FEDERAL STATE BUDGETARY EDUCATIONAL INSTITUTION
OF HIGHER EDUCATION «BASHKIR STATE MEDICAL UNIVERSITY»
OF THE MINISTRY OF HEALTHCARE OF THE RUSSIAN FEDERATION
(FSBEI HE BSMU MOH Russia)

T.V. Victorova K.V. Danilko

Cytology&Genetics

Manual

Ufa — 2019

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The manual «Cytology&Genetics» is prepared according to requirements of federal state educational standard of the highest educations (Moscow, 2015) on discipline «Biology». This textbook has been written from many years' experience of teaching general biology for Bashkir State Medical University students.

In this manual the questions of organization of biological systems at the molecular, cellular and organismal levels are presented. By revealing the mechanisms of biological processes, biology promotes understanding of the pathological disturbances that occur in the human body, and provides means both for combating diseases, and for preventing and curing them. The special attention is paid to the importance of modern achievements in the field of biology and genetics for development of modern medicine.

The manual is recommended for independent out-of-class work for the 1-st year medical students.

Рекомендовано Координационным советом по области образования «Здравоохранение и медицинские науки» в качестве учебного пособия для использования в образовательных учреждениях, реализующих основные образовательные программы высшего образования по направлению подготовки специалитета по специальности 31.05.01 «Лечебное дело».

Recommended by the Coordination Council on Education "Health and Medical Sciences" as a training tool for use in educational institutions that implement basic educational programs of higher education in the direction of training a specialist in the specialty 31.05.01 «General Medicine». Contents

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INTRODUCTION

The modern medicine is characterized by prompt accumulation of new information on fundamental and system bases of activity. A necessary condition of progress of modern medicine is the high level of development of biology including such disciplines as such as cytology, genetics, theory of evolution, parasitology and ecology. In the manual brought to your attention in biology the main place is allocated for the description of the biological regularities peculiar to all live organisms. At the same time, authors tried to open these processes taking into account features of a human body as studying of the person as the biosocial subject with emphasis on its biological characteristics, represents the paramount importance for formation of medical thinking at first-year students of medical universities of Russian.

In the manual "Cytology&Genetics", biology recognizes the cell as the basic unit of life and genes as the basic unit of heredity. Biology is a natural science concerned with the study of life and living organisms, including their structure, function, growth, evolution, distribution, and taxonomy. Modern biology is a vast and eclectic field, composed of many branches and disciplines.

Disciplines of biology are defined by the scale at which organisms are studied, the kinds of organisms studied, and the methods used to study them: cellular biology examines the basic building-block of all life, the cell; molecular biology studies the complex interactions among biological molecules; biochemistry examines the rudimentary chemistry of life; physiology examines the physical and chemical functions of tissues, organs, and organ systems of an organism; evolutionary biology examines the processes that produced the diversity of life; and ecology examines how organisms interact in their environment.

Biology today is the foundation of all branches of science concerned with the study of life and its multiformity, including the life of man, and, consequently, it also underlies medicine, and medical theory and practice. The special attention is paid to the characteristic of hereditary material both prokaryotic

and eukaryotic organisms, to problems of genetic freight of populations and genetic predisposition of people to multiple-factorial human diseases. It is also the firm foundation on which modern public health protection is based.

Certain branches of biology (physiology, parasitology, and microbiology) are especially important in medicine, while medical workers must be particularly concerned with the branches that deal with the individual development of man.

By revealing the mechanisms of biological processes, biology promotes understanding of the pathological disturbances that occur in the human body, and provides means both for combating diseases, and for preventing and curing them.

It has now become impossible to treat a patient without taking into account his biological features (height, development, age), and the study of purely medical disciplines has become impossible without a grounding in general biology.

CHAPTER 1. THE SCIENCE OF BIOLOGY

BIOLOGY IS THE SCIENCE OF LIFE

Properties of Life. In its broadest sense, ***biology is the study of living things — the science of life***. Living things come in an astounding variety of shapes and forms, and biologists study life in many different ways. They live with gorillas, collect fossils, and listen to whales. They isolate viruses, grow mushrooms, and examine the structure of fruit flies. They read the messages encoded in the long molecules of heredity and count how many times a hummingbird's wings beat each second. What makes something "alive"? Anyone could deduce that a galloping horse is alive and a car is not, but *why?* We cannot say, "If it moves, it's alive," because a car can move, and gelatin can wiggle in a bowl. They certainly are not alive. What characteristics *do* define life? All living organisms share five basic characteristics:

1. Order. All organisms consist of one or more cells with highly ordered structures: atoms make up molecules, which construct cellular organelles, which are contained within cells. This hierarchical organization continues at higher levels in multicellular organisms and among organisms.

2. Sensitivity. All organisms respond to stimuli. Plants grow toward a source of light, and your pupils dilate when you walk into a dark room.

3. Growth, development, and reproduction. All organisms are capable of growing and reproducing, and they all possess hereditary molecules that are passed to their offspring, ensuring that the offspring are of the same species. Although crystals also "grow," their growth does not involve hereditary molecules.

4. Regulation. All organisms have regulatory mechanisms that coordinate the organism's internal functions. These functions include supplying cells with nutrients, transporting substances through the organism, and many others.

5. Homeostasis. All organisms maintain relatively constant internal conditions, different from their environment, a process called homeostasis.

All living things share certain key characteristics: order, sensitivity, growth, development and reproduction, regulation, and homeostasis.

Hierarchical organization of living things. Life is highly organized — from small and simple to large and complex, within cells, within multicellular organisms, and among organisms. Considered in terms of levels-of-organization, the science of biology can be said to consist of other disciplines focusing on particular levels. Thus one speaks of molecular biology, cell biology, biology of organisms, population biology, and community biology (Fig. 1.1).

All living things is composed of some 92 elements.

1) Elements and Atoms. Each element is made up of just one type of atom. An atom has a weight, which is dependent on the number of protons and neutrons in the nucleus, and its chemical properties are dependent on the number of electrons in the outer shell.

2) Molecules and Compounds. Atoms react with one another by forming ionic bonds or covalent bonds. Ionic bonds are an attraction between charged ions. Atoms share electrons in covalent bonds, which can be single, double, or triple bonds.

3) Water and Living Things. Water, acids, and bases are important inorganic molecules. The polarity of water accounts for it being the universal solvent; hydrogen bonding accounts for it boiling at 100°C and freezing at 0°C. Because it is slow to heat up and slow to freeze, water is liquid at the temperature of living things. Pure water has a neutral pH; acids increase the hydrogen ion concentration [H^+] but decrease the pH, and bases decrease the hydrogen ion concentration [H^+] but increase the pH of water.

4) Molecules of Life. The chemistry of carbon accounts for the chemistry of organic compounds. Carbohydrates, lipids, proteins, and nucleic acids are molecules with specific functions in cells.

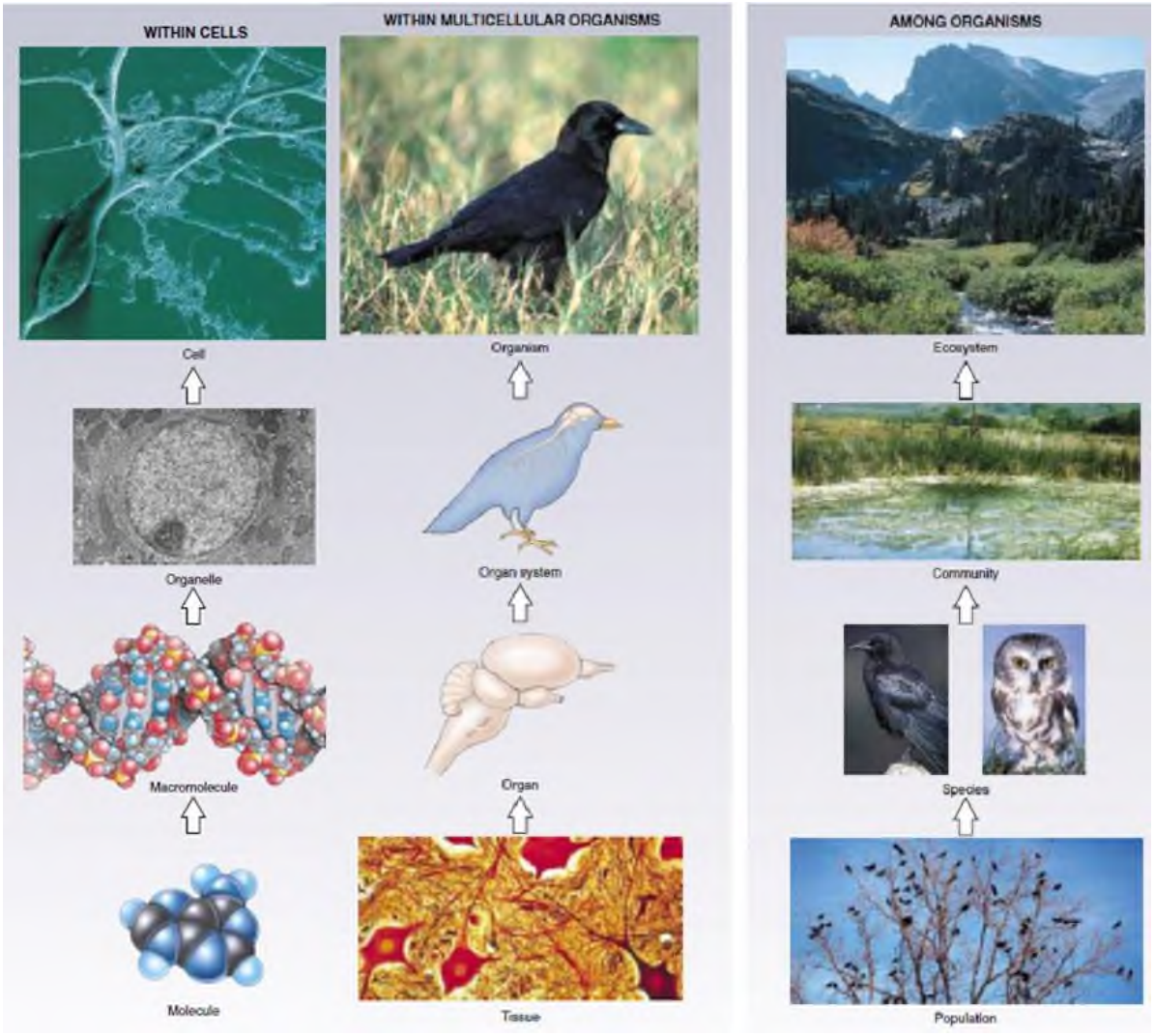


FIGURE 1.1. Hierarchical organization of living things. Life is highly organized from small and simple to large and complex, within cells, within multicellular organisms, and among organisms.

5) Carbohydrates. Glucose is the 6-carbon sugar most utilized by cells for "quick" energy. Like the rest of the macromolecules to be studied, condensation synthesis joins two or more sugars, and a hydrolysis reaction splits the bond. Plants store glucose as starch, and animals store glucose as glycogen. Humans cannot digest cellulose, which forms plant cell walls.

6) Lipids. Lipids are varied in structure and function. Fats and oils, which function in long-term energy storage, contain glycerol and three fatty acids. Fatty acids can be saturated or unsaturated. Plasma membranes contain phospholipids that have a polarized end. Certain hormones are derived from cholesterol, a complex ring compound.

7) Proteins. The primary structure of a polypeptide is its own particular sequence of the possible 20 types of amino acids. The secondary structure is often an alpha - helix. The tertiary structure occurs when a polypeptide bends and twists into a three-dimensional shape. A protein can contain several polypeptides, and this accounts for a possible quaternary structure.

8) Nucleic Acids. Nucleic acids are polymers of nucleotides. Each nucleotide has three components: a sugar, a base, and phosphate (phosphoric acid). DNA, which contains the sugar deoxyribose, is the genetic material that stores information for its own replication and for the order in which amino acids are to be sequenced in proteins. DNA, with the help of RNA, specifies protein synthesis. ATP, with its unstable phosphate bonds, is the energy currency of cells. Hydrolysis of ATP to ADP+P releases energy that is used by the cell to do metabolic work.

THE DIVERSITY OF LIFE: THE NATURE OF VIRUSES

Viral Structure. All viruses have the same basic structure: a core of nucleic acid surrounded by protein. Individual viruses contain only a single type of nucleic acid, either DNA or RNA. The DNA or RNA genome may be linear or circular, and single-stranded or double-stranded. Viruses are frequently classified by the nature of their genomes. RNA-based viruses are known as **retroviruses.** Nearly all viruses form a protein sheath, or **capsid,** around their nucleic acid core. The capsid is composed of one to a few different protein molecules repeated many times (Fig. 1.2).

In some viruses, specialized enzymes are stored within the capsid. Many animal viruses form an **envelope** around the capsid rich in proteins, lipids, and glycoprotein molecules. While some of the material of the envelope is derived from the host cell's membrane, the envelope does contain proteins derived from viral genes as well. Viruses occur in virtually every kind of organism that has been investigated for their presence. However, each type of virus can replicate in only a very limited number of cell types. The suitable cells for a particular

virus are collectively referred to as its **host range.** The size of the host range reflects the coevolved histories of the virus and its potential hosts.

A recently discovered herpes-virus turned lethal when it expanded its host range from the African elephant to the Indian elephant, a situation made possible through cross-species contacts between elephants in zoos.

Some viruses wreak havoc on the cells they infect; many others produce no disease or other outward sign of their infection.

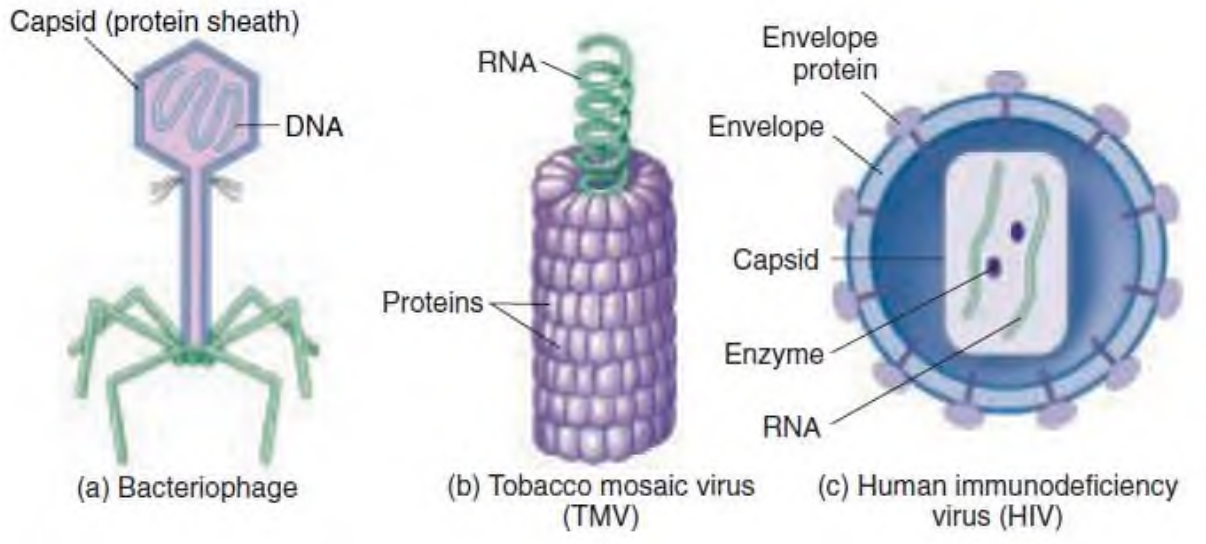


FIGURE 1.2. The structure of a bacterial, plant, and animal virus. (*a*) Bacterial viruses, called bacteriophages, often have a complex structure. (*b*) TMV infects plants and consists of 2130 identical protein molecules (*purple*) that form a cylindrical coat around the single strand of RNA (*green*). The RNA backbone determines the shape of the virus and is protected by the identical protein molecules packed tightly around it. (*c*) In the human immunodeficiency virus (HIV), the RNA core is held within a capsid that is encased by a protein envelope.

Still other viruses remain dormant for years until a specific signal triggers their expression. A given organism often has more than one kind of virus. This suggests that there may be many more kinds of viruses than there are kinds of organisms - perhaps millions of them. Only a few thousand viruses have been described at this point.

The diversity of life: the nature of cell organisms

Biologists categorize all living things into six major groups called kingdoms: archaebacteria, eubacteria, protists, fungi, plants, and animals.

Bacteria Are Simple Cells. Prokaryotes, the bacteria, are the simplest organisms. Prokaryotic cells are small, consisting of cytoplasm surrounded by a plasma membrane and encased within a rigid cell wall, with no distinct interior compartments (Fig. 1.3).

Most bacteria are encased by a strong **cell wall** composed of *peptidoglycan.* A prokaryotic cell is like a oneroom cabin in which eating, sleeping, and watching TV all occur in the same room. They are encased by an exterior wall composed of carbohydrates cross-linked by short polypeptides, and some are propelled by rotating flagella. Generalized cell organization of a acterium. Some bacteria have hairlike growths on the outside of the cell called pili.

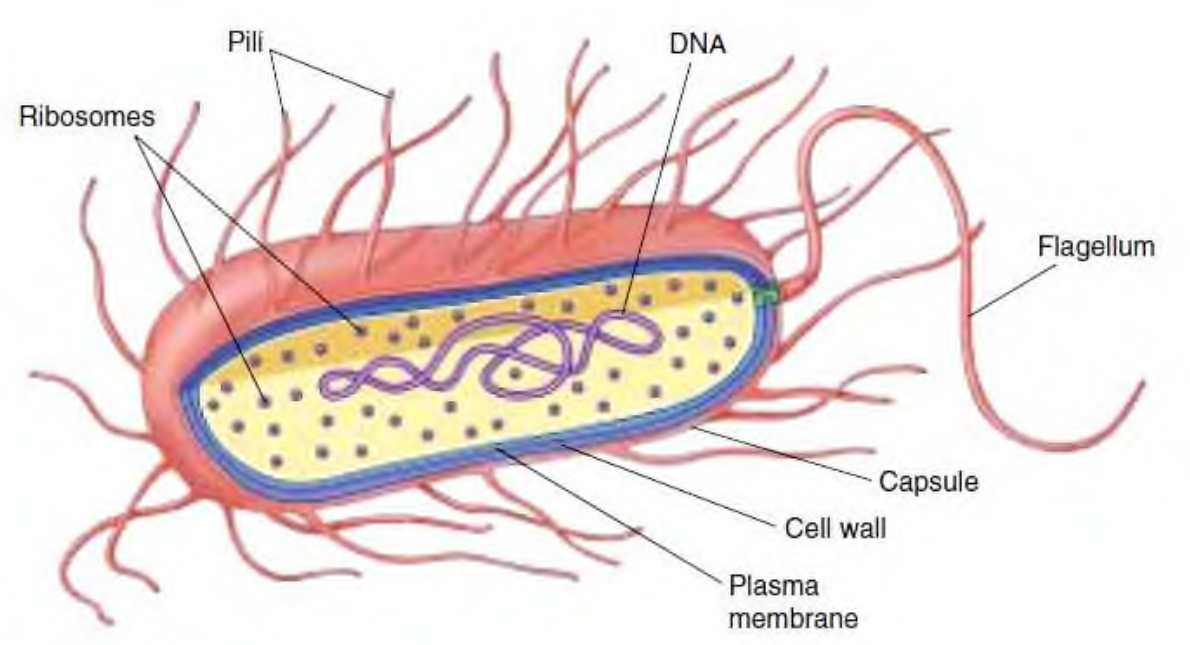


FIGURE 1.3. Structure of a bacterial cell. Generalized cell organization of a bacterium. Some bacteria have hairlike growths on the outside of the cell called pilli.

