Chapter 16

The Molecular Basis of Inheritance

Key Concepts

16.1 DNA is the genetic material

16.2 Many proteins work together in DNA replication and repair

Framework

This chapter outlines the key evidence that was gathered to establish DNA as the molecule of inheritance. Watson and Crick's double helix, with its rungs of specifically paired nitrogenous bases and twisting side ropes of phosphate and sugar groups, provided the three-dimensional model that explained DNA's ability to encode a great variety of information and produce exact copies of itself through semiconservative replication. The replication of DNA is an extremely fast and accurate process involving many enzymes and proteins.

Chapter Review

Deoxyribonucleic acid, or DNA, is the genetic material. Nucleic acids' ability to direct their own replication allows for the precise copying and transmission of DNA to all the cells in the body and from one generation to the next. DNA encodes the blueprints that direct and control the biochemical, anatomical, physiological, and behavioral traits of organisms.

16.1 DNA is the genetic material

***The Search for the Genetic Material:* Scientific Inquiry**

Chromosomes were shown to carry hereditary information and to consist of proteins and DNA. Until the 1940s, most scientists believed that proteins were the genetic material because of their specificity and heterogeneity. The role of DNA in heredity was first established through work with microorganisms—bacteria and viruses.

In 1928 F. Griffith was working with two strains of *Streptococcus pneumoniae.* When he mixed the remains of heat-killed pathogenic bacteria with harmless bacteria, some bacteria were changed into disease-causing bacteria. These bacteria had incorporated external genetic material in a process called **transformation,** which results in a change in genotype and phenotype.

O. Avery worked for more than a decade to identify the transforming agent by purifying chemicals from heat-killed pathogenic cells. In 1944 Avery, McCarty, and MacLeod announced that DNA was the molecule that transformed the bacteria.

Viruses consist of little more than DNA, or sometimes RNA, contained in a protein coat. They reproduce by infecting a cell and commandeering that cell's metabolic machinery. **Bacteriophages,** or **phages,** are viruses that infect bacteria. In 1952 A. Hershey and M. Chase showed that DNA was the genetic material of a phage known as T2 that infects the bacterium *Escherichia coli (E. coli).*

E. Chargaff, in 1947, reported that the ratio of nitrogenous bases in the DNA from various organisms was species specific. Chargaff also determined that the number of adenines and thymines was approximately equal, and the number of guanines and cytosines was also equal in the DNA from all the organisms he studied. The A=T and G=C properties of DNA became known as *Chargaff's rules.*

Circumstantial evidence that DNA is the genetic material came from the observation that a eukaryotic cell doubles its DNA content prior to mitosis and that diploid cells have twice as much DNA as haploid gametes of the same organism.

■ INTERACTIVE QUESTION 16.1

Hershey and Chase devised an experiment using radioactive isotopes to determine whether the phage's DNA or protein entered the bacteria and was the genetic material of T2 phage.

a. How did they label phage protein?

b. How did they label phage DNA?

Separate samples of *E. coli* were infected with the differently labeled T2 cells, then blended and centrifuged to isolate the bacterial cells from the lighter viral particles.

c. Where was the radioactivity found in the samples with labeled phage protein?

d. In the samples with labeled phage DNA?

e. What did Hershey and Chase conclude from these results?

Building a Structural Model of DNA: **Scientific Inquiry**

By the early 1950s, the arrangement of covalent bonds in a nucleic acid polymer was established, but the three-dimensional structure of DNA was yet to be determined.

