strands untwisted. Notice that the strands are oriented in opposite directions. **(c)** The tight stacking of the base pairs is clear in this computer model. Van der Waals attractions between the stacked pairs play a major role in holding the molecule together.

The Watson-Crick Model of DNA (1953)

Sugar-Phosphate backbone (two; outside)

Nitrogenous base-pairs (inside)

A + T

G + C

Held together by hydrogen bonds (H-bonds)

(after Klug & Cummings 1997)

E. DNA Replication

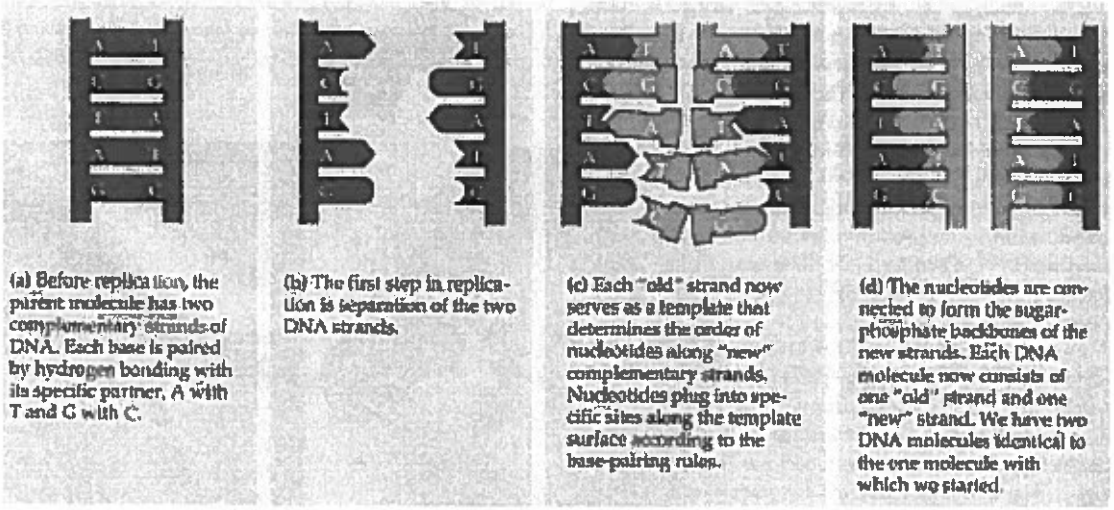
In April 1953, Watson and Crick's new model for DNA structure, the double helix, was published in the British journal Nature. This model of DNA structure suggested a *templace* mechanism for DNA replication.

DNA replication happens during **Interphase** (specifically **S phase**) in the cell cycle. The general mechanism for DNA replication is conceptually simple, but the actual process:

- *Is complex.* The helical molecule must untwist while it copies its two antiparallel strands simultaneously. This requires the cooperation of over a dozen enzymes and other proteins.
- *Is extremely rapid.* It takes only a few hours to copy the 6 billion bases of a single human cell.
- *Is accurate.* Only about one in a billion nucleotides is incorrectly paired.

Watson and Crick proposed that during DNA replication:

1. The two DNA strands separate (double-stranded DNA "*unzips*"). The hydrogen bonds between A-T and C-G weaken and the DNA splits into two strands.
2. Each strand is a template for assembling a complementary strand.
3. Nucleotides line up singly along the template strand in accordance with the complementary base-pairing rules (A-T and G-C).
4. Enzymes (**polymerases**) link the nucleotides together at their sugar-phosphate groups.
5. Errors are fixed – other enzymes check for mistakes in the replication then mistakes are *snipped out* (by yet other enzymes) and fixed by **polymerase**.



(a) Before replication, the parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.

(b) The first step in replication is separation of the two DNA strands.

(c) Each "old" strand now serves as a template that determines the order of nucleotides along "new" complementary strands. Nucleotides plug into specific sites along the template surface according to the base-pairing rules.

(d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each DNA molecule now consists of one "old" strand and one "new" strand. We have two DNA molecules identical to the one molecule with which we started.