Bacteria are very important in the economy of living organisms. They harvest light in photosynthesis, break down dead organisms and recycle their components, cause disease, and are involved in many important industrial processes.

Eukaryotic Cells Have Complex Interiors. Eukaryotic cells (Fig. 1.4) are far more complex than prokaryotic cells. The hallmark of the eukaryotic cell is compartmentalization. The interiors of eukaryotic cells contain numerous **organelles,** membrane-bounded structures that close off compartments within which multiple biochemical processes can proceed simultaneously and independently. Plant cells often have a large membrane-bounded sac called a **central vacuole,** which stores proteins, pigments, and waste materials. Both plant and animal cells contain **vesicles,** smaller sacs that store and transport a variety of materials. Inside the nucleus, the DNA is compact units called **chromosomes.** All eukaryotic cells are supported by an internal protein scaffold, the **cytoskeleton.** While the cells of animals and some protists lack cell walls, the cells of fungi, plants, and many protists have strong **cell walls** composed of cellulose or chitin fibers embedded in a matrix of other polysaccharides and proteins. This composition is very different from the peptidoglycan that makes up bacterial cell walls. Let's now examine the structure and function of the internal components of eukaryotic cells in more detail. Most mature plant cells contain large central vacuoles which occupy a major portion of the internal volume of the cell.

Multicellular organisms usually consist of many small cells rather than a few large ones because small cells function more efficiently. They have a greater relative surface area, enabling more rapid communication between the center of the cell and the environment.

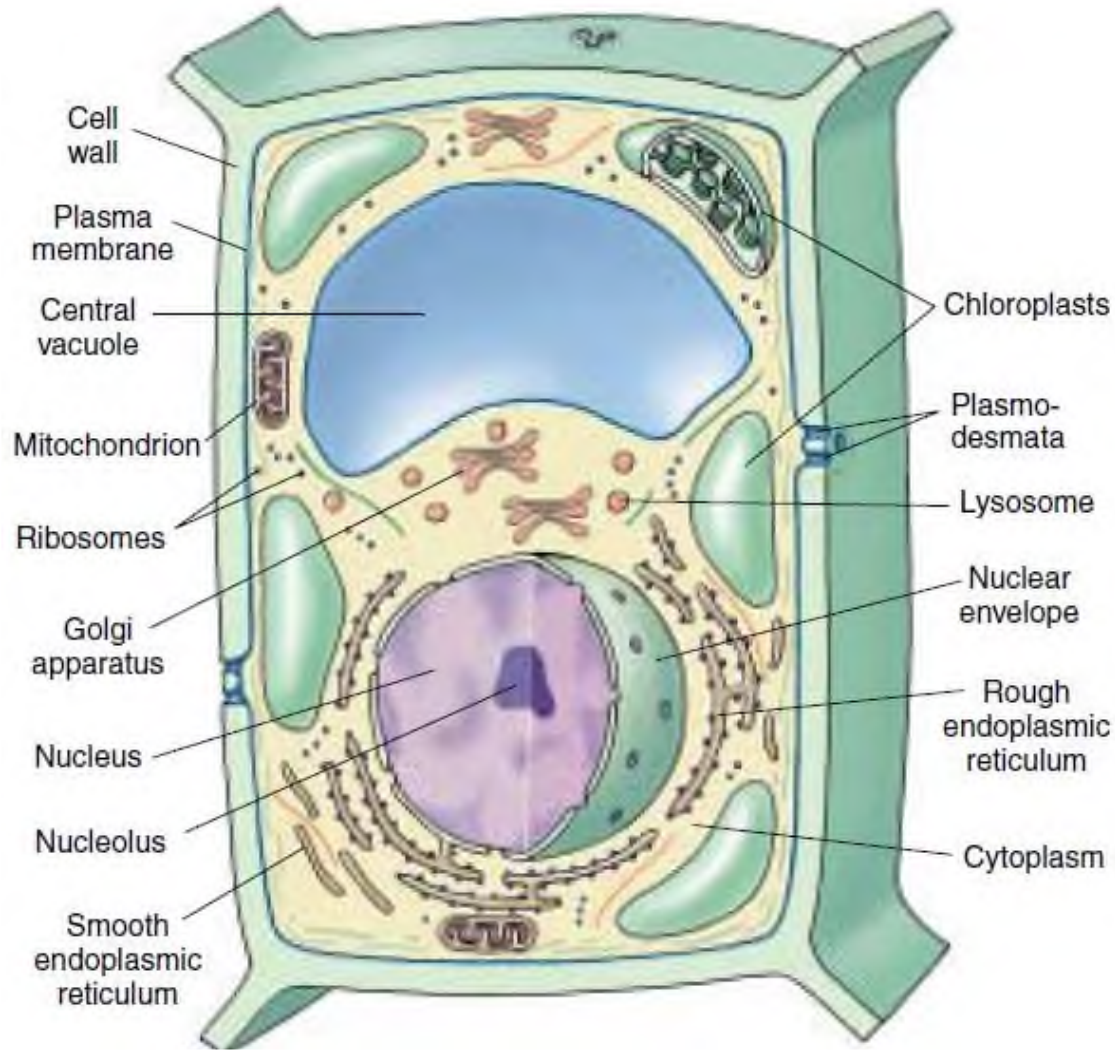


FIGURE 1.4. Structure of a plant cell. A generalized illustration of a plant cell. Most mature plant cells contain large central vacuoles which occupy a major portion of the internal volume of the cell.

Key Questions:

1. What are the key characteristics of living things?
2. Why are viruses not considered to be living organism?
3. What is a bacteriophage?
4. How do many animal viruses penetrate the host cell?
5. How are prokaryotes different from eukaryotes in terms of their cell walls, interior organization, and flagella?
6. What is the structure of the bacterial cell?
7. Why is it so much more difficult to treat a viral infection than a bacterial one?
8. What is the apparent origin of organelles found in almost all eukaryotes?

Examples of Review questions

NN	Questions	Right answers
1	THE MAIN CHARACTERISTICS OF LIVING ORGANISMS ARE ___ . 1) self-reproduction, specificity of organization, regularize of structure; 2) discretion and integrity, growth and development, metabolism and energy metabolism; 3) irritability, movement, heredity and variability; 4) all answers are correct.	**4**
2	UNLIKE EUKARYOTES, PROKARYOTES LACK ___ . 1) a plasma membrane. 2) DNA. 3) ribosomes. 4) nuclei. 5) molecular motors.	**4**
3	WHICH OF THE FOLLOWING CHARACTERISTICS DISTINGUISHES PROKARYOTES FROM EUKARYOTES? 1) Eukaryotes have a nucleus, while prokaryotes do not. 2) Prokaryotes lack ribosomes, which are found in eukaryotes. 3) Prokaryotes do not contain DNA, but eukaryotes do. 4) Eukaryotic organisms are much more widespread than prokaryotes. 5) Prokaryotes produce antibiotics, eukaryotes do not.	**1**
4	WHICH IS PRESENT IN BOTH PROKARYOTIC CELLS AND IN EUKARYOTIC PLANT CELLS? 1) Chloroplasts 2) Cell walls 3) Nucleus 4) Mitochondria 5) Microtubules	**2**

CHAPTER 2. EUKARYOTIC CELLS ORGANIZATION

2.1. BIOLOGICAL MEMBRANE STRUCTURE AND FUNCTIONS

BIOLOGICAL MEMBRANES ARE FLUID LAYERS OF LIPID

A eukaryotic cell contains many membranes. While they are not all identical, they share the same fundamental architecture. Cell membranes are assembled from four components:

1. Lipid bilayer. Every cell membrane is composed of a phospholipid bilayer. The other components of the membrane are enmeshed within the bilayer, which provides a flexible matrix and, at the same time, imposes a barrier to permeability.

2. Transmembrane proteins. A major component of every membrane is a collection of proteins that float on or in the lipid bilayer. These proteins provide passageways that allow substances and information to cross the membrane. Many membrane proteins are not fixed in position; they can move about, as the phospholipid molecules do. Some membranes are crowded with proteins, while in others, the proteins are more sparsely distributed.

3. Network of supporting fibers. Membranes are structurally supported by intracellular proteins that reinforce the membrane's shape. For example, a red blood cell has a characteristic biconcave shape because a scaffold of proteins called spectrin links proteins in the plasma membrane with actin filaments in the cell's cytoskeleton. Membranes use networks of other proteins to control the lateral movements of some key membrane proteins, anchoring them to specific sites.

4. Exterior proteins and glycolipids. Membrane sections assemble in the endoplasmic reticulum, transfer to the Golgi complex, and then are transported to the plasma membrane. The endoplasmic reticulum adds chains of sugar molecules to membrane proteins and lipids, creating a "sugar coating" called the glycocalyx that extends from the membrane on the outside of the cell only. Different cell types exhibit different varieties of these glycoproteins and glycolipids on their surfaces, which act as cell identity markers.

The fluid mosaic model of the plasma membrane. A variety of proteins protrude through the plasma membrane of animal cells, and nonpolar regions of the proteins tether them to the membrane's nonpolar interior. The three principal classes of membrane proteins are transport proteins, receptors, and cell surface markers. Carbohydrate chains are often bound to the extracellular portion of these proteins, as well as to the membrane phospholipids. These chains serve as distinctive identification tags, unique to particular cells.

The fluid mosaic model proposes that membrane proteins are embedded within the lipid bilayer (Fig. 2.1).

A phospholipid is a composite molecule similar to a triacylglycerol, except that only two fatty acids are bound to the glycerol backbone; a phosphorylated alcohol occupies the third position on the backbone. Because the phosphorylated alcohol usually extends from one end of the molecule and the two fatty acid chains extend from the other, phospholipids are often diagrammed as a polar head with two nonpolar hydrophobic tails.

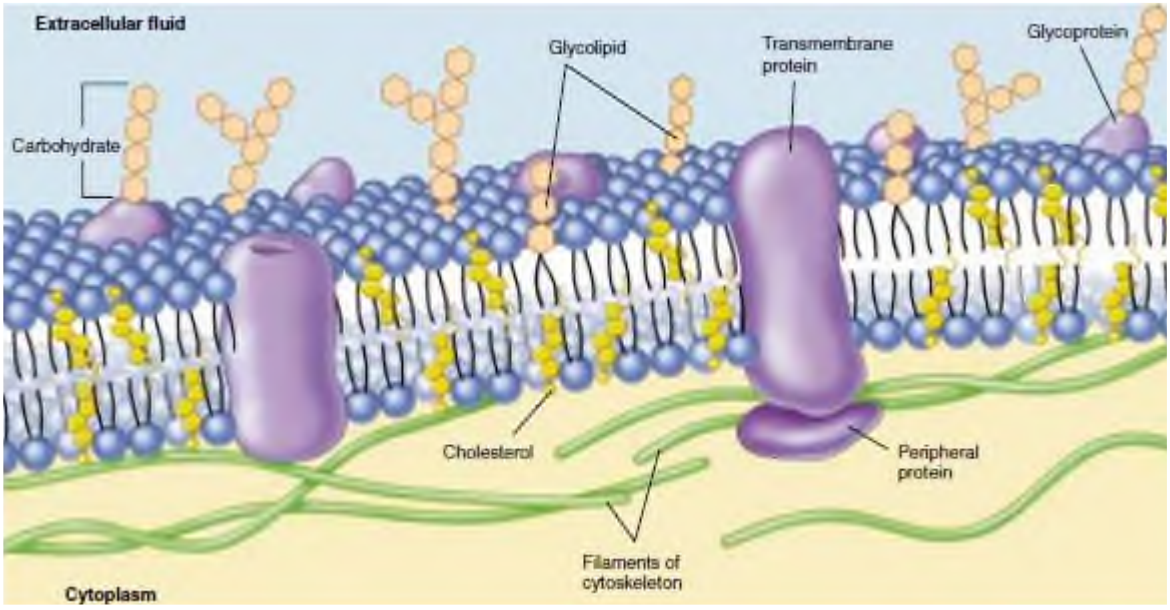


FIGURE 2.1. The fluid mosaic model of the plasma membrane. A variety of proteins protrude through the plasma membrane of animal cells, and nonpolar regions of the proteins tether them to the membrane's nonpolar interior. The three principal classes of membrane proteins are transport proteins, receptors, and cell surface markers. Carbohydrate chains are often bound to the extracellular portion of these proteins, as well as to the membrane phospholipids. These chains serve as distinctive identification tags, unique to particular cells.

The basic foundation of biological membranes is a lipid bilayer, which forms spontaneously. In such a layer, the nonpolar hydrophobic tails of phospholipid molecules point inward, forming a nonpolar barrier to water-soluble molecules. The lipid bilayer is liquid like a soap bubble, rather than solid like a rubber balloon.

Membranes are composed of a lipid bilayer within which proteins are anchored. The plasma membrane is a complex assembly of proteins enmeshed in a fluid array of phospholipid molecules. This enormously flexible design permits a broad range of interactions with the environment, some directly involving membrane proteins. Though cells interact with their environment through their plasma membranes in many ways, we will focus on six key classes of membrane protein (Fig. 2.2).

Functions of plasma membrane proteins. The many proteins embedded within a membrane carry out a host of functions, many of which are associated with transport of materials or information across the membrane. Membrane proteins act as transporters, enzymes, cell surface receptors, and cell surface markers, as well as aiding in cell-to-cell adhesion and securing the cytoskeleton:

1. Transporters. Membranes are very selective, allowing only certain substances to enter or leave the cell, either through channels or carriers. In some instances, they take up molecules already present in the cell in high concentration.

A channel protein. This transmembrane protein mediates photosynthesis in the bacterium *Halobacterium halobium*. The protein traverses the membrane seven times with hydrophobic helical strands that are within the hydrophobic center of the lipid bilayer. The helical regions form a channel across the bilayer through which protons are pumped by the retinal chromophore (green).

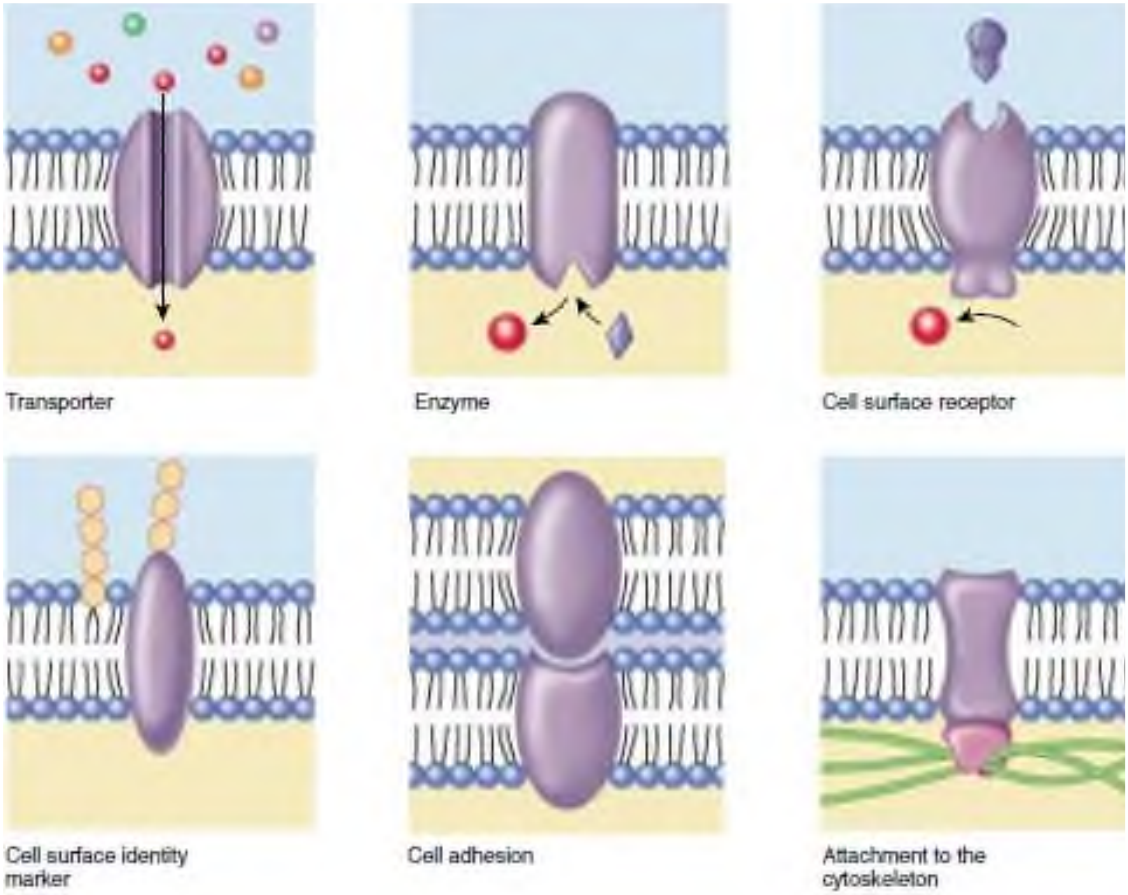


FIGURE 2.2. Functions of plasma membrane proteins. Membrane proteins act as transporters, enzymes, cell surface receptors, and cell surface markers, as well as aiding in cell-to-cell adhesion and securing the cytoskeleton.

A pore protein. The bacterial transmembrane protein porin creates large open tunnels called pores in the outer membrane of a bacterium. Sixteen strands of β-pleated sheets run antiparallel to each other, creating a β barrel in the bacterial outer cell membrane. The tunnel allows water and other materials to pass through the membrane.

2. Enzymes. Cells carry out many chemical reactions on the interior surface of the plasma membrane, using enzymes attached to the membrane.

3. Cell surface receptors. Membranes are exquisitely sensitive to chemical messages, detecting them with receptor proteins on their surfaces that act as antennae.

4. Cell surface identity markers. Membranes carry cell surface markers that identify them to other cells. Most cell types carry their own ID tags, specific combinations of cell surface proteins characteristic of that cell type.