In X-ray crystallography, an X-ray beam passed through a substance produces an X-ray diffraction photo with a pattern of spots that a crystallographer interprets as information about three-dimensional molecular structure. J. Watson saw an X-ray photo produced by R. Franklin that indicated the helical shape of DNA. He deduced that the width of the helix suggested that it consisted of two strands, thus the term **double helix.**

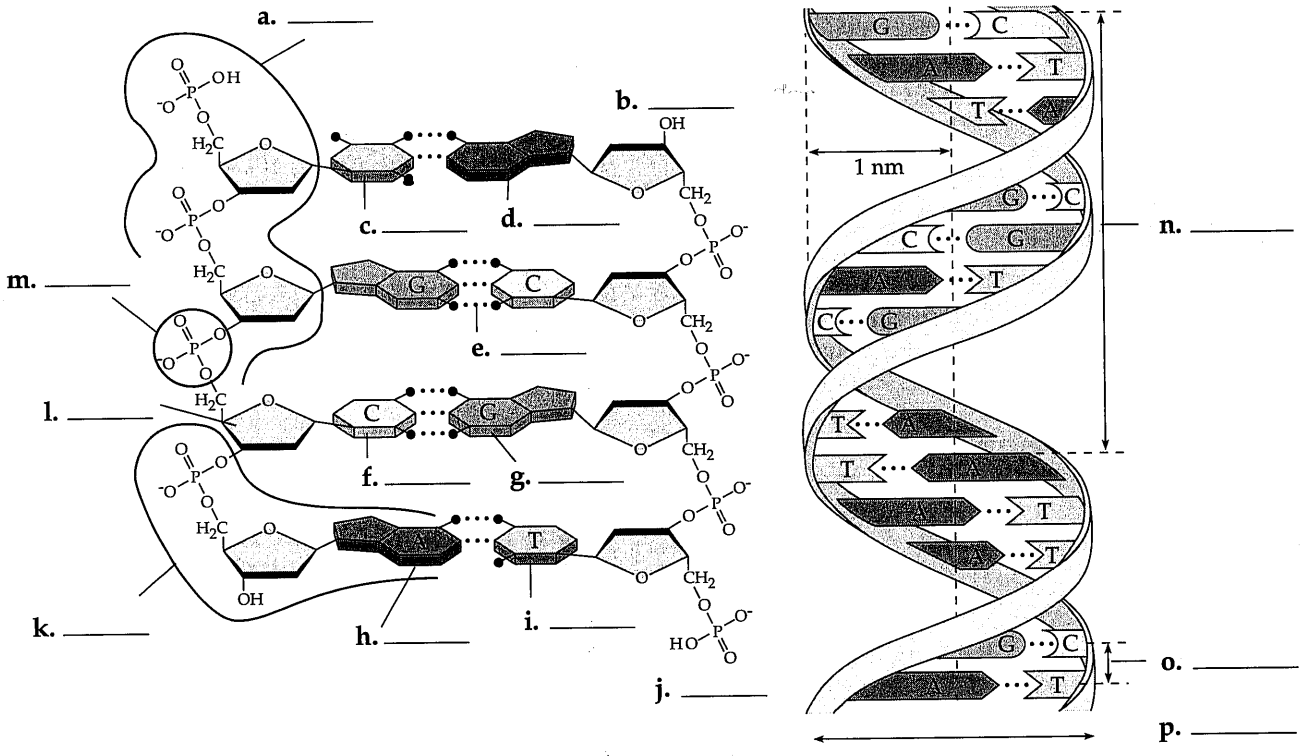
Watson and F. Crick constructed wire models to build a double helix that would conform to the X-ray measurements and the known chemistry of DNA. They arrived at a model that paired the nitrogenous bases on the inside of the helix with the sugar-phosphate chains on the outside. The helix makes one full turn every 3.4 nm; thus ten layers of nucleotide pairs, stacked 0.34 nm apart, are present in each turn of the helix.

To produce the molecule's uniform 2-nm width, a purine base must pair with a pyrimidine. The molecular arrangements of the side groups of the bases permit two hydrogen bonds to form between adenine and thymine and three hydrogen bonds between guanine and cytosine. This complementary pairing explains Chargaff's Rules. Van der Waals attractions between the closely stacked bases help to hold the molecule together.

In 1953 Watson and Crick published a paper in *Nature* reporting the double helix as the molecular model for DNA.

■ INTERACTIVE QUESTION 16.2

Review the structure of DNA by labeling the following diagrams.