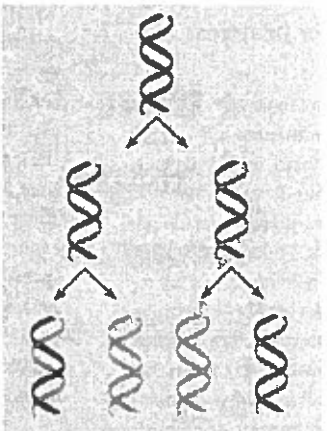
FIGURE 16.7 • A model for DNA replication: the basic concept. In this simplification, a short segment of DNA has been untwisted to convert the double helix to a two-dimensional version of the molecule that resembles a ladder. The rails of the ladder are the sugar-phosphate backbones of the two DNA strands. The rungs are the pairs of nitrogenous bases. Simple shapes are used to symbolize the four kinds of bases. Dark blue represents DNA strands originally present in the parent cell. Newly synthesized DNA is represented by light blue.

What is semiconservative DNA replication?

Watson and Crick's model is a **semiconservative** model for DNA replication.

- They predicted that when a double helix replicates, each of the two daughter molecules will have one old or conserved strand from the parent molecule and one newly created strand.

- In the late 1950s, Matthew Meselson and Franklin Stahl provided the experimental evidence to support the semiconservative model of DNA replication.



(b) **Semiconservative model:** The two strands of the parental molecule separate, and each functions as a template for synthesis of a new complementary strand.

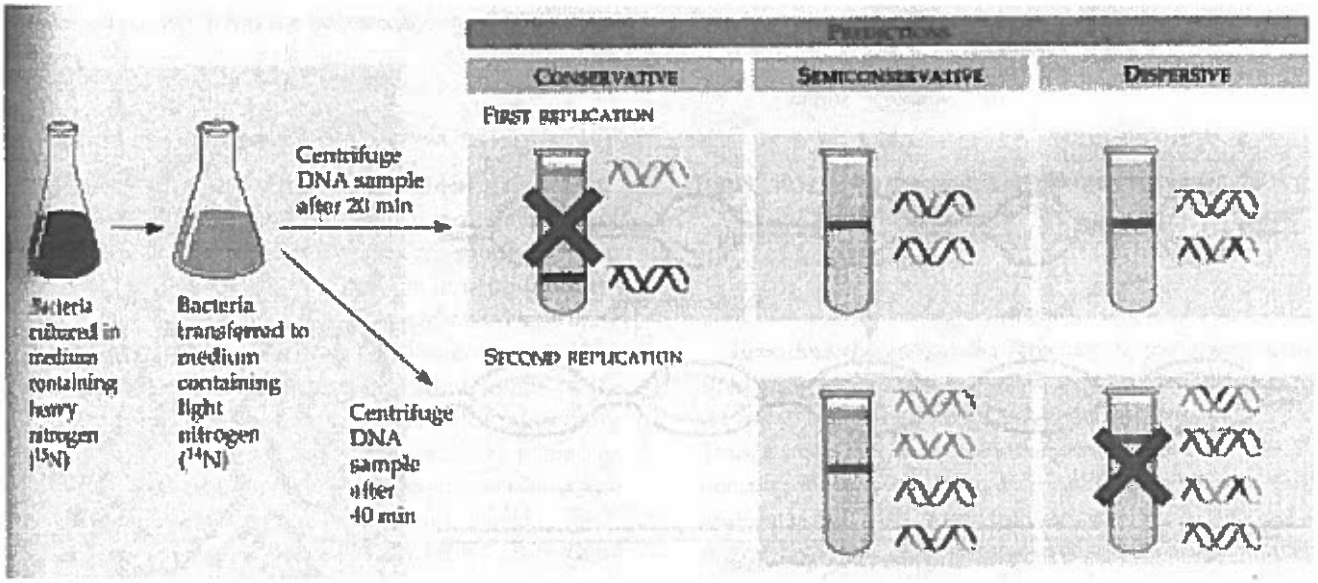


FIGURE 16.9 • The Meselson-Stahl experiment tested three hypotheses of DNA replication. Meselson and Stahl cultured *E. coli* for several generations on a medium containing a heavy isotope of nitrogen, ^{15}N. The bacteria incorporated the heavy nitrogen into their nucleotides and then into their DNA. The scientists then transferred the bacteria to a medium containing ^{14}N, the lighter, more common isotope of nitrogen. Thus, any new DNA that the bacteria synthesized would be lighter than the "old" DNA made in the ^{15}N medium. Meselson and Stahl could distinguish DNA of different densities by centrifuging DNA extracted from the bacteria. The centrifuge tubes in this drawing represent the results predicted by the three hypotheses in FIGURE 16.8. The first replication in the ^{14}N medium produced a band of hybrid (^{15}N-^{14}N) DNA. This result eliminated the conservative hypothesis. A second replication produced both light and hybrid DNA, a result that eliminated the dispersive hypotheses and supported the semiconservative hypothesis.