5. Cell adhesion proteins. Cells use specific proteins to glue themselves to one another. Some act like Velcro, while others form a more permanent bond.

6. Attachments to the cytoskeleton. Surface proteins that interact with other cells are often anchored to the cytoskeleton by linking proteins.

How nonpolar regions lock proteins into membranes. A spiral helix of nonpolar amino acids (red) extends across the nonpolar lipid interior, while polar (purple) portions of the protein protrude out from the bilayer. The protein cannot move in or out because such a movement would drag nonpolar segments of the protein into contact with water.

The many proteins embedded within a membrane carry out a host of functions, many of which are associated with transport of materials or information across the membrane.

Plasma membranes are supported by a network of fibers and coated on the exterior with cell identity markers.

The fluid mosaic model proposes that membrane proteins are embedded within the lipid bilayer. Membranes are composed of a lipid bilayer within which proteins are anchored. Plasma membranes are supported by a network of fibers and coated on the exterior with cell identity markers.

PASSIVE TRANSPORT ACROSS MEMBRANES MOVES DOWN THE CONCENTRATION GRADIENT

Diffusion. Molecules and ions dissolved in water are in constant motion, moving about randomly. This random motion causes a net movement of these substances from regions where their concentration is high to regions where their concentration is lower, a process called **diffusion** (Fig. 2.3). Net movement driven by diffusion will continue until the concentrations in all regions are the same. You can demonstrate diffusion by filling a jar to the brim with ink, capping it, placing it at the bottom of a bucket of water, and then carefully removing the cap. The ink molecules will slowly diffuse out from the jar until

there is a uniform concentration in the bucket and the jar. This uniformity in the concentration of molecules is a type of equilibrium.

Diffusion is the net movement of substances to regions of lower concentration as a result of random spontaneous motion. It tends to distribute substances uniformly. Membrane transport proteins allow only certain molecules and ions to diffuse through the plasma membrane.

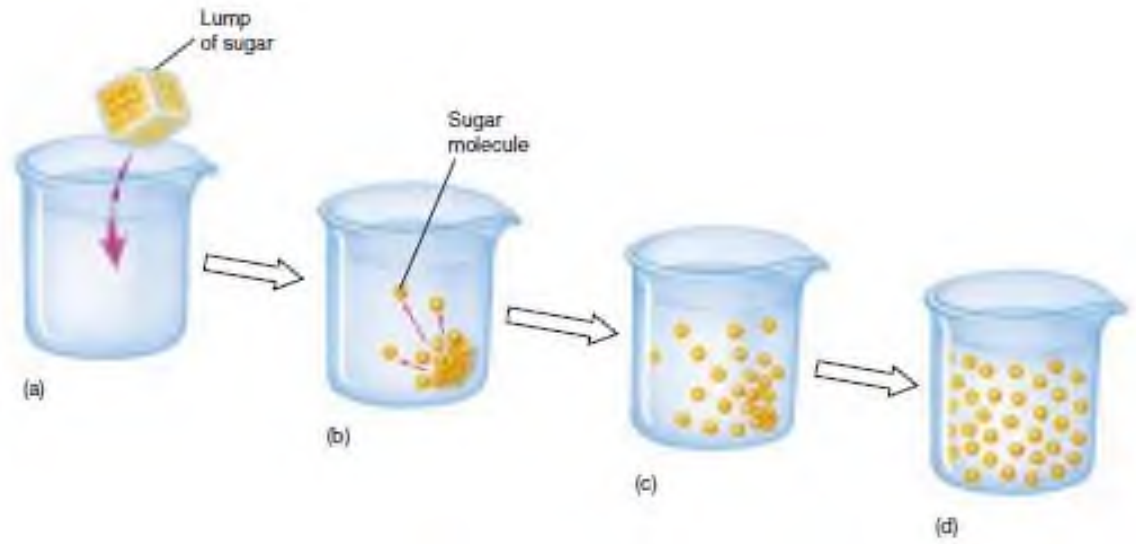


FIGURE 2.3. Diffusion. If a lump of sugar is dropped into a beaker of water (*a*), its molecules dissolve (*b*) and diffuse (*c*). Eventually, diffusion results in an even distribution of sugar molecules throughout the water (*d*).

Facilitated diffusion is a carrier-mediated transport process. Carriers, another class of membrane proteins, transport ions as well as other solutes like sugars and amino acids across the membrane. Like channels, carriers are specific for a certain type of solute and can transport substances in either direction across the membrane. Unlike channels, however, they facilitate the movement of solutes across the membrane by physically binding to them on one side of the membrane and releasing them on the other. Again, the direction of the solute's net movement simply depends on its *concentration gradient* across the membrane. If the concentration is greater in the cytoplasm, the solute is more likely to bind to the carrier on the cytoplasmic side of the membrane and be released on the extracellular side. This will cause a net movement from inside to outside. If the concentration is greater in the extracellular fluid, the net movement will be from outside to inside. Thus, the net movement always occurs

from areas of high concentration to low, just as it does in simple diffusion, but carriers facilitate the process. For this reason, this mechanism of transport is sometimes called **facilitated diffusion** (Fig. 2.4).

Facilitated diffusion provides the cell with a ready way to prevent the buildup of unwanted molecules within the cell or to take up needed molecules, such as sugars, that may be present outside the cell in high concentrations. Facilitated diffusion has three essential characteristics:

1. It is specific. Any given carrier transports only certain molecules or ions.

2. It is passive. The direction of net movement is determined by the relative concentrations of the transported substance inside and outside the cell.

3. It saturates. If all relevant protein carriers are in use, increases in the concentration gradient do not increase the transport rate.

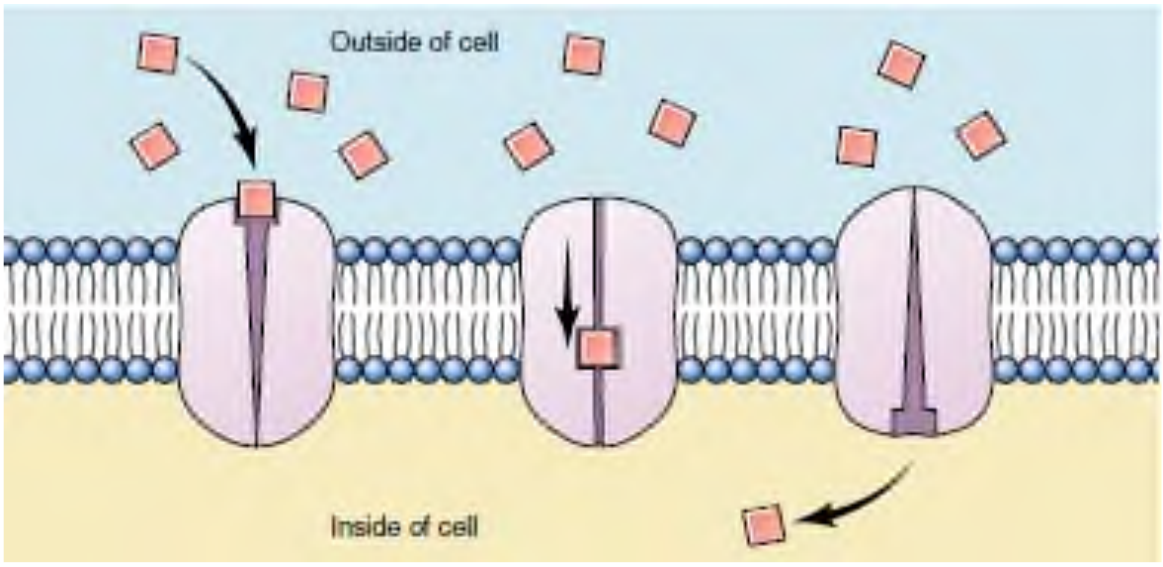


FIGURE 2.4. Facilitated diffusion is a carrier-mediated transport process. Molecules bind to a receptor on the extracellular side of the cell and are conducted through the plasma membrane by a membrane protein.

Facilitated diffusion is the transport of molecules and ions across a membrane by specific carriers in the direction of lower concentration of those molecules or ions.

Molecules bind to a receptor on the extracellular side of the cell and are conducted through the plasma membrane by a membrane protein.

Osmosis. Osmosis is the diffusion of water, but not solutes, across a membrane (Fig. 2.5). In a hyperosmotic solution water moves out of the cell

toward the higher concentration of solutes, causing the cell to shrivel. In an isosmotic solution, the concentration of solutes on either side of the membrane is the same.Osmosis still occurs, but water diffuses into and out of the cell at the same rate, and the cell doesn't change size. In a hypoosmotic solution the concentration of solutes is higher within the cell than without, so the net movement of water is into the cell. In a hyperosmotic solution water moves out of the cell toward the higher concentration of solutes, causing the cell to shrivel. In an isosmotic solution, the concentration of solutes on either side of the membrane is the same. Osmosis still occurs, but water diffuses into and out of the cell at the same rate, and the cell doesn't change size. In a hypoosmotic solution the concentration of solutes is higher within the cell than without, so the net movement of water is into the cell.

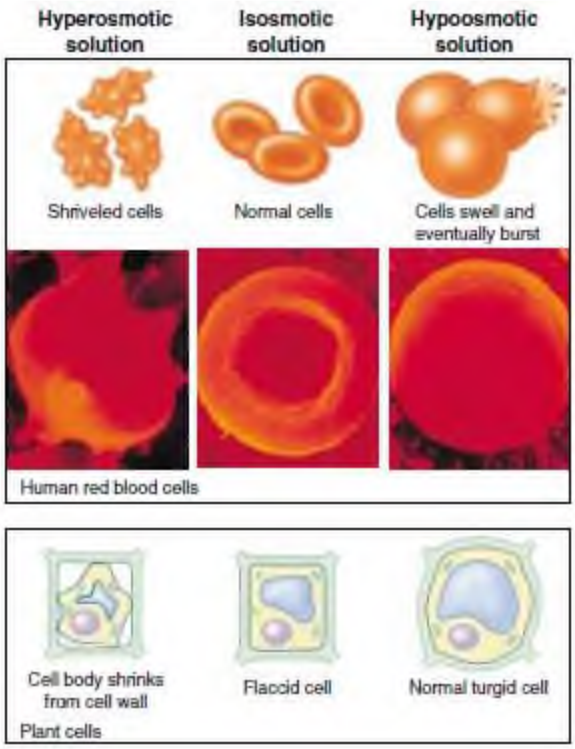


FIGURE 2.5. Osmosis. In a hyperosmotic solution water moves out of the cell toward the higher concentration of solutes, causing the cell to shrivel. In an isosmotic solution, the concentration of solutes on either side of the membrane is the same. Osmosis still occurs, but water diffuses into and out of the cell at the same rate, and the cell doesn't change size. In a hypoosmotic s lution the concentration of solutes is higher within the cell than without, so the net movement of water is into the cell.

How solutes create osmotic pressure. Charged or polar substances are soluble in water because they form hydrogen bonds with water molecules clustered around them. When a polar solute (illustrated here with urea) is added to the solution on one side of a membrane, the water molecules that gather around each urea molecule are no longer free to diffuse across the membrane; in effect, the polar solute has reduced the number of free water molecules on that side of

the membrane increasing the osmotic pressure. Because the hypoosmotic side of the membrane (on the right, with less solute) has more unbound water molecules than the hyperosmotic side (on the left, with more solute), water moves by diffusion from the right to the left.

Turgor. Most plant cells are hyperosmotic to their immediate environment, containing a high concentration of solutes in their central vacuoles. The resulting internal hydrostatic pressure, known as **turgor pressure,** presses the plasma membrane firmly against the interior of the cell wall, making the cell rigid. The newer, softer portions of trees and shrubs depend on turgor pressure to maintain their shape, and wilt when they lack sufficient water.

Active transport across membranes is powered by energy from ATP.

Active transport moves a solute across a membrane up its concentration gradient, using protein carriers driven by the expenditure of chemical energy.

The sodium-potassium pump. The sodium-potassium pump works through a series of conformational changes in the transmembrane protein (Fig. 2.6):

Step 1. Three sodium ions bind to the cytoplasmic side of the protein, causing the protein to change its conformation.

Step 2. In its new conformation, the protein binds a molecule of ATP and cleaves it into adenosine diphosphate and phosphate (ADP + Pi). ADP is released, but the phosphate group remains bound to the protein. The protein is now phosphorylated.

Step 3. The phosphorylation of the protein induces a second conformational change in the protein. This change translocates the three Na^+ across the membrane, so they now face the exterior. In this new conformation, the protein has a low affinity for Na^+, and the three bound Na^+ dissociate from the protein and diffuse into the extracellular fluid.

Step 4. The new conformation has a high affinity for K^+, two of which bind to the extracellular side of the protein as soon as it is free of the Na^+.

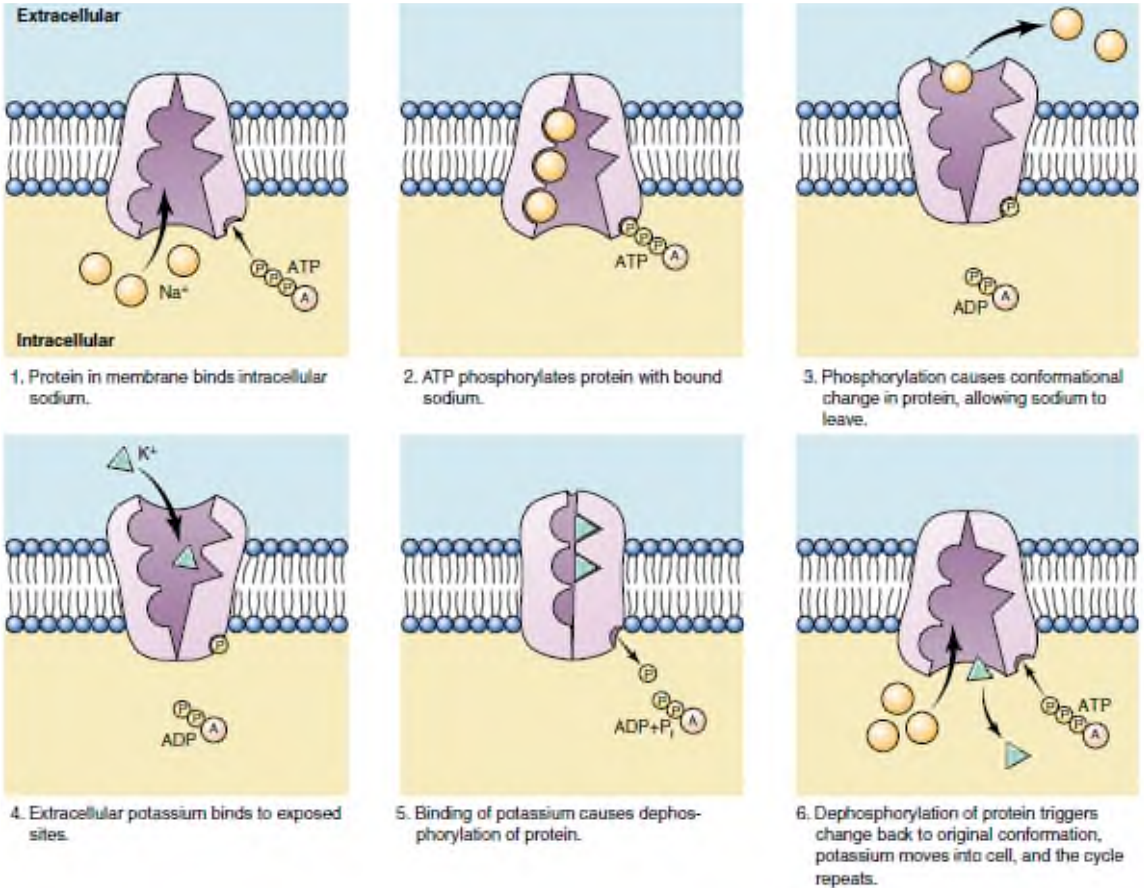


FIGURE 2.6. The sodium-potassium pump. The protein channel known as the sodium-potassium pump transports sodium (Na^+) and potassium (K^+). ions across the cell membrane. For every three Na^+ that are transported out of the cell, two K^+ are transported into the cell. The sodium-potassium pump is fueled by ATP.

Step 5. The binding of the K^+ causes another conformational change in the protein, this time resulting in the dissociation of the bound phosphate group.

Step 6. Freed of the phosphate group, the protein reverts to its original conformation, exposing the two K^+ to the cytoplasm. This conformation has a low affinity for K^+, so the two bound K^+ dissociate from the protein and diffuse into the interior of the cell. The original conformation has a high affinity for Na^+; when these ions bind, they initiate another cycle. Three Na^+ leave the cell and two K+.

Cotransport through a coupled transport protein. A membrane protein transports sodium ions into the cell, down their concentration gradient, at the same time it transports a sugar molecule into the cell. The gradient driving the Na^+ entry is so great that sugar molecules can be brought in *against* their concentration gradient.

Endocytosis. Cells import bulk materials by engulfing them with their plasma membranes in a process called endocytosis; similarly, they extrude or secrete material through exocytosis (Fig. 2.7). Both phagocytosis (*a*) and pinocytosis (*b*) are forms of endocytosis.

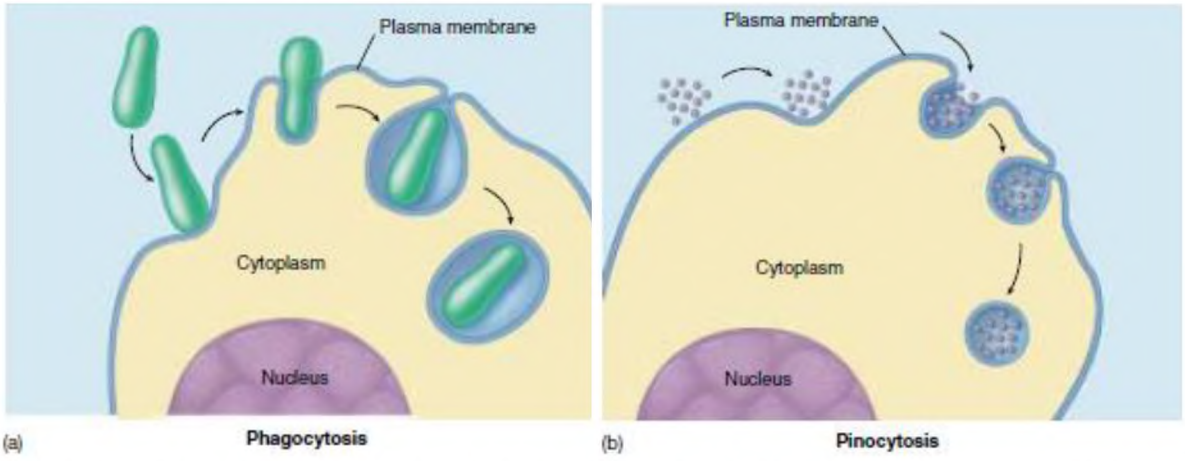


FIGURE 2.7. Endocytosis. Both phagocytosis (*a*) and pinocytosis (*b*) are forms of endocytosis.