16.2 Many proteins work together in DNA replication and repair

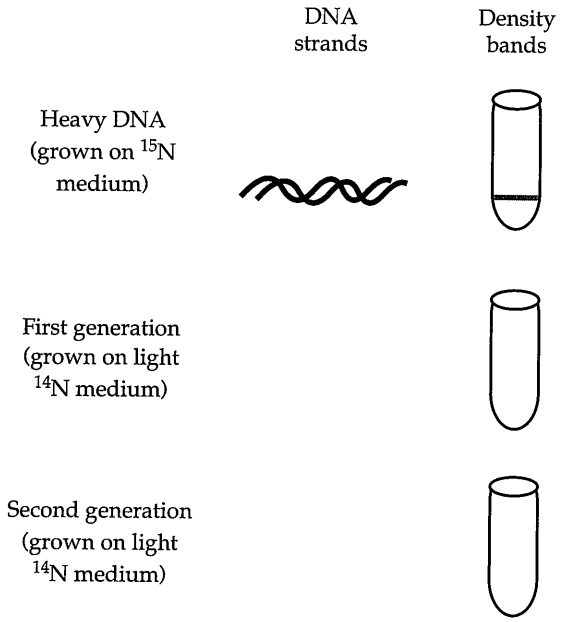
The Basic Principle: Base Pairing to a Template Strand Watson and Crick noted that the base-pairing rule of DNA sets up a mechanism for its replication. Each side of the double helix is an exact complement to the other. When the two sides of a DNA molecule separate in the replication process, each strand serves as a template for rebuilding a double-stranded molecule.

The **semiconservative model** of DNA replication predicts that the two daughter DNA molecules each have one parental strand and one newly formed strand. In contrast, a conservative model predicts that the parent double helix re-forms and the duplicated molecule is totally new, whereas a dispersive model predicts that all four strands of the two DNA molecules are a mixture of parental and new DNA.

M. Meselson and F. Stahl tested these models by growing *E. coli* in a medium with ^{15}N, a heavy isotope that the bacteria incorporated into their nitrogenous bases. Cells with labeled DNA were transferred to a medium with the lighter isotope, ^{14}N. Samples were removed after one and two generations of bacterial growth, and the DNA was extracted and centrifuged. The resulting locations of the density bands in the centrifuge tubes confirmed the semiconservative model of DNA replication.

■ INTERACTIVE QUESTION 16.3

Using different colors for heavy (parental) and light (new) strands of DNA, sketch the results of two replication cycles when *E. coli* were moved from medium containing ^{15}N to ^{14}N medium. Show the resulting density bands in the centrifuge tubes.



***DNA Replication:* A Closer Look** Replication begins at special sites, called **origins of replication,** where proteins that initiate replication bind to a specific sequence of nucleotides and separate the two strands to form a replication "bubble." Replication proceeds in both directions in the two Y-shaped **replication forks.**

Enzymes called **DNA polymerases** connect nucleotides to the growing end of the new DNA strand. DNA polymerase III and I are involved in replication in *E. coli;* at least 11 different DNA polymerases have been discovered so far in eukaryotes. A nucleoside triphosphate lines up with its complementary base on the template strand; it loses two phosphate groups and the hydrolysis of this pyrophosphate to two inorganic phosphates ($\textcircled{P}_i$) provides the energy for polymerization.

The two strands of a DNA molecule are antiparallel; their sugar-phosphate backbones run in opposite directions. The deoxyribose sugar of each nucleotide is connected to its own phosphate group at its 5′ carbon and connects to the phosphate group of the adjacent nucleotide by its 3′ carbon. Thus a strand of DNA has polarity, with a 5′ end where the first nucleotide's phosphate group is exposed, and a 3′ end where the nucleotide at the other end has a hydroxyl group attached to its 3′ carbon.

■ INTERACTIVE QUESTION 16.4

Look back to Interactive Question 16.2 and label the 5′ and 3′ ends of both strands of the DNA molecule.