Receptor-mediated endocytosis. Cells that undergo receptor-mediated endocytosis have pits coated with the protein clathrin that initiate endocytosis when target molecules bind to receptor proteins in the plasma membrane. A coated pit appears in the plasma membrane of a developing egg cell, covered with a layer of proteins (80,000×). When an appropriate collection of molecules gathers in the coated pit, the pit deepens and seals off to form a coated vesicle, which carries the molecules into the cell.

Exocytosis. Proteins and other molecules are secreted from cells in small packets called vesicles, whose membranes fuse with the plasma membrane, releasing their contents to the cell surface. (*b*) A transmission electron micrograph showing exocytosis.

Key Questions:

1. Plasma membrane structure (the Fluid-Mosaic Model).
2. Biological membranes are fluid layers of lipid.
3. The Phospholipid Bilayer.
4. The main functions of cell membrain.
5. Kinds and functions of Membrane Proteins.

6. Passive transport across cell membrane:

   6.1.Diffusion

   6.2.Facilitated diffusion

   6.3.Osmosis

   6.4. Active transport across cell membrane (on example of Na^+/K^+ pump).

7. Exocytosis

8. Endocytosis (phagocytosis and pinocytosis).

Examples of Review questions

NN	Questions	Right answers
1	THE MAJOR FACTOR LIMITING CELL SIZE IS THE ___ . 1) concentration of water in the cytoplasm. 2) need for energy. 3) presence of membranous organelles. 4) ratio of surface area to volume. 5) composition of the plasma membrane.	**5**
2	PHOSPHOLIPIDS ___ . 1) have hydrophilic fatty acid tails. 2) have charged, hydrophobic head groups. 3) form lipid bilayers. 4) form droplets within the cytoplasm. 5) all of the above.	**3**
3	THE FUNCTIONS OF CELL PROTEINS: 1) structural, catalyst, transportable; 2) protective, contractive, regulative; 3) receptive, energy, toxic; 4) all answers are correct	**4**
4	TRANSPORT OF SUBSTANCES ACROSS THE PLASMA MEMBRANE UP THEIR CONCENTRATION GRADIENTS IS PERFORMED: 1) diffusion; 2) osmosis; 3) active transport; 4) endocytosis.	**1**
5	PHAGOCYTES KILL HARMFUL BACTERIA BY ___ . 1) endocytosis. 2) producing antibodies. 3) complement. 4) T cell stimulation. 5) inflammation.	**1**

2.2. CELL CYTOPLASM STRUCTURE.

STRUCTURE OF EUKARYOTIC CELLS

Eukaryotic cells contain membrane-bounded organelles that carry out specialized functions (Fig.2.8, Table.2.1).

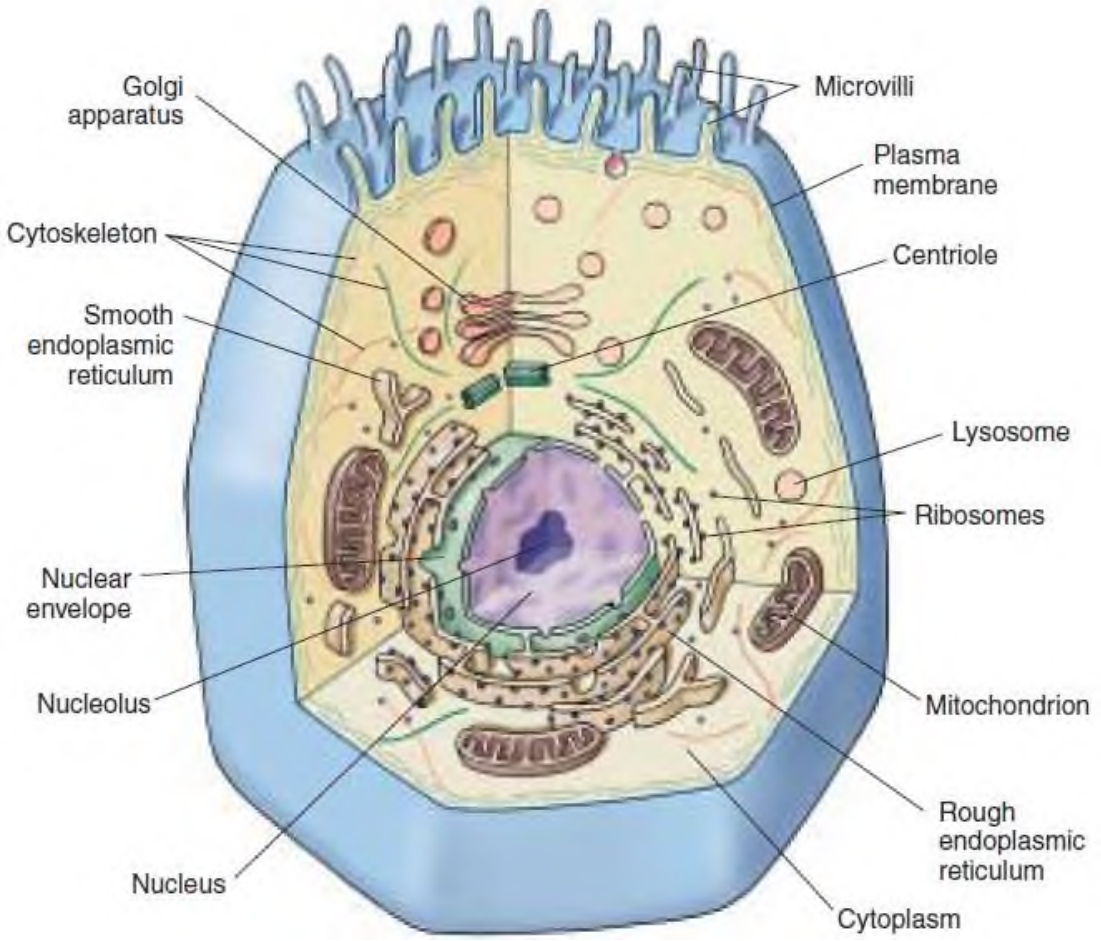


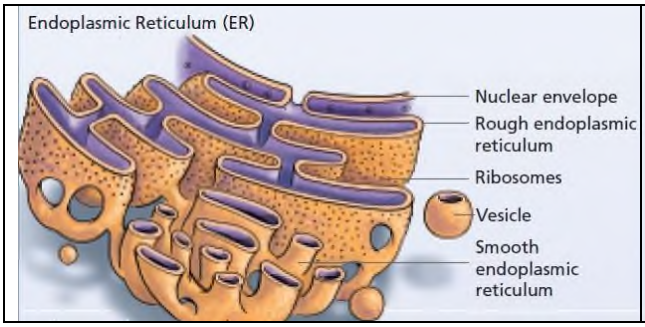
FIGURE 2.8. Structure of an animal cell.

Table 2.1.

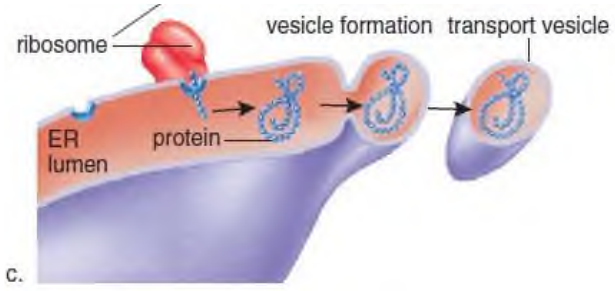
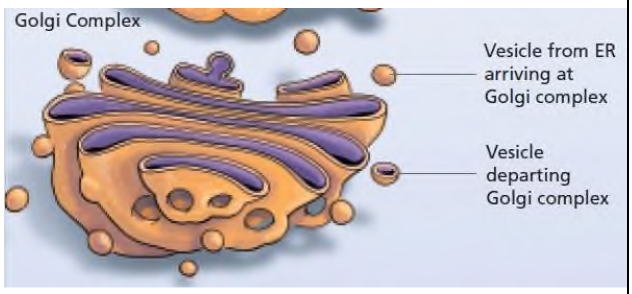
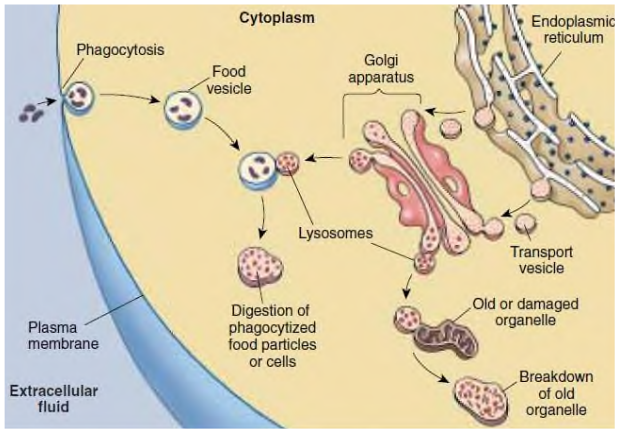
Eukaryotic Cell Structure and their Function

Table 2.1. Eukaryotic Cell Structure and their Function

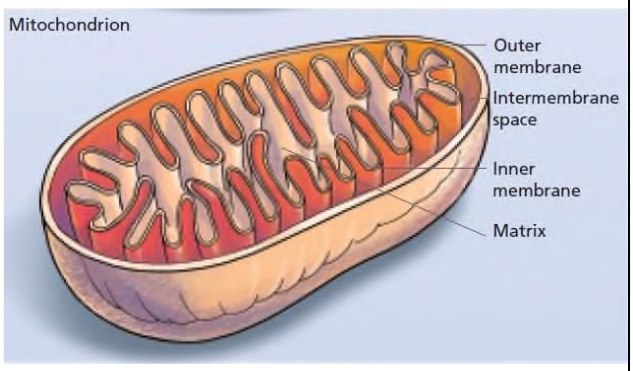
Structure	Description	Function
Cell wall	Outer layer of cellulose or chitin; or absent	Protection; support
Cytoskeleton	Network of protein filaments	Structural support, cell movement
Flagella (cilia)	Cellular extensions with *9 +2* arrangement of pairs *of* microtubules	Motility or moving fluids over surfaces
Plasma membrane	Lipid bilayer with embedded proteins	Regulates what passes into and out of cell; cell-to-cell recognition
Endoplasmic reticulum	Network of internal membranes	Forms compartments and vesicles; participates in protein and lipid synthesis
Nucleus	Structure (usually spherical) surrounded by double membrane that contains chromosomes	Control center of cell; directs protein synthesis and cell reproduction
Golgi apparatus	Stacks of flattened vesicles	Packages proteins for export from cell; forms secretory vesicles
Lysosomes	Vesicles derived from Golgi apparatus that contain hydrolytic digestive enzymes	Digest worn-out organelles and cell debris; play role in cell death
Microbodies	Vesicles formed from incorporation of lipids and proteins containing oxidative and other enzymes	Isolate particular chemical activities from rest of cell
Mitochondria	Bacteria-like elements with double membrane	"Power plants" of the cell; sites of oxidative metabolism
Chloroplasts	Bacteria-like elements with membranes containing chlorophyll, a photosynthetic pigment	Sites of photosynthesis
Chromosomes	Long threads of DNA that form a complex with protein	Contain hereditary information
Nucleolus	Site of genes for rRNA synthesis	Assembles ribosomes
Ribosomes	Small, complex assemblies of protein and RNA, often bound to endoplasmic reticulum	Sites of protein synthesis



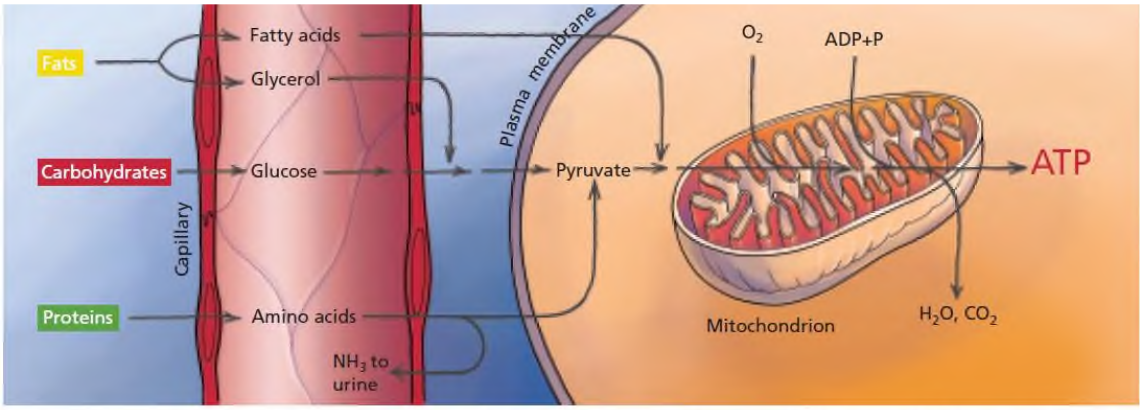
The ER is a large network of membranes that begins at the nuclear envelope and extends into the cytoplasm. ER with ribosomes attached is called rough ER. Rough ER manufactures proteins that will be secreted from the cell

	or that will become part of the plasma membrane. ER without ribosomes attached is called smooth ER. The function of the smooth ER depends on cell type but includes tasks such as detoxifying harmful substances and synthesizing lipids. Vesicles are pinched-off pieces of membrane that transport substances to Golgi complex or plasma membrane. On the rough ER a protein made at a ribosome moves into the lumen of the system and eventually is packaged in a transport vesicle for distribution inside the cell.
	The Golgi complex (GC) is a stack of membranous sacs. Vesicles from the ER fuse with the GC and empty their protein contents. The proteins are then modified, sorted, and sent to the correct destination in new transport vesicles that bud off from one of the sacs.
	A lysosome is a membrane-enclosed sac of digestive enzymes that degrade proteins, carbohydrates, and fats. Lysosomes roam around the cell, and engulf targeted molecules and organelles for recycling. Only animal cells contain lysosomes. Lysosomes that are not functioning actively do not maintain an acidic internal pH and are called *primary lysosomes.* When a primary lysosome fuses with a food vesicle or other organelle, its pH falls and its arsenal of hydrolytic enzymes is activated; it is then called a *secondary lysosome.* In addition to breaking down organelles and other structures within cells, lysosomes also eliminate other cells that the cell has engulfed in a process called *phagocytosis,* a specific type of

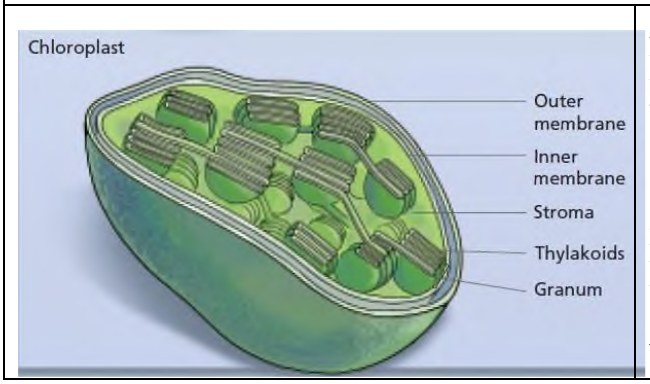
endocytosis. When a white blood cell, for example, phagocytizes a passing pathogen, lysosomes fuse with the resulting "food vesicle," releasing their enzymes into the vesicle and degrading the material within.



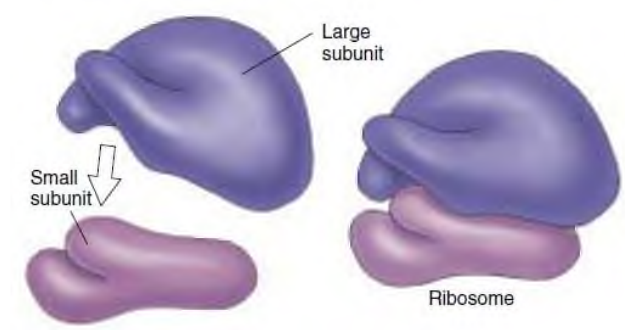
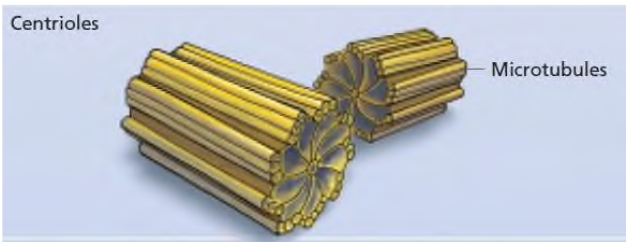
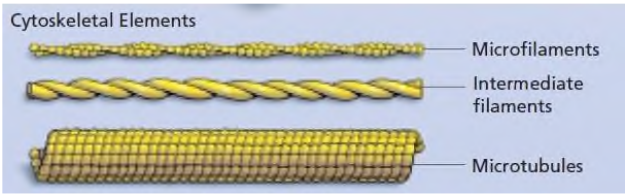
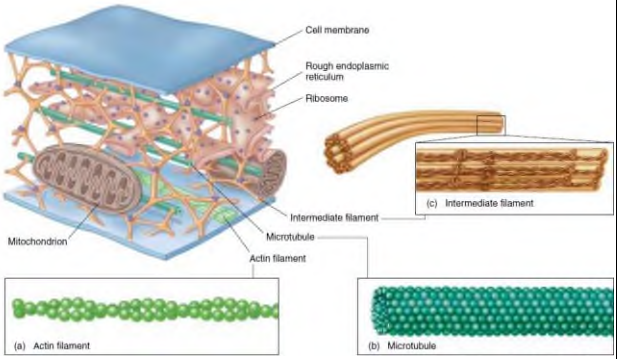
Mitochondria are energy-producing organelles composed of two membranes. The inner and outer mitochondrial membranes are separated by the intermembrane space. The highly convoluted inner membrane carries many of the proteins involved in producing ATP. The outer membrane regulates traffic into and out of the mitochondrion. The matrix of the mitochondrion is the location of many of the reactions of cellular respiration.

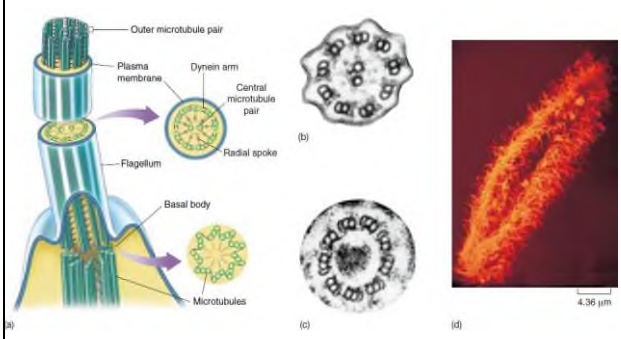


Cellular respiration. Carbohydrates, proteins, and fats are broken down inside a mitochondrion. The energy released by their digestion is used to synthesize ATP.



An important organelle present only in plant cells, the chloroplast uses the sun's energy to convert carbon dioxide and water into sugars. Each chloroplast has an outer membrane, an inner membrane, a liquid material called the stroma, and a network of flattened membranes called thylakoids that stack

	on one another to form structures called grana (singular granum).
	Ribosomes consist of a large and a small subunit composed of rRNA and protein. The individual subunits are synthesized in the nucleolus and then move through the nuclear pores to the cytoplasm, where they assemble. Ribosomes serve as sites of protein synthesis.
	Centrioles are barrel-shaped rings composed of nine microtubule triplets. Microtubules help move chromosomes around when a cell divides. Centrioles are involved in microtubule formation during cell division and the formation of cilia and flagella. Centrioles are present only in animal cells.
 	Cytoskeletal elements are protein fibers in the cytoplasm that give shape to a cell, hold and move organelles (including transport vesicles), and are typically involved in cell movement. **Molecules that make up the cytoskeleton**. (*a*) Actin filaments. Actin filaments are made of two strands of the fibrous protein actin twisted together and usually occur in bundles. Actin filaments are ubiquitous, although they are concentrated below the plasma membrane in bundles known as stress fibers, which may have a contractile function. (*b*) Microtubules. Microtubules are composed of 13 stacks of tubulin protein subunits arranged side by side to form a tube. Microtubules are comparatively stiff cytoskeletal elements that serve to organize metabolism and intracellular transport in the nondividing cell. (*c*) Intermediate filaments. Intermediate filaments are composed of overlapping stag-

	gered tetramers of protein. This molecular arrangement allows for a ropelike structure that imparts tremendous mechanical strength to the cell.
	**Flagella and cilia.** (*a*) A eukaryotic flagellum originates directly from a basal body. (*b*) The flagellum has two microtubules in its core connected by radial spokes to an outer ring of nine paired microtubules with dynein arms. (*c*) The basal body consists of nine microtubule triplets connected by short protein segments. The structure of cilia is similar to that of flagella, but cilia are usually shorter. (*d*) The surface of this *Paramecium* is covered with a dense forest of cilia.

The central vacuole. A plant's central vacuole stores dissolved substances and can increase in size to increase the surface area of a plant cell. Plant cells store substances in a large central vacuole, and encase themselves within a strong cellulose cell wall.

Cell walls in plants. Plant cell walls are thicker, stronger, and more rigid than those of bacteria. Primary cell walls are laid down when the cell is young. Thicker secondary cell walls may be added later when the cell is fully grown

Key Questions:

1. What are the three principles of the cell theory?

2. Cell membrane organelles, classification.

3. What is the endoplasmic reticulum? What is its function? How does rough ER differ from smooth ER?

4. What is the function of the Golgi apparatus? How do the substances released by the Golgi apparatus make their way to other locations in the cell?

5. Lysosomes: Intracellular Digestion Centers. Peroxisomes.

6. What types of eukaryotic cells contain mitochondria? What function do mitochondria perform?

7. What unique metabolic activity occurs in chloroplasts?

8. What is the function of the ribosomes?

9. What cellular functions do centrioles participate in?

10. What kinds of cytoskeleton fibers are stable and which are changeable?

11. How do cilia compare with eukaryotic flagella?

12. What are differences between animal and plant cells?

Examples of Review questions

NN	Questions	Right answers
1	MEMBRANE ORGANELLES OF THE CELL ARE: 1) microtubules, a cell center 2) endoplasmic reticulum, vacuoles, centrioles 3) endoplasmic reticulum, mitochondria, lysosomes, vacuoles 4) plastids, ribosomes, vacuoles	**3**
2	ON THE RIBOSOMES PERFORMED OF SYNTESIS: 1) carbohydrates, lipids 2) ATP, NADP 3) polypeptides 4) all hormones	**3**
3	THE GOLGI APPARATUS___ . 1) is found only in animals 2) is found in prokaryotes 3) is the appendage that moves a cell around in its environment 4) is a site of rapid ATP production 5) packages and modifies proteins	**5**
4	THE CYTOSKELETON CONSISTS OF ___ . 1) cilia, flagella, and microfilaments. 2) cilia, microtubules, and microfilaments. 3) internal cell walls. 4) microtubules, intermediate filaments, and microfilaments. 5) calcified microtubules.	**4**
5	THE MAIN DIFFERENCES BETWEEN PLANT AND ANIMAL CELLS. THE CELLS OF PLANTS CONTAIN: 1) nucleus, lysosomes, vacuoles; 2) mitochondria, plastids, ribosomes; 3) cell-wall, chloroplasts, vacuoles; 4) cell membrane, ribosomes, vacuoles.	**3**

2.3. THE NUCLEUS STRUCTURE

THE NUCLEUS

The nucleus of a eukaryotic cell contains the cell's hereditary apparatus and isolates it from the rest of the cell (Fig. 2.9).

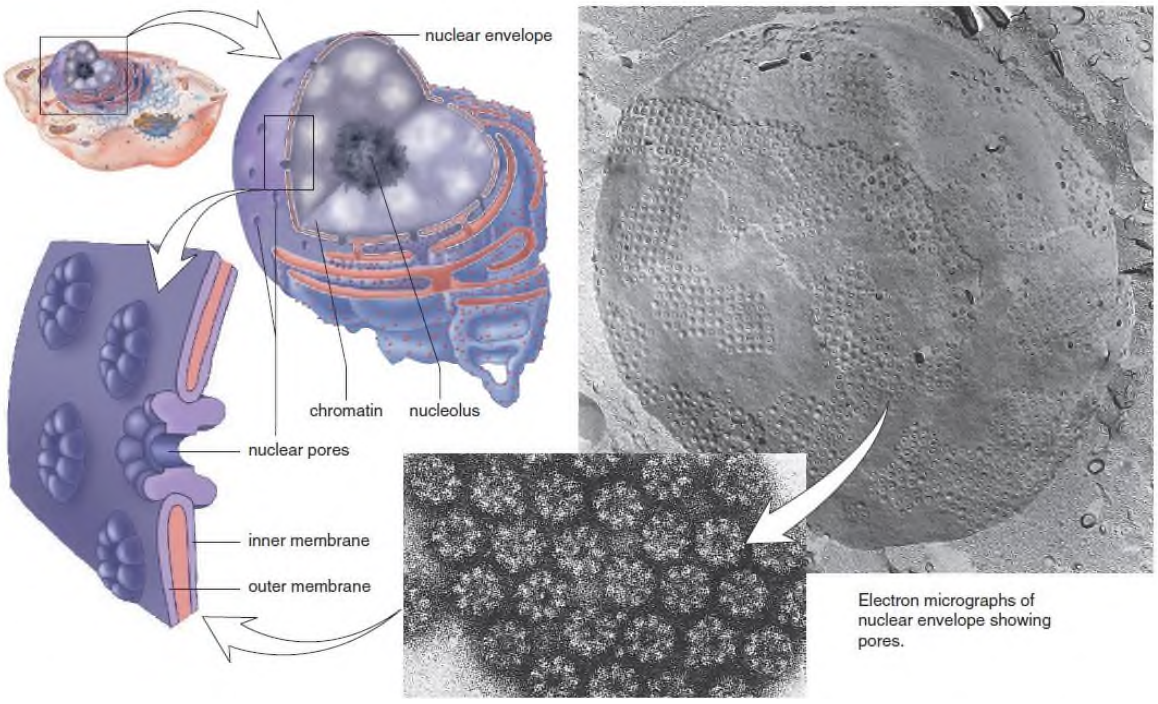


FIGURE 2.9. The nucleus and the nuclear envelope. The nucleus contains chromatin. chromatin has a special region called the nucleolus, which is where rRNA is produced and ribosomal subunits are assembled. The nuclear envelope contains pores, as shown in this micrograph of a freeze-fractured nuclear envelope. Each pore is lined by a complex of eight proteins.

The surface of the nucleus is bounded by *two* phospholipid bilayer membranes called a nuclear envelope, enclosing a fluid-filled interior containing the chromosomes. Scattered over the surface of the nuclear envelope, like craters on the moon, are shallow depressions called **nuclear pores.**

In both bacteria and eukaryotes, DNA contains the hereditary information specifying cell structure and function. However, unlike the circular DNA of bacteria, the DNA of eukaryotes is divided into several linear **chromosomes.** Except when a cell is dividing, its chromosomes are fully extended into

threadlike strands, called **chromatin,** of DNA complexed with protein (Fig. 2.10).

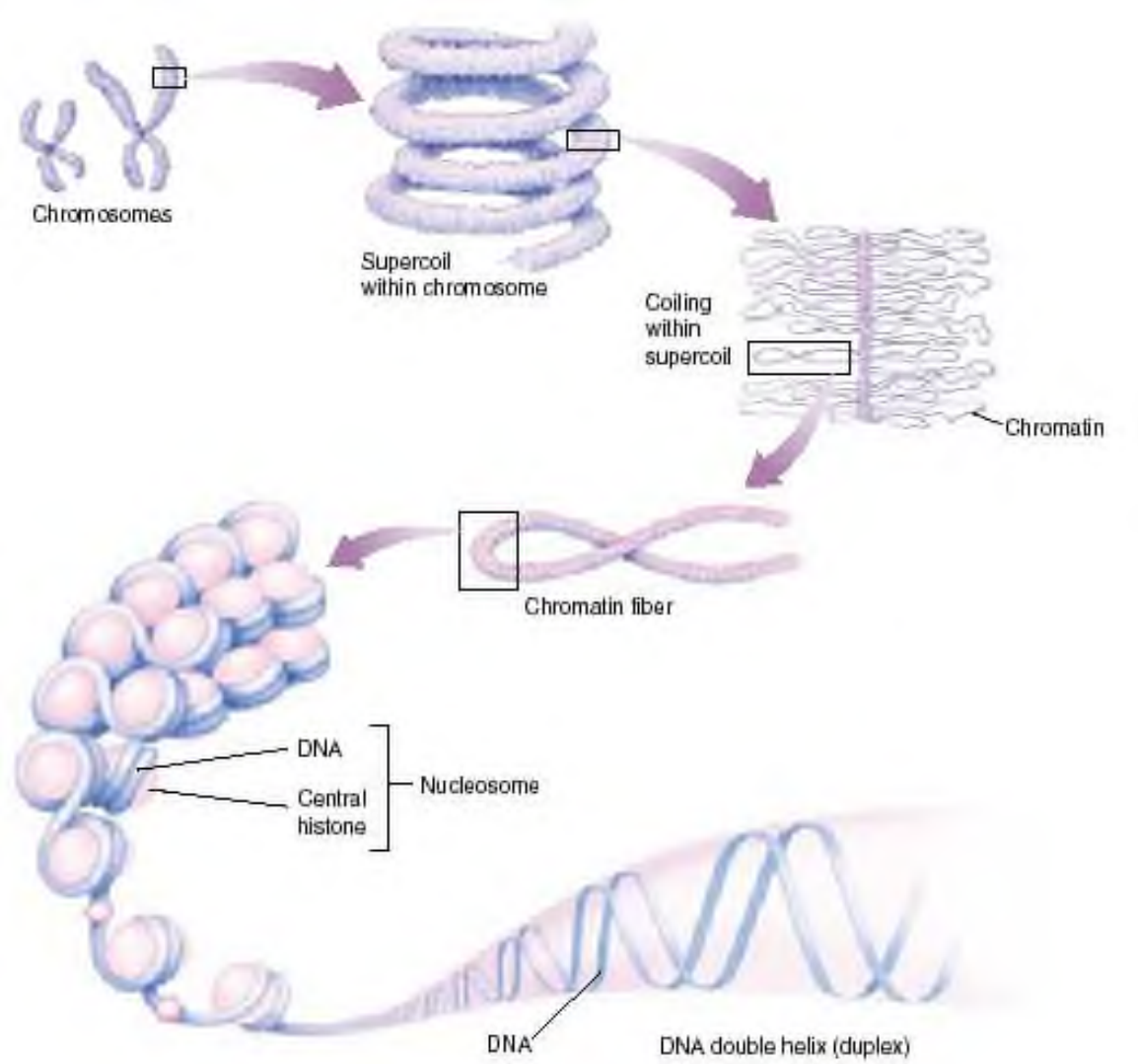


FIGURE 2.10. Levels of eukaryotic chromosomal organization. Nucleotides assemble into long double strands of DNA molecules. These strands require further packaging to fit into the cell nucleus. The DNA duplex is tightly bound to and wound around proteins called *histones.*The DNA-wrapped histones are called *nucleosomes.* The nucleosomes then coalesce into *chromatin* fibers, ultimately coiling around into *supercoils* that make up the form of DNA recognized as a *chromosome.*

This open arrangement allows proteins to attach to specific nucleotide sequences along the DNA. Without this access, DNA could not direct the day-to-day activities of the cell. The chromosomes are associated with packaging proteins called **histones.** When a cell prepares to divide, the DNA coils up around the histones into a highly condensed form. In the initial stages of this

condensation, units of histone can be seen with DNA wrapped around like a sash. Called **nucleosomes,** these initial aggregations resemble beads on a string. Coiling continues until the DNA is in a compact mass. Under a light microscope, these fully condensed chromosomes are readily seen in dividing cells as densely staining rods. After cell division, eukaryotic chromosomes uncoil and can no longer be individually distinguished with a light microscope. Uncoiling the chromosomes into a more extended form permits enzymes to makes RNA copies of DNA. Only by means of these RNA copies can the information in the DNA be used to direct the synthesis of proteins.

Chromosomes may differ widely in appearance. They vary in size, staining properties, the location of the *centromere* (a constriction found on all chromosomes), the relative length of the two arms on either side of the centromere, and the positions of constricted regions along the arms. With the exception of the **gametes** (eggs or sperm) and a few specialized tissues, every cell in a human body is **diploid (2*n*).** This means that the cell contains two nearly identical copies of each of the 23 types of chromosomes, for a total of 46 chromosomes. The **haploid (1*n*)** gametes contain only one copy of each of the 23 chromosome types, while certain tissues have unusual numbers of chromosomes—many liver cells, for example, have two nuclei, while mature red blood cells have no nuclei at all. The two copies of each chromosome in body cells are called **homologous chromosomes,** or **homologues** (Greek *homologia,* "agreement"). Before cell division, each homologue replicates,producing two identical **sister chromatids** joined at the **centromere,** a condensed area found on all eukaryotic chromosomes (Fig. 2.11).

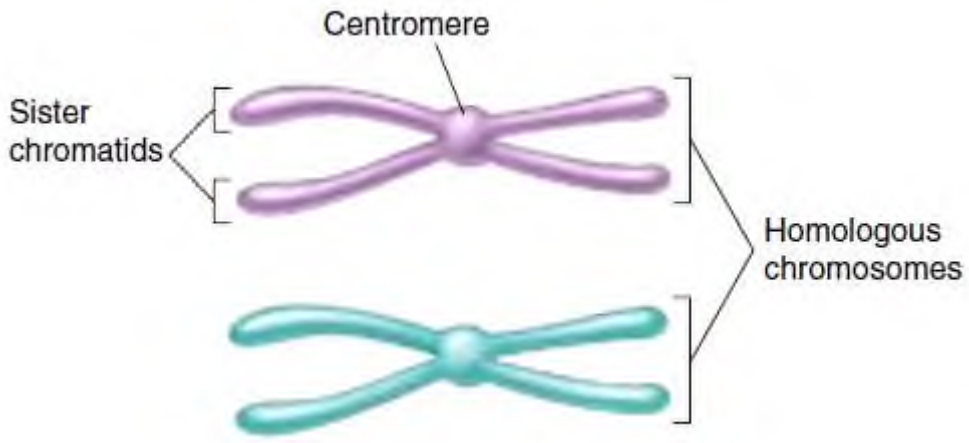


FIGURE 2.11. The difference between homologous chromosomes and sister chromatids.

The particular array of chromosomes that an individual possesses is called its **karyotype**. Karyotypes show marked differences among species and sometimes even among individuals of the same species. The 24 human chromosome types are numbered from largest to smallest—1 to 22—although chromosome 21 is actually the smallest. The other two chromosomes are the X and the Y. Early attempts to sizeorder chromosomes resulted in generalized groupings because many of the chromosomes are of similar size. Centromere position is one distinguishing feature of chromosomes. A chromosome is **metacentric** if the centromere divides it into two arms of approximately equal length. It is **submetacentric** if the centromere establishes one long arm and one short arm, and **acrocentric** if it pinches off only a small amount of material toward one end (Fig. 2.12). Some species have telocentric chromosomes that have only one arm, but humans do not. The long arm of a chromosome is designated *q*, and the short arm *p*, where *p* stands for "petite."

Heterochromatin. The general material that collectively composes a chromosome is called **chromatin** by cytogeneticists. When chromosomes are treated with chemicals that react with DNA, such as Feulgen stain, distinct regions with different staining characteristics become visible. Densely staining regions are called **heterochromatin.**

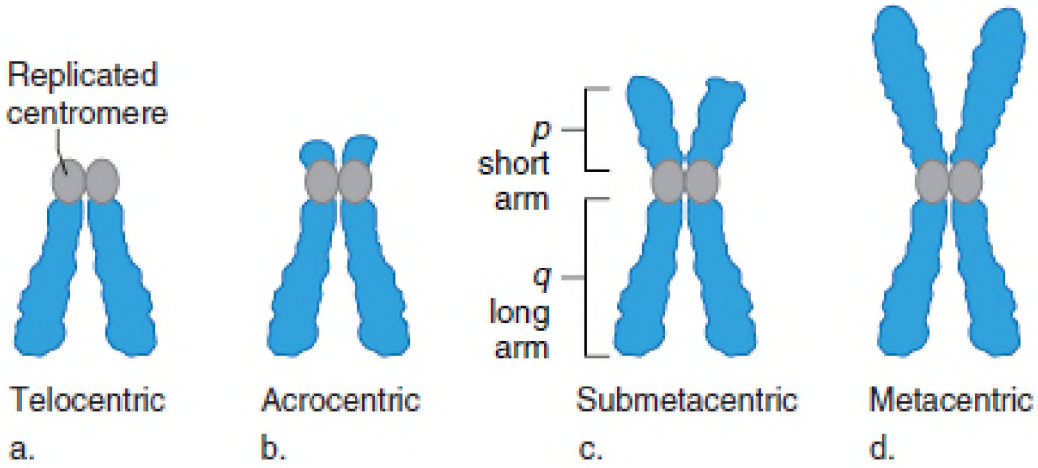


FIGURE 2.12. Centromere position is used to distinguish chromosomes. (*a*) A telocentric chromosome has the centromere at one end. (*b*) An acrocentric chromosome has the centromere near an end. (*c*) A submetacentric chromosome's centromere creates a long arm (*q*) and a short arm (*p*). (*d*) A metacentric chromosome's centromere creates equalsized arms.