DNA polymerases add nucleotides only to the 3′ end of growing strands; DNA is replicated in a 5′→3′ direction. Because the DNA strands run in an antiparallel direction, the simultaneous synthesis of both strands presents a problem. The **leading strand** is the new 5′ → 3′ strand being formed along the template by DNA polymerase III (DNA pol III) in the progressing replication fork. The **lagging strand** is created as a series of short segments, called **Okazaki fragments,** that are formed in the 5′→3′ direction away from the replication fork. An enzyme called **DNA ligase** joins the sugar-phosphate backbones of the fragments.

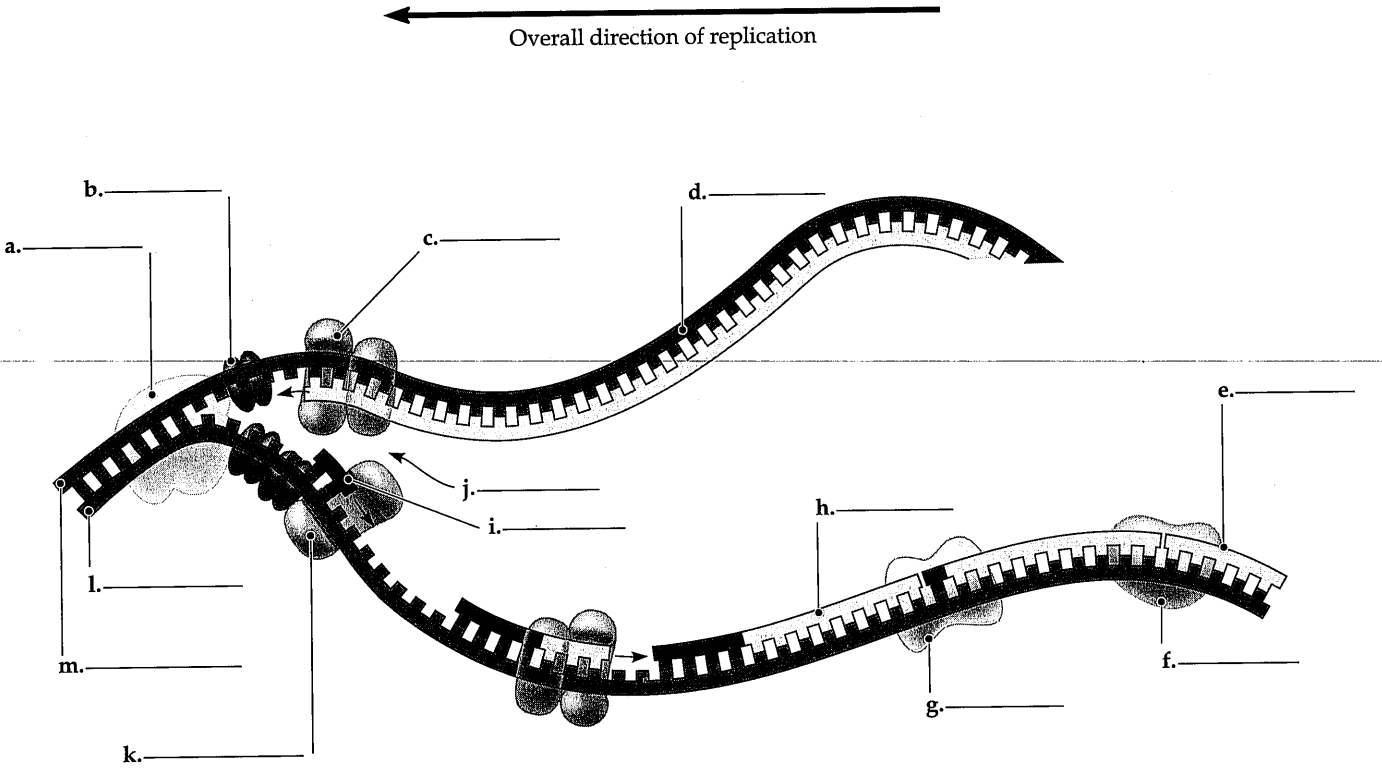
DNA polymerases cannot initiate synthesis of a DNA strand; they can only add nucleotides to an existing chain that is attached to a template strand. An enzyme called **primase** joins about 10 RNA nucleotides to form the **primer** needed to start the chain. Each fragment on the lagging strand requires a primer. A continuous strand of DNA is produced after DNA polymerase I (DNA pol I) replaces the RNA primer with DNA nucleotides and ligase joins the fragments.

An enzyme called **helicase** unwinds the helix and separates the parent strand at replication forks, and **single-strand binding proteins** keep the separated strands apart while they serve as templates. **Topoisomerase** helps relieve the strain from the twisting of DNA strands in front of helicase.

The various proteins that function in DNA replication form a large complex. In eukaryotic cells, many such complexes may anchor to the nuclear matrix and the DNA polymerase molecules may pull the parental DNA through them.

■ INTERACTIVE QUESTION 16.5

In this diagram showing the replication of DNA, label the following items: leading and lagging strands, Okazaki fragment, DNA pol III, DNA pol I, DNA ligase, helicase, primase, single-strand binding proteins, RNA primer, replication fork, and 5′ and 3′ ends of parental DNA.



Proofreading and Repairing DNA Initial pairing errors in nucleotide placement may occur as often as 1 per 100,000 base pairs. The amazing accuracy of DNA replication (one error in ten billion nucleotides) is achieved as DNA polymerases check each newly added nucleotide against its template and replace incorrect nucleotides. Other enzymes also fix incorrectly paired nucleotides, called **mismatch repair.**

Reactive chemicals, radioactive emissions, X-rays, and UV light may alter DNA molecules. Many different types of DNA repair enzymes (130 have been identified in humans so far) may correct these changes. In **nucleotide excision repair,** the damaged strand is cut out by a **nuclease** and the gap is correctly filled through the action of DNA polymerase and ligase. In skin cells, nucleotide excision repair frequently corrects thymine dimers caused by ultraviolet rays of sunlight.

Replicating the Ends of DNA Molecules Because DNA polymerase cannot attach nucleotides to the 5′ end of a daughter DNA strand, repeated replications cause a progressive shortening of linear DNA molecules. Multiple repetitions of a short nucleotide sequence at the ends of chromosomes, called **telomeres,** protect an organism's genes from being shortened during successive DNA replications.

The shortening of telomeres may limit cell division. In germ line cells, however, the enzyme **telomerase,** which contains an RNA template for the telomere

sequence, lengthens telomeres. Some somatic cancer cells and "immortal" strains of cultured cells produce telomerase and are thus capable of unregulated cell division.

Word Roots

helic- = a spiral (*helicase:* an enzyme that untwists the double helix of DNA at the replication forks)
liga- = bound or tied (*DNA ligase:* a linking enzyme for DNA replication)
-phage = to eat (*bacteriophages:* viruses that infect bacteria)
semi- = half (*semiconservative model:* type of DNA replication in which the replicated double helix consists of one old strand, derived from the old molecule, and one newly made strand)
telos- = an end (*telomere:* the protective structure at each end of a eukaryotic chromosome)
trans- = across (*transformation:* a phenomenon in which external DNA is assimilated by a cell)

Structure Your Knowledge

1. Summarize the evidence and techniques Watson and Crick used to deduce the double-helix structure of DNA.
2. Review your understanding of DNA replication by describing the key enzymes and proteins (in the order of their functioning) that direct replication.