Poorly staining regions are said to be **euchromatin.** The distinction is the result of the degree of compactness, or coiling, of the DNA in the chromosome. The position of much of the heterochromatin on the chromosome is constant and is, in this sense, a hereditary feature. We now know that most of the active genes are located in euchromatin. Euchromatin stains less densely because it is packed less tightly, and the general idea is that the looser packing makes genes more accessible for transcription and hence gene activity. The question of how euchromatin and heterochromatin are maintained in more or less constant position is under current investigation.

Euchromatin contains most of the active genes. Heterochromatin is more condensed and densely staining.

Telomeres are the ends of chromosomes. Generally there is no visible structure that represents the telomere, but at the DNA level it can be distinguished by the presence of distinct nucleotide sequences. The ends of chromosomes represent a special challenge to the chromosomal replication mechanism, and this problem is overcome by the presence at the tips of chromosomes of tandem arrays of simple DNA sequences that do not encode an RNA or a protein product. For example, in the ciliate *Tetrahymena* there is repetition of the sequence TTGGGG, and in humans the repeated sequence is TTAGGG.

CHAPTER 3. CELL CYCLE, AND MITOSIS

PHASES OF THE CELL CYCLE

The increased size and more complex organization of eukaryotic genomes over those of bacteria required radical changes in the process by which the two replicas of the genome are partitioned into the daughter cells during cell division. This division process is diagrammed as a **cell cycle,** consisting of five phases (Fig.3.1).

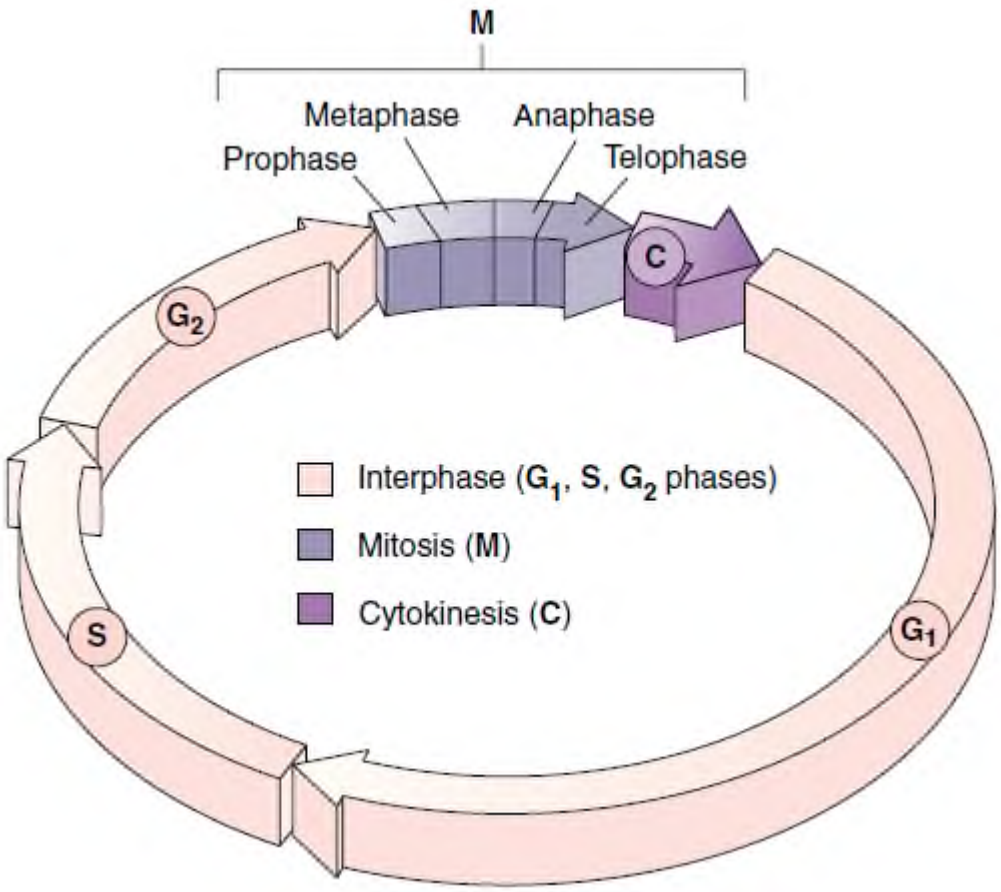


FIGURE 3.1. The cell cycle. Each wedge represents one hour of the 22-hour cell cycle in human cells growing in culture. G_1 represents the primary growth phase of the cell cycle, S the phase during which a replica of the genome is synthesized, and G_2 the second growth phase.

The process of cell division is diagrammed as a **cell cycle,** consisting of five phases. The Five Phases of Cell Cycle are G1, S, G2, M, C. G_1 is the primary growth phase of the cell. Most of the variation in the length of the cell cycle from one organism or tissue to the next occurs in the **G_1 phase**. Cells often pause in G_1 before DNA replication and enter a resting state called **G_0 phase;**

they may remain in this phase for days to years before resuming cell division. At any given time, most of the cells in an animal's body are in G_0 phase. Some, such as muscle and nerve cells, remain there permanently; others, such as liver cells, can resume G_1 phase in response to factors released during injury. For many organisms, this encompasses the major portion of the cell's life span. **S** is the phase in which the cell synthesizes a replica of the genome. **G_2 phase** is the second growth phase, in which preparations are made for genomic separation. During this phase, mitochondria and other organelles replicate, chromosomes condense, and microtubules begin to assemble at a spindle. G1, S, and G2 together constitute **interphase,** the portion of the cell cycle between cell divisions. **M (mitosis)** is the phase of the cell cycle in which the microtubular apparatus assembles, binds to the chromosomes, and moves the sister chromatids apart. This process is the essential step in the separation of the two daughter genomes. We will discuss mitosis as it occurs in animals and plants, where the process does not vary much (it is somewhat different among fungi and some protists). Although mitosis is a continuous process, it is traditionally subdivided into four stages: prophase, metaphase, anaphase, and telophase. **C phase** is the phase of the cell cycle when the cytoplasm divides, creating two daughter cells. This phase is called **cytokinesis.** In animal cells, the microtubule spindle helps position a contracting ring of actin that constricts like a drawstring to pinch the cell in two. In cells with a cell wall, such as plant cells, a plate forms between the dividing cells.

CONTROL OF THE CELL CYCLE

Cells use a centralized control system to check whether proper conditions have been achieved before passing three key "checkpoints" in the cell cycle.

Cell growth is assessed at the G_1 checkpoint. Located near the end of G_1, just before entry into S phase, this checkpoint makes the key decision of whether the cell should divide, delay division, or enter a resting stage. In yeasts, where researchers first studied this checkpoint, it is called START. If

conditions are favorable for division, the cell begins to copy its DNA, initiating S phase. The G_1 checkpoint is where the more complex eukaryotes typically arrest the cell cycle if environmental conditions make cell division impossible, or if the cell passes into G_0 for an extended period.

The success of DNA replication is assessed at the G_2 checkpoint. The second checkpoint, which occurs at the end of G_2, triggers the start of M phase. If this checkpoint is passed, the cell initiates the many molecular processes that signal the beginning of mitosis.

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

Early research into the cell cycle involved fusing cells in different stages of the cycle (such as the G_1, S, and G_2 phases) to determine whether the cytoplasmic components of one cell would affect the behavior of the other. Results of these experiments led to the discovery of a protein complex called the **maturation-promoting factor (MPF)** because of its role in causing oocytes to mature. It is now also referred to as the **mitosis-promoting factor** since it initiates the mitosis phase of the cell cycle. Further research has shown that MPF is made of two proteins, one that oscillates in quantity during the cell cycle and one whose quantity is constant. The oscillating component is referred to as **cyclin;** the constant gene product is an enzyme controlled by the *cdc2* gene (*cdc* stands for *c*ell *d*ivision *c*ycle) called Cdc2p. Cdc2p is a kinase, an enzyme that phosphorylates other proteins, transferring a phosphate group from ATP to an amino acid of the protein it is acting on. (Phosphorylation controls many of the

processes in mitosis and in metabolism in general; for example, the nuclear membrane begins to break down when its subunits are phosphorylated).

Because the Cdc2p kinase works when combined with cyclin, it is referred to as a **cyclindependent kinase (CDK).** Several of these kinasecyclin combinations control stages of the cell cycle; the cyclin of the mitosis- promoting factor is called cyclin B. In general, cylin-dependent kinases are regulated by phosphorylation and dephosphorylation, cyclin levels, and activation or deactivation of inhibitors. Normally, Cdc2p remains at high levels in the cell but does not initiate mitosis for two reasons. First, phosphate groups block its active site, the place on the enzyme that actually does the phosphorylating. Second, the enzyme can only function when it combines with a molecule of cyclin B, the protein that oscillates during the cell cycle.

Cyclin B is at very low levels when mitosis ends. During ensuing cell growth, numbers of cyclin B molecules increase, combining with Cdc2p proteins until a critical quantity is reached. However, Cdc2p-cyclin B complexes are still not active. That requires the product of another gene to dephosphorylate the Cdc2p-cyclin B complex. At that point, the Cdc2p-cyclin B complex goes into action, initiating the changes that begin mitosis (Fig. 3.2).

Presumably the cell is ready for mitosis at that point, having gone through G_1, S, and G_2 phases (which we will discuss in detail later in the chapter). Once mitosis has been initiated, cyclin B, along with other proteins that have served their purpose by this point in the cell cycle, breaks down with the help of a protein complex called the **anaphase-promoting complex (APC),** also called the **cyclosome.** The cyclosome works by attaching a **ubiquitin** molecule to the proteins that are to be broken down. Cdc2p is then phosphorylated to block its active site. The cell now completes mitosis and enters G_1; quantities of cyclin B are very low, and virtually no functioning Cdc2p-cyclin B remains. Thus, active Cdc2p is the kinase that controls the initiation of mitosis.

Some points in the cell cycle, such as the initiation of mitosis, can be delayed until all necessary conditions are in place. These **checkpoints** allow the

cell to make sure that various events have been "checked off" as completed before the next phase begins. **Surveillance mechanisms** that involve dozens of proteins, many just discovered, oversee these checkpoints. In the cell cycle, three checkpoints involve cyclin-dependent kinases; each has its own specific cyclin that initiates either the G_1, S, or mitosis phase.

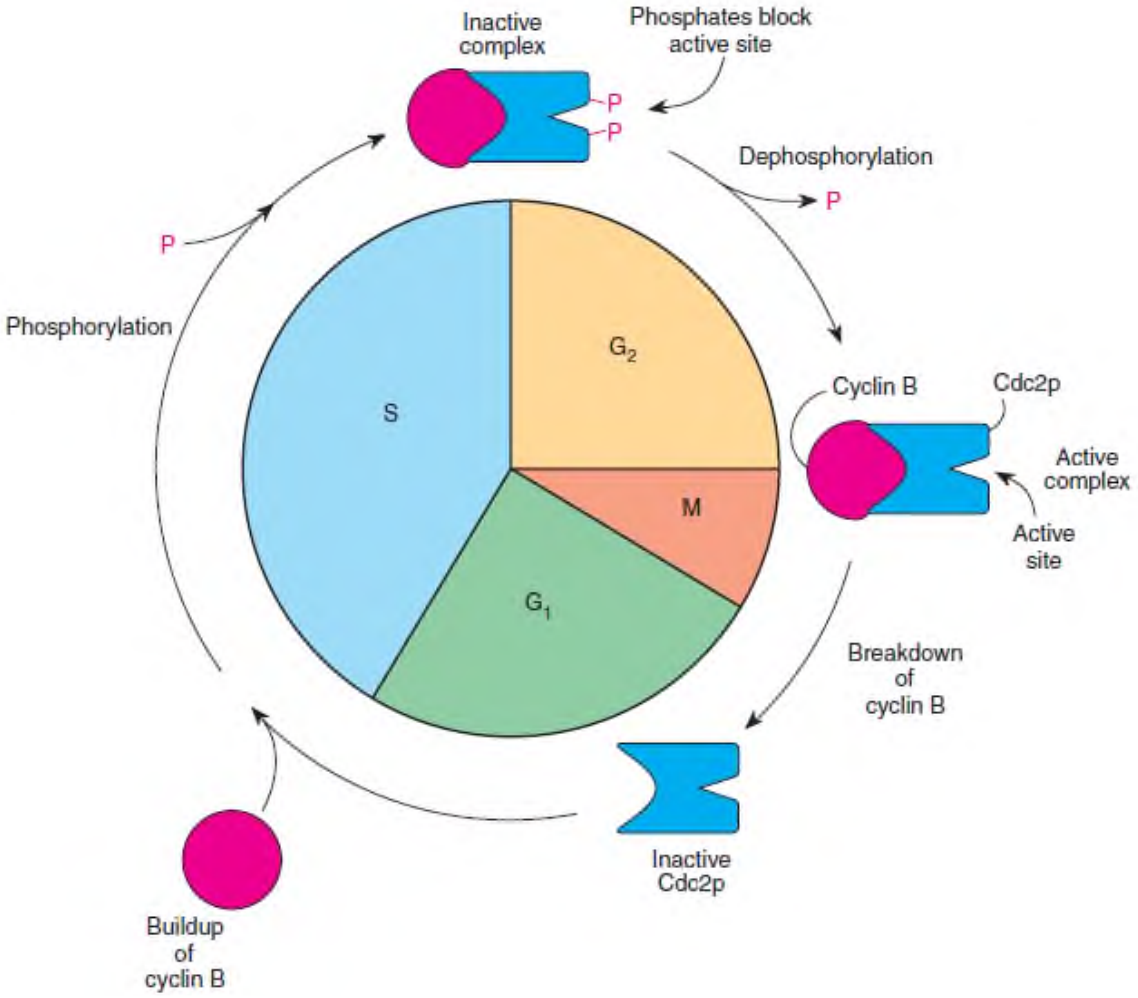


FIGURE 3.2. The proteins Cdc2p (CDK1) and cyclin B combine to form the maturation-promoting (or mitosis-promoting) factor. During mitosis, cyclin B is broken down. During G_1 and S phases, cyclin B builds up and combines with Cdc2P, which is then phosphorylated at the active site to render it inactive. Dephosphorylation, a process that begins to take place only after DNA replication is finished, produces an active maturation-promoting factor.

In addition, other checkpoints that don't involve cyclin-dependent kinases occur at other transition phases in the cell cycle. Cell cycle control is of particular interest because the cell cycle routinely halts if there is genetic damage, giving the cell a chance to repair the damage before committing to cell division.

If the damage is too extreme, the cell can enter a programmed cell death sequence. If these mechanisms fail, cancer may result. The genetic control of the cell cycle is one of the most active areas of current research.

Mitosis is assessed at the M checkpoint. Occurring at metaphase, the third checkpoint triggers the exit from mitosis and cytokinesis and the beginning of G_1.

A complex of two proteins triggers passage through cell cycle checkpoints. Cdk is a protein kinase that activates numerous cell proteins by phosphorylating them. Cyclin is a regulatory protein required to activate Cdk; in other words, Cdk does not function unless cyclin is bound to it.

How cell cycle control works. As the cell cycle passes through the G_1 and G_2 checkpoints, Cdk becomes associated with different cyclins and, as a result, activates different cellular processes. At the completion of each phase, the cyclins are degraded, bringing Cdk activity to a halt until the next set of cyclins appears.

Two groups of proteins, cyclins and Cdks, interact to regulate the cell cycle. Cells also receive protein signals called growth factors that affect cell division.

Controlling the Cell Cycle in Multicellular Eukaryotes. The cells of multicellular eukaryotes are not free to make individual decisions about cell division, as yeast cells are. The body's organization cannot be maintained without severely limiting cell proliferation, so that only certain cells divide, and only at appropriate times. The way that cells inhibit individual growth of other cells is apparent in mammalian cells growing in tissue culture: a single layer of cells expands over a culture plate until the growing border of cells comes into contact with neighboring cells, and then the cells stop dividing. If a sector of cells is cleared away, neighboring cells rapidly refill that sector and then stop dividing again. How are cells able to sense the density of the cell culture around them? Each growing cell apparently binds minute amounts of positive regulatory signals called **growth factors,** proteins that stimulate cell division

(such as MPF). When neighboring cells have used up what little growth factor is present, not enough is left to trigger cell division in any one cell.

Most eukaryotic cells repeat a process of growth and division referred to as the cell cycle. The cycle can vary in length from a few minutes to several years.

MITOSIS

We will discuss **mitosis** as it occurs in animals and plants, where the process does not vary much (it is somewhat different among fungi and some protists). As an aid in describing the events of mitosis, the process is divided into four phases: prophase, metaphase, anaphase, and telophase (Fig. 3.3). Although the stages of mitosis are depicted as if they were separate, they are actually continuous, and one stage flows from the other with no noticeable interruption.

Prophase. The events of **prophase** indicate that nuclear division is about to occur. The two pairs of centrioles outside the nucleus begin moving away from each other toward opposite ends of the nucleus. Spindle fibers appear between the separating centriole pairs, the nuclear envelope begins to fragment, and the nucleolus begins to disappear. The chromosomes are now visible. Each is composed of two sister chromatids held together at a centromere. Spindle fibers attach to the centromeres as the chromosomes continue to shorten and to thicken. During prophase, chromosomes are randomly placed in the nucleus.

At the end of prophase, a cell has a fully formed spindle. A **spindle** has poles, asters, and fibers. The **asters** are arrays of short microtubules that radiate from the poles, and the fibers are bundles of microtubules that stretch between the poles. Microtubule organizing centers (MTOC) are associated with the centrioles at the poles. These centers organize microtubules when the cell is not dividing; it is likely that they also organize the spindle. Centrioles may assist in this function, but their location at the poles of a spindle could be simply to ensure that each daughter cell receives a pair of centrioles.

Metaphase. During **metaphase,** the nuclear envelope is fragmented, and the spindle occupies the region formerly occupied by the nucleus. The chromosomes are now at the equator (center) of the spindle. Metaphase is characterized by a fully formed spindle, and the chromosomes, each with two sister chromatids, are aligned at the equator.

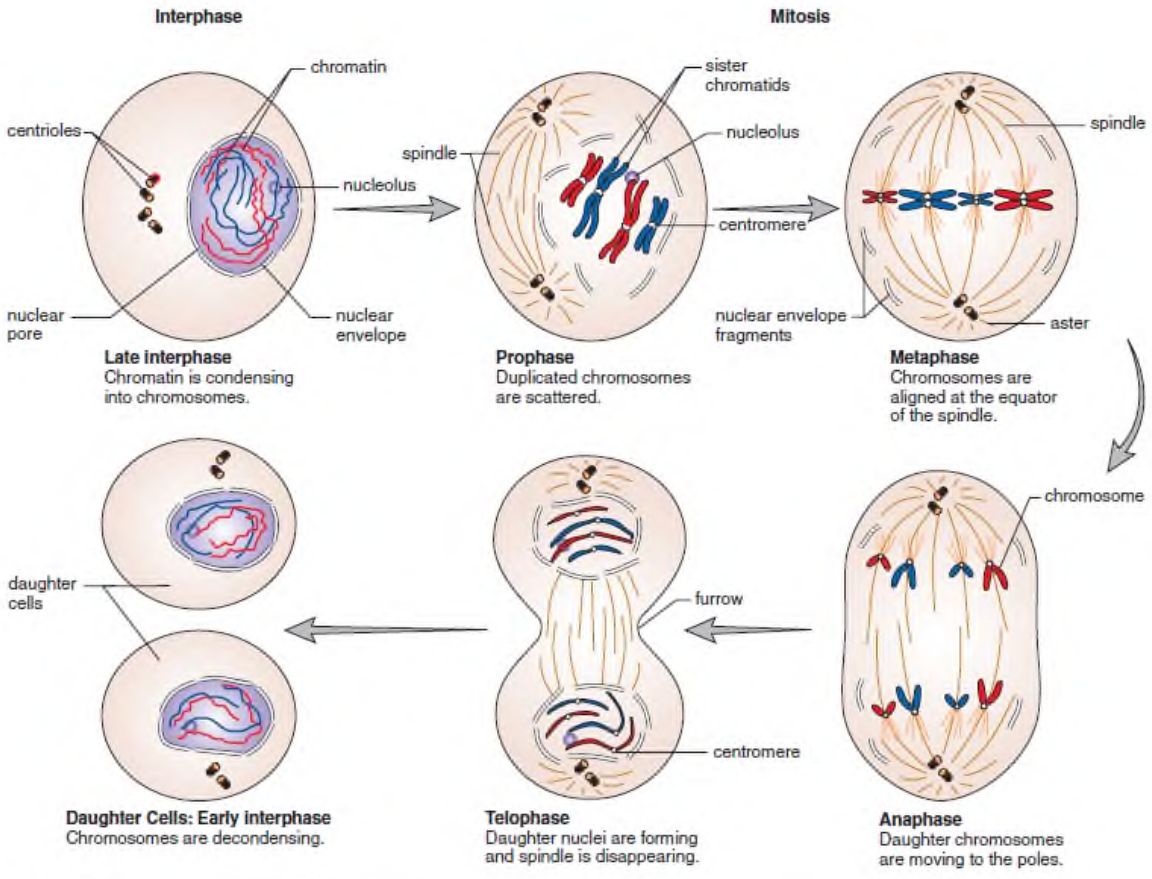


FIGURE 3.3. Stages of Mitosis. The blue chromosomes were inherited from one parent, and the red – from other.

Anaphase. At the start of **anaphase,** the sister chromatids separate. *Once separated, the chromatids are called chromosomes.* Separation of the sister chromatids ensures that each cell receives a copy of each type of chromosome and thereby has a full complement of genes. During anaphase, the daughter chromosomes move to the poles of the spindle. Anaphase is characterized by the diploid number of chromosomes moving toward each pole.

The spindle brings about chromosomal movement. Two types of spindle fibers are involved in the movement of chromosomes during anaphase. One type extends from the poles to the equator of the spindle; there they overlap. As

mitosis proceeds, these fibers increase in length, and this helps push the chromosomes apart. The chromosomes themselves are attached to other spindle fibers that simply extend from their centromeres to the poles. These fibers get shorter and shorter as the chromosomes move toward the poles. Therefore, they pull the chromosomes apart.

Spindle fibers, as stated earlier, are composed of microtubules. Microtubules can assemble and disassemble by the addition or subtraction of tubulin (protein) subunits. This is what enables spindle fibers to lengthen and shorten and what ultimately causes the movement of the chromosomes.

Telophase. **Telophase** begins when the chromosomes arrive at the poles. During telophase, the chromosomes become indistinct chromatin again. The spindle disappears as nucleoli appear, and nuclear envelope components reassemble in each cell. Telophase is characterized by the presence of two daughter nuclei.

Cytokinesis is division of the cytoplasm and organelles. In animal cells, a slight indentation called a **cleavage furrow** passes around the circumference of the cell. Actin filaments form a contractile ring, and as the ring gets smaller and smaller, the cleavage furrow pinches the cell in half. As a result, each cell becomes enclosed by its own plasma membrane.

Following mitosis, each daughter cell is 2n. When the sister chromatids separate during anaphase, each newly forming cell receives the same number and kinds of chromosomes as the parental cell.

Key Questions:

1. The nucleus structure.
2. Nuclear envelope.
3. Pore complex.
4. Karyoplasm.
5. Nucleulus structure: fiber center (rDNA), fiber component (rRNA), granule component (smool and large subunits of ribosomes).

6. Chromatin.

7. The eukaryotic chromosomes structure.

8. What are the differences between prokaryotic and eukaryitic chromosomes?

9. What are the differences between chromotin and chromosome?

10. Levels of DNA packing in chromatin: nucleosomal, supercoils, loop-shaped, and chromosomal.

11. Heterochromatin and euchromatin.

12. The chromosome structure: short arm (p), long arm (q), centromere, NOR, satellites.

13. Morphology of chromosomes: methacentric, submethacentric, acrocentric and telocentric.

14. What is the karyotype? Characteristics of human karyotype.

15. What are the sister chromatides, and homologous.

16. What are the differences between chromosome sets in somatic cells and in gamets?

17. What phases of the cell cycle?

18. What happens to the chromosomes during cell cycle?

19. What aspects of the cell cycle are controlled by the G1, G2, and M checkpoints? How are cyclins and cyclin-dependent

20. protein kinases involved in cell cycle regulation at checkpoints?

21. What is Mitosis? What phases of Mitosis?

Examples of Review questions

NN	Questions	Right answers
1	IN THE CELL'S NUCLEUS ARE PERFOMED: 1) photosynthesis and chemosynthesis 2) biosynthesis of proteins, hydrocarbons and lipids 3) synthesis of DNA and RNA 4) synthesis of ATP, DNA and proteins	**3**
2	NUCLEOSOMES ___ . 1) are made of chromosomes 2) consist entirely of DNA 3) consist of DNA wound around a histone core 4) are present only during mitosis 5) are present only during prophase	**3**
3	THE SET OF CHROMOSOMES IN SOMATIC CELLS IS CALLED: 1) genotype 2) phenotyhe 3) karyotype 4) metaphase	**3**
4	DNA REPLICATION (S PHASE) OCCURS____. 1) between G1 and G2 of interphase 2) during G2 3) during prophase of mitosis 4) between metaphase and anaphase	**1**
5	A CELL THAT BEGINS MITOSIS WITH 46 CHRO-MOSOMES PRODUCES DAUGHTER CELLS WITH _____. 1) 13 chromosomes 2) 23 chromosomes 3) 26 chromosomes 4) 46 chromosomes	**4**

CHAPTER 4. MEIOSIS AND GAMETOGENESIS

MEIOSIS

Meiosis, which requires two nuclear divisions, results in *four daughter cells, each having one of each kind of chromosome and therefore half the number of chromosomes as the parental cell.* The parental cell has the 2n number of chromosomes, while the daughter cells have the n number of chromosomes. Therefore, meiosis is often called reduction division. The daughter cells that result from meiosis go on to become the gametes.

Overview of Meiosis: 2n – n. Meiosis results in four daughter cells because it consists of two divisions, called meiosis I and meiosis II. Before meiosis I begins, each chromosome has duplicated and is composed of two sister chromatids. The parental cell is 2n. When a cell is 2n, the chromosomes occur in pairs. For example, the 46 chromosomes of humans occur in 23 pairs. These pairs are called **homologous chromosomes.**

During meiosis I, the homologous chromosomes of each pair come together and line up side-by-side due to a means of attraction still unknown. This so-called **synapsis** results in a **tetrad,** an association of four chromatids that stay in close proximity until they separate. During synapsis, nonsister chromatids may exchange genetic material. The exchange of genetic material between chromatids is called **crossing-over.** Crossing-over is significant because it recombines the genes of the parental cell and increases the variability of the gametes and therefore the offspring (Fig. 4.1).

Following synapsis during meiosis I, the homologous chromosomes of each pair separate. This separation means that one chromosome from each homologous pair will be found in each daughter cell. There are no restrictions as to which chromosome goes to each daughter cell, and therefore, all possible combinations of chromosomes may occur within the gametes. Following meiosis I, the daughter cells have half the number of chromosomes, and the chromosomes are still duplicated.

During meiosis I, homologous chromosomes separate, and the daughter cells receive one of each pair. The daughter cells are not genetically identical. The chromosomes are still composed of two chromatids.

When meiosis II begins, the chromosomes are still duplicated. Therefore, no duplication of chromosomes is needed between meiosis I and meiosis II. The chromosomes are composed of two sister chromatids. During meiosis II, the sister chromatids separate in each of the cells from meiosis I. Each of the resulting four daughter cells has the haploid number of chromosomes. Following meiosis, the daughter cells are not genetically identical to the parental cell. Following duplication of chromosomes, the parental cell undergoes two divisions, meiosis I and meiosis II. During meiosis I, homologous chromosomes separate, and during meiosis II, chromatids separate. The final daughter cells are haploid.

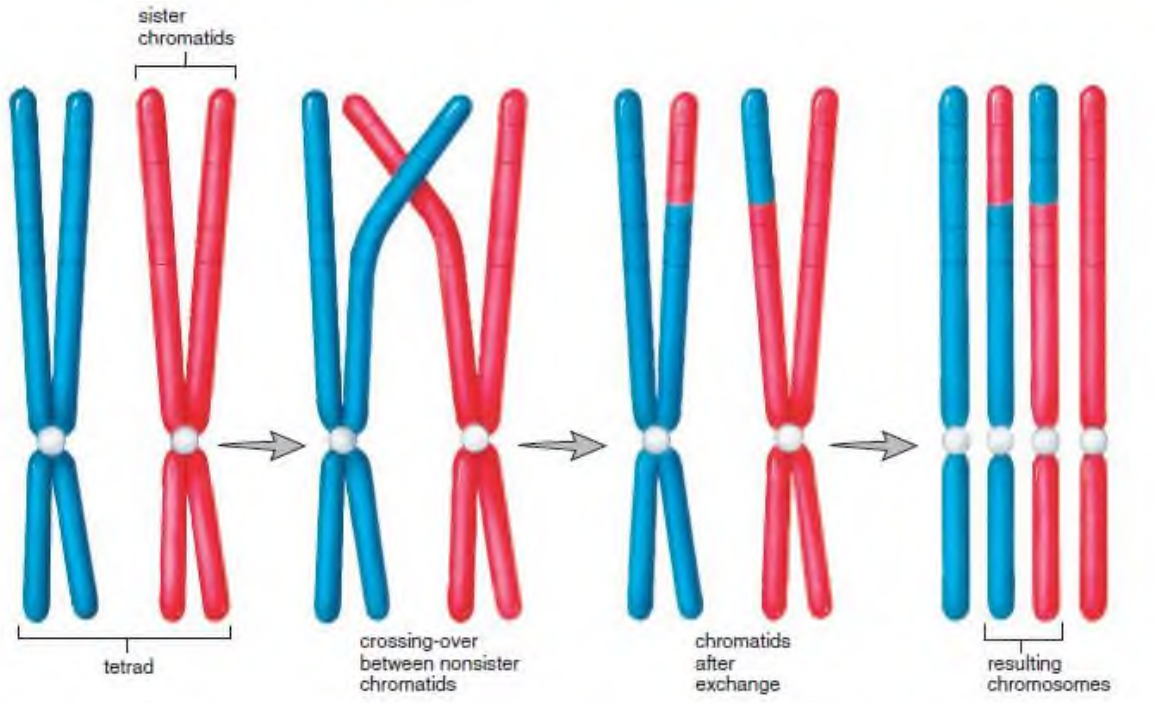


FIGURE 4.1. Crossing-over. When homologous chromosomes are in synapsis, the nonsister chromatids exchange genetic material. The illustration shows only one crossover per chromosome pair, but the average is slightly more than two per chromosome pair in humans. Following crossing-over, the sister chromatids may no longer be identical and instead may have different combinations of genes.

During meiosis II, the sister chromatids separate, and the resulting four daughter cells are each haploid. Meiosis involves two cell divisions. During meiosis I, tetrads form and crossing-over occurs. Homologous chromosomes separate, and each daughter cell receives one of each kind of chromosome. During meiosis II, the sister chromatids separate, and there are four daughter cells, each with the haploid number of chromosomes.

Meiosis I and meiosis II.

a) During meiosis I, homologous chromosomes undergo synapsis and then separate so that each daughter cell has only one chromosome from each original homologous pair. For simplicity's sake, the results of crossing-over have not been depicted. Notice that each daughter cell is haploid and each chromosome still has two chromatids.

b) During meiosis II, sister chromatids separate. Each daughter cell is haploid, and each chromosome consists of one chromatid.

The Importance of Meiosis. Because of meiosis, the chromosomal number stays constant in each generation of humans. In humans, meiosis occurs in the testes and ovaries during the production of the gametes. When a haploid sperm fertilizes a haploid egg, the new individual has the diploid number of chromosomes. There are three ways the new individual is assured a different combination of genes than either parent has:

1. Crossing-over recombines the genes on the sister chromatids of homologous pairs of chromosomes.

2. Following meiosis, gametes have all possible combinations of chromosomes.

3. At fertilization, recombination of chromosomes occurs because the sperm and egg carry varied combinations of chromosomes.

Meiosis and fertilization ensure that the chromosomal number stays constant in each generation and that the new individual has a different combination of chromosomes and genes than either parent.

A comparison of meiosis and mitosis. Meiosis involves two nuclear divisions with no DNA replication between them (Fig. 4.2).

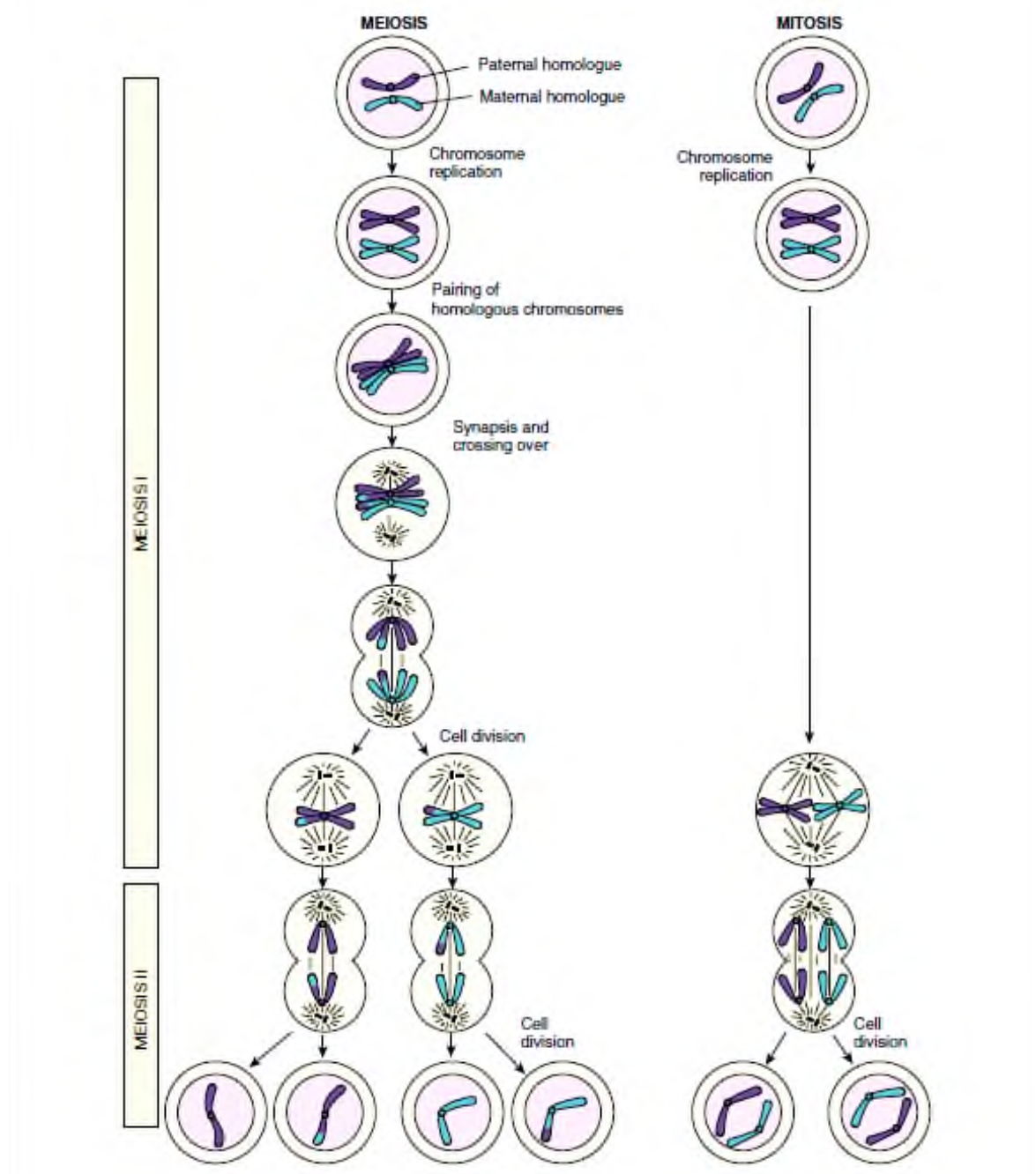


FIGURE 4.2. A comparison of meiosis and mitosis.

Meiosis involves two nuclear divisions with no DNA replication between them. It thus produces four daughter cells, each with half the original number of chromosomes. Crossing over occurs in prophase I of meiosis. Mitosis involves a single nuclear division after DNA replication. It thus produces two daughter cells, each containing the original number of chromosomes.

It thus produces four daughter cells, each with half the original number of chromosomes. Crossing over occurs in prophase I of meiosis. Mitosis involves a single nuclear division after DNA replication. It thus produces two daughter cells, each containing the original number of chromosomes. Parthenogenesis is a form of asexual reproduction that is practiced by many insects and some lizards. Among mammals, the sex is determined by the presence of a Y chromosome in males and its absence in females.

Meiosis is a part of spermatogenesis and oogenesis; therefore, both sperm and egg are haploid.

GAMETOGENESIS. SPERMATOGENESIS

Meiosis is a part of **spermatogenesis,** production of sperm, and **oogenesis,** production of eggs. Spermatogenesis and oogenesis occur in the sex organs—the testes in males and the ovaries in females. The gametes appear differently in the two sexes (Fig. 4.3), and meiosis is different, too.