Test Your Knowledge

MULTIPLE CHOICE: *Choose the one best answer.*

1. One of the reasons most scientists believed proteins were the carriers of genetic information was that
   a. proteins were more heat stable than nucleic acids.
   b. the protein content of duplicating cells always doubled prior to division.
   c. proteins were much more complex and heterogeneous molecules than nucleic acids.
   d. early experimental evidence pointed to proteins as the hereditary material.
   e. proteins were found in DNA.
2. Transformation involves
   a. the uptake of external genetic material, often from one bacterial strain to another.
   b. the creation of a strand of RNA from a DNA molecule.
   c. the infection of bacterial cells by phage.
   d. the type of semiconservative replication shown by DNA.
   e. the replication of DNA along the lagging strand.
3. The DNA of an organism has thymine as 20% of its bases. What percentage of its bases would be guanine?
   a. 20% c. 40% e. 80%
   b. 30% d. 60%
4. In his work with pneumonia-causing bacteria, Griffith found that
   a. DNA was the transforming agent.
   b. the pathogenic and harmless strains mated.
   c. heat-killed harmless cells could cause pneumonia when mixed with heat-killed pathogenic cells.
   d. some heat-stable chemical was transferred to harmless cells to transform them into pathogenic cells.
   e. a T2 phage transformed harmless cells to pathogenic cells.
5. When T2 phages are grown with radioactive sulfur,
   a. their DNA is tagged.
   b. their proteins are tagged.
   c. their DNA is found to be of medium density in a centrifuge tube.
   d. they transfer their radioactivity to *E. coli* chromosomes when they infect the bacteria.
   e. their excision enzymes repair the damage caused by the radiation.
6. Meselson and Stahl
   a. provided evidence for the semiconservative model of DNA replication.
   b. were able to separate phage protein coats from *E. coli* by using a blender.
   c. found that DNA labeled with ^{15}N was of intermediate density.
   d. grew *E. coli* on labeled phosphorus and sulfur.
   e. found that DNA composition was species specific.

7. Watson and Crick concluded that each base could not pair with itself because
   a. there would not be room for the helix to make a full turn every 3.4 nm.
   b. the uniform width of 2 nm would not permit two purines or two pyrimidines to pair together.
   c. the bases could not be stacked 0.34 nm apart.
   d. identical bases could not hydrogen-bond together.
   e. they would be on antiparallel strands.

8. The joining of nucleotides in the polymerization of DNA requires energy from
   a. DNA polymerase.
   b. the hydrolysis of the terminal phosphate group of ATP.
   c. RNA nucleotides.
   d. the hydrolysis of GTP.
   e. the hydrolysis of the pyrophosphates removed from nucleoside triphosphates.

9. Continuous elongation of a new DNA strand along one strand of DNA
   a. requires the action of DNA ligase as well as polymerase.
   b. occurs because DNA ligase can only elongate in the 5′→ 3′ direction.
   c. occurs on the leading strand.
   d. occurs on the lagging strand.
   e. a, b, and c are correct.

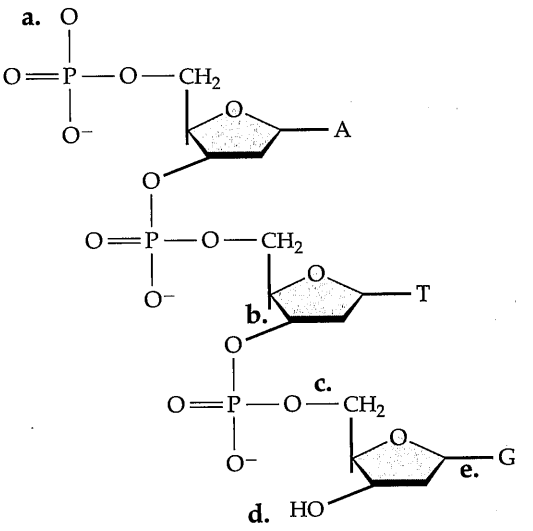
10. Which of the following statements about DNA polymerase is *incorrect?*
    a. It forms the bonds between complementary base pairs.
    b. It is able to proofread and correct for errors in base pairing.
    c. It is unable to initiate synthesis; it requires an RNA primer.
    d. It only works in the 5′→ 3′ direction.
    e. It is found in eukaryotes and prokaryotes.

11. Thymine dimers—covalent links between adjacent thymine bases in DNA—may be induced by UV light. When they occur, they are repaired by
    a. excision enzymes (nucleases).
    b. DNA polymerase.
    c. ligase.
    d. primase.
    e. a, b, and c are all needed.

12. How does DNA synthesis along the lagging strand differ from that on the leading strand?
    a. Nucleotides are added to the 5′ end instead of the 3′ end.
    b. Ligase is the enzyme that polymerizes DNA on the lagging strand.
    c. An RNA primer is needed on the lagging strand but not on the leading strand.
    d. Okazaki fragments, which each grow 5′→ 3′, must be joined along the lagging strand.
    e. Helicase synthesizes Okazaki fragments, which are then joined by ligase.

13. Which of the following enzymes or proteins is paired with an incorrect or inaccurate function?
    a. Helicase—unwind and separate parental double helix
    b. Telomerase—add telomere repetitions to end of chromosomes
    c. Single-strand binding protein—hold strands of unwound DNA apart and straight
    d. Nuclease—cut out (excise) damaged DNA strand
    e. Primase—form DNA primer to start replication

Use the following diagram to answer Questions 14 through 17.



14. Which letter indicates the 5′ end of this single DNA strand?
    a. b. c. d. e.

15. At which letter would the next nucleotide be added?
    a. b. c. d. e.

16. Which letter indicates a phosphodiester bond formed by DNA polymerase?

    a. **b.** **c.** **d.** **e.**

17. The base sequence of the DNA strand made from this template would be (from top to bottom)

    a. A T C.
    b. C G A.
    c. T A C.
    d. U A C.
    e. A T G.

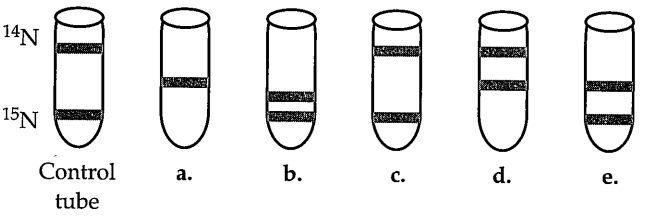
18. T2 phage is grown in *E. coli* with radioactive phosphorus and then allowed to infect other *E. coli*. The culture is blended to separate the viral coats from the bacterial cells and centrifuged. Which of the following best describes the expected results of such an experiment?

    a. Both viral and bacterial DNA are labeled; radioactivity is found in the supernatant.
    b. Both viral and bacterial proteins are labeled; radioactivity is present in both the supernatant and the pellet.
    c. Viral proteins are labeled; radioactivity is found in the supernatant but not in the pellet.
    d. Viral DNA is labeled; radioactivity is found in the pellet.
    e. The virus destroyed the bacteria; no pellet is formed.

19. What are telomeres, and what do they do?

    a. ever-shortening tips of chromosomes that may signal cells to stop dividing at maturity
    b. highly repetitive sequences at tips of chromosomes that protect the lagging strand during replication
    c. repetitive sequences of nucleotides at the centromere region of a chromosome
    d. enzymes that are present in germ-line cells that allow these cells to undergo repeated divisions
    e. Both a and b are correct.

20. You are trying to support your hypothesis that DNA replication is *conservative*; i.e., parental strands separate; complementary strands are made, but these new strands join together to make a new DNA molecule, and the parental strands rejoin. You take *E. coli* that had grown in a medium containing only heavy nitrogen (^{15}N) and transfer a sample to a medium containing light nitrogen (^{14}N). After allowing time for only one DNA replication, you centrifuge a sample and compare the density band(s) formed with control bands for bacteria grown on either normal ^{14}N or ^{15}N medium. Which band location would support your hypothesis of *conservative* DNA replication?



Bands after one replication on ^{14}N

21. Using the experiment explained in question 20, which centrifuge tube would represent the band distribution obtained after one replication showing that DNA replication is *semiconservative?*