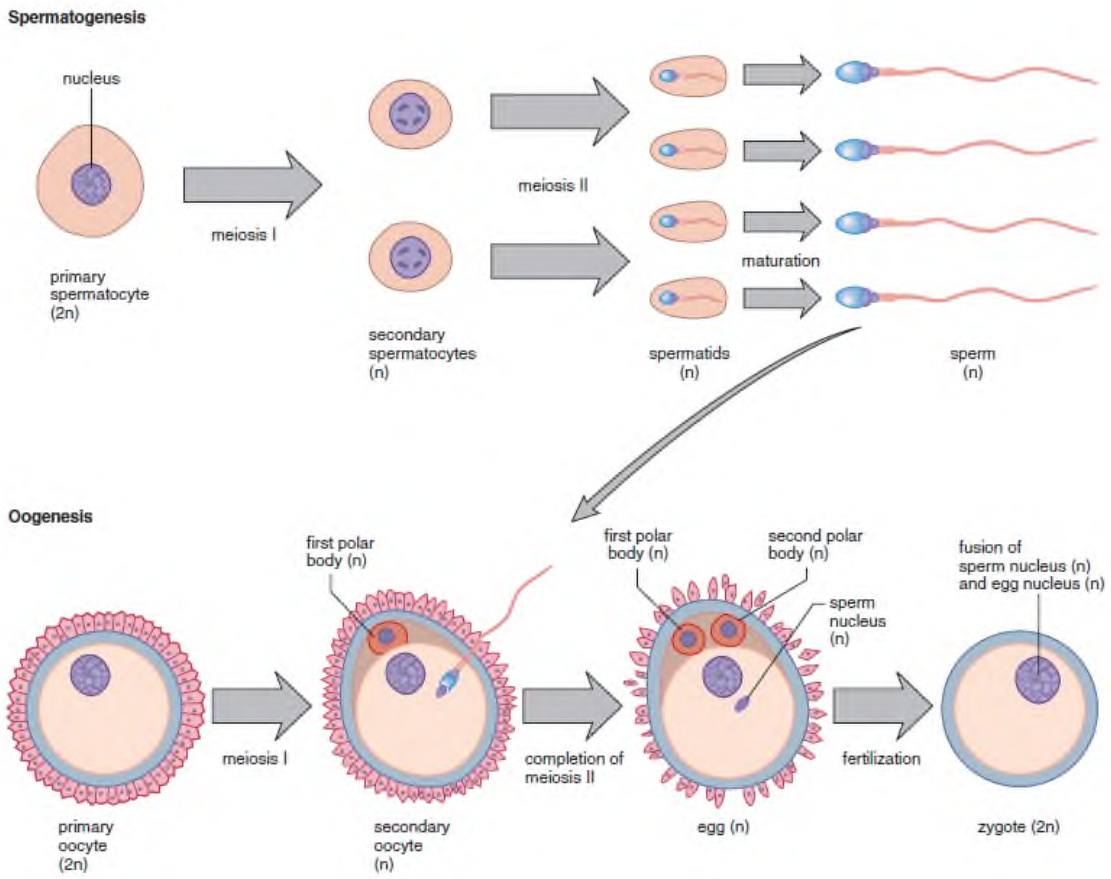


FIGURE 4.3. Spermatogenesis and oogenesis.

The process of meiosis in males always results in four cells that become sperm. Meiosis in females produces only one egg. Meiosis I results in one large cell called a secondary oocyte and one polar body. After meiosis II, there is one egg and two (or possibly three) polar bodies. **Polar bodies** are products of oogenesis that contain chromosomes but little cytoplasm. The plentiful cytoplasm of the egg serves as a source of nutrients and cellular organelles for the developing embryo. Spermatogenesis, once started, continues to completion, and mature sperm result. In contrast, oogenesis does not necessarily go to completion. Only if a sperm fertilizes the secondary oocyte does it undergo meiosis II and become an egg. Regardless of this complication, however, both the sperm and the egg contribute the haploid number of chromosomes to the zygote (fertilized egg). In humans, each gamete contributes 23 chromosomes.

Spermatogenesis, which occurs in the testes of males, produces sperm (Fig. 4.4).

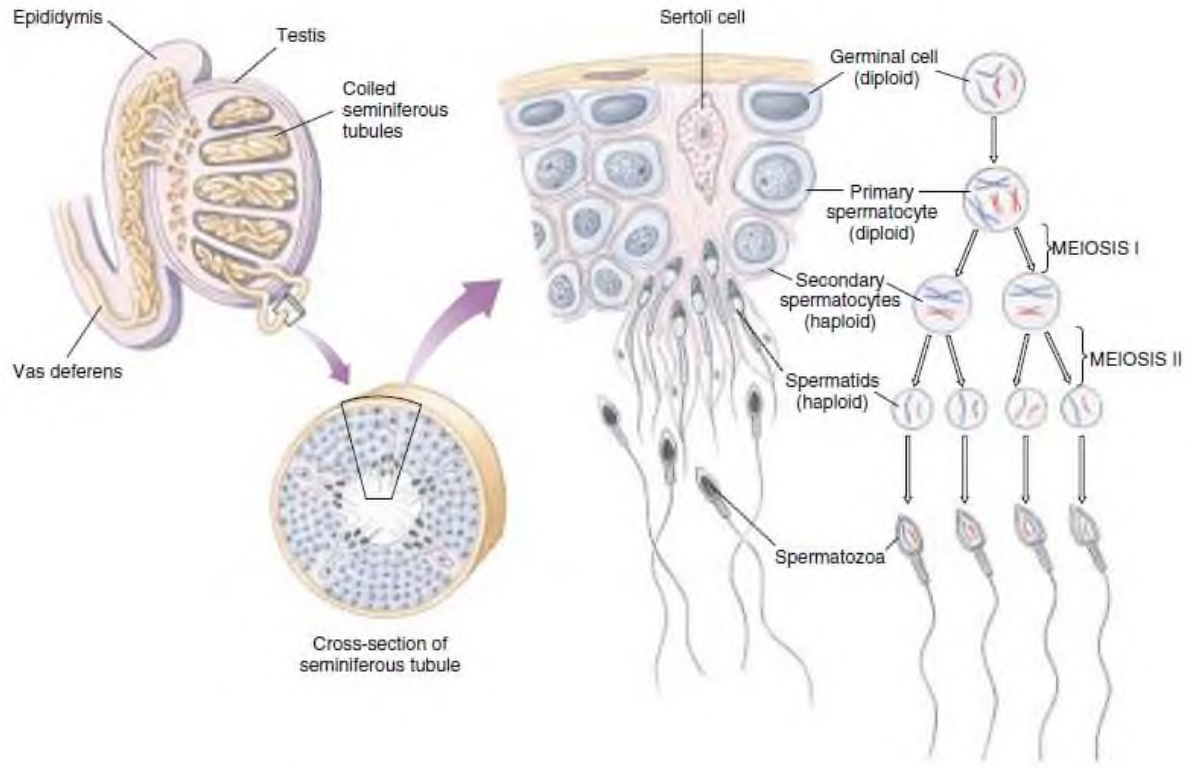


FIGURE 4.4. The testis and spermatogenesis.

Inside the testis, the seminiferous tubules are the sites of spermatogenesis. Germinal cells in the seminiferous tubules give rise to spermatozoa by meiosis. Sertoli cells are non-germinal cells within the walls of the seminiferous tubules. They assist spermatogenesis in several ways, such as helping to convert spermatids into spermatozoa. A primary spermatocytes are diploid. At the end of the first meiotic division, homologous chromosomes have separated, and two haploid secondary spermatocytes form. The second meiotic division separates the sister chromatids and results in the formation of four haploid spermatids.

Spermatozoa, or sperm, are relatively simple cells, consisting of a head, body, and tail (Fig. 4.5).

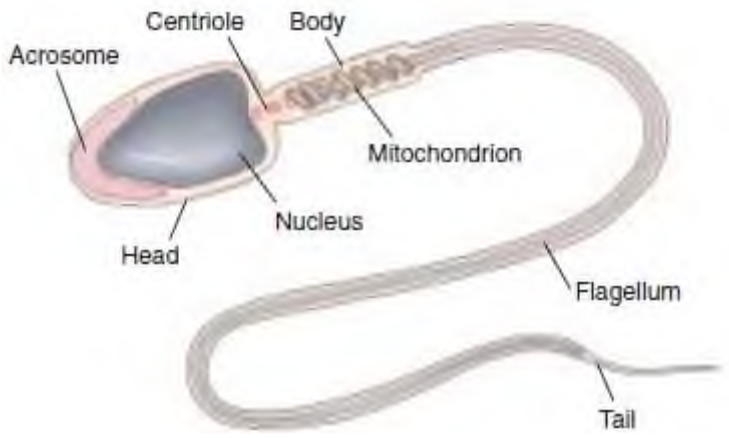


FIGURE 4.5. Human sperm. Sperm leave the testes and pass through the epididymis and vas deferens; the ejaculatory duct merges with the urethra, which empties at the tip of the penis.

Spermatogenesis produces four viable sperm, whereas oogenesis produces one egg and at least two polar bodies. Notice that oogenesis does not go to completion unless the secondary oocyte is fertilized. In humans, both sperm and egg have 23 chromosomes each; therefore, following fertilization, the zygote has 46 chromosomes.

GAMETOGENESIS. OOGENESIS

Oogenesis, which occurs in the ovaries of females, produces eggs.

Menstrual and Estrous Cycles. At birth, a female's ovaries contain some 2 million follicles, each with an ovum that has begun meiosis but which is arrested in prophase of the first meiotic division. At this stage, the ova are

called primary oocytes. Some of these primary-oocyte-containing follicles are stimulated to develop during each cycle. The human menstrual (Latin *mens*, "month") cycle lasts approximately one month (28 days on the average) and can be divided in terms of ovarian activity into a follicular phase and luteal phase, with the two phases separated by the event of ovulation.

Follicular Phase. During the follicular phase, a few follicles are stimulated to grow under FSH stimulation, but only one achieves full maturity as a tertiary, or Graafian, follicle. This follicle forms a thin-walled blister on the surface of the ovary (Fig. 4.6). The primary oocyte within the Graafian follicle completes the first meiotic division during the follicular phase. Instead of forming two equally large daughter cells, however, it produces one large daughter cell, the secondary oocyte, and one tiny daughter cell, called a polar body. Thus, the secondary oocyte acquires almost all of the cytoplasm from the primary oocyte, increasing its chances of sustaining the early embryo should the oocyte be fertilized.

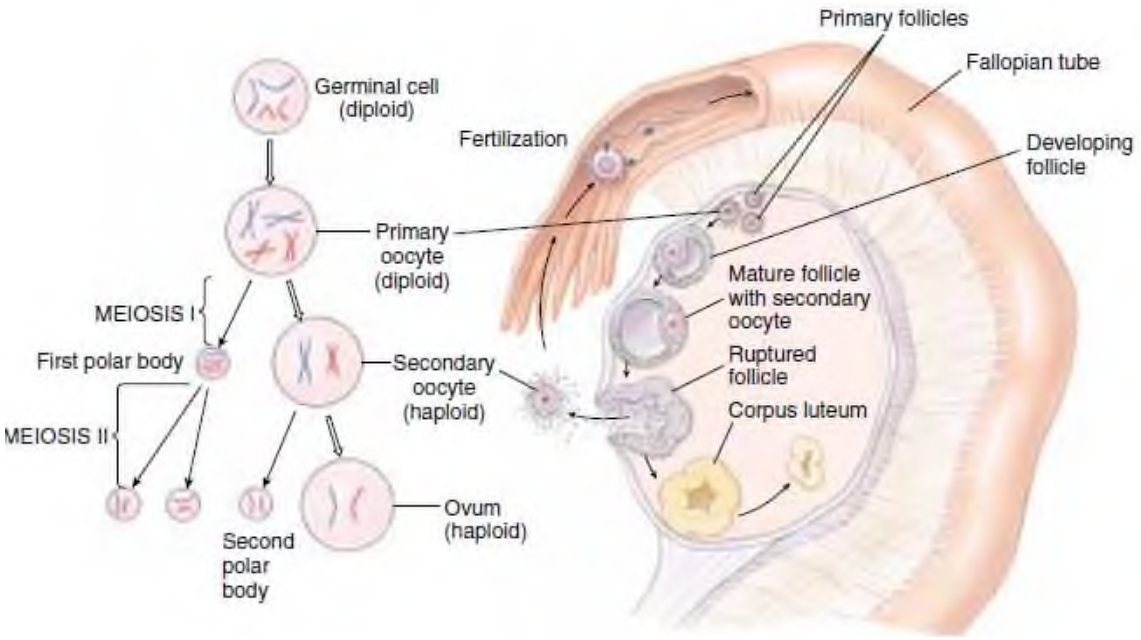


FIGURE 4.6. The meiotic events of oogenesis in humans. A primary oocyte is diploid. At the completion of the first meiotic division, one division product is eliminated as a polar body, while the other, the secondary oocyte, is released during ovulation. The secondary oocyte does not complete the second meiotic division until after fertilization; that division yields a second polar body and a single haploid egg, or ovum. Fusion of the haploid egg with a haploid sperm during fertilization produces a diploid zygote.

The polar body, on the other hand, often disintegrates. The secondary oocyte then begins the second meiotic division, but its progress is arrested at metaphase II. It is in this form that the egg cell is discharged from the ovary at ovulation, and it does not complete the second meiotic division unless it becomes fertilized in the fallopian tube.

An egg cell released from the ovary in ovulation is drawn by fimbria into the fallopian tube, which conducts the egg cell to the lining of the uterus, or endometrium, where it implants if fertilized. If fertilization does not occur, the corpus luteum regresses at the end of the cycle and the resulting fall in estradiol and progesterone secretion cause menstruation to occur in humans and apes. During the follicular phase the granulosa cells secrete increasing amounts of estradiol, which stimulates the growth of the endometrium. Hence, this portion of the cycle is also referred to as the **proliferative phase** of the endometrium. During the luteal phase of the cycle, the combination of estradiol and progesterone cause the endometrium to become more vascular, glandular, and enriched with glycogen deposits.

Because of the endometrium's glandular appearance, this portion of the cycle is known as the **secretory phase** of the endometrium (Fig. 4.7). In the absence of fertilization, the corpus luteum triggers its own atrophy, or regression, toward the end of the luteal phase. It does this by secreting hormones (estradiol and progesterone) that inhibit the secretion of LH, the hormone needed for its survival. In many mammals, atrophy of the corpus luteum is assisted by luteolysin, a paracrine regulator believed to be a prostaglandin.

The ovarian follicles develop under FSH stimulation, and one follicle ovulates under LH stimulation. During the follicular and luteal phases, the hormones secreted by the ovaries stimulate the development of the endometrium, so an embryo can implant there if fertilization has occurred. A secondary oocyte is released from an ovary at ovulation, and it only completes meiosis if it is fertilized.

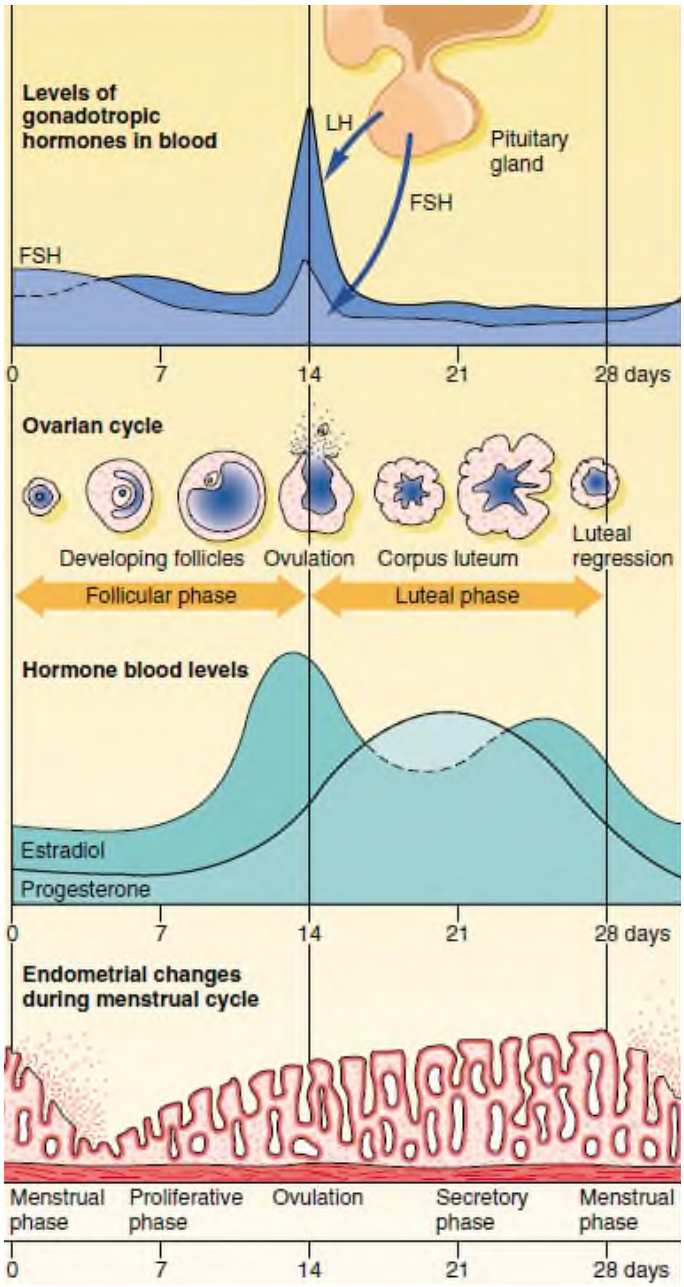


FIGURE 4.7. The human menstrual cycle. The growth and thickening of the endometrial (uterine) lining is stimulated by estradiol and progesterone. The decline in the levels of these two hormones triggers menstruation, the sloughing off of built-up endometrial tissue.

The disappearance of the corpus luteum results in an abrupt decline in the blood concentration of estradiol and progesterone at the end of the luteal phase, causing the built-up endometrium to be sloughed off with accompanying bleeding. This process is called menstruation, and the portion of the cycle in which it occurs is known as the **menstrual phase** of the endometrium.

The journey of an egg. Produced within a follicle and released at ovulation, an egg is swept into a fallopian tube and carried along by waves of ciliary motion in the tube walls. Sperm journeying upward from the vagina fertilize the egg within the fallopian tube. The resulting zygote undergoes several mitot-

ic divisions while still in the tube, so that by the time it enters the uterus, it is a hollow sphere of cells called a blastocyst. The blastocyst implants within the wall of the uterus, where it continues its development (The egg and its subsequent stages have been enlarged for clarification.).

A sperm must penetrate a layer of granulosa cells and then a layer of glycoprotein called the zona pellucida, before it reaches the oocyte membrane (Fig. 4.8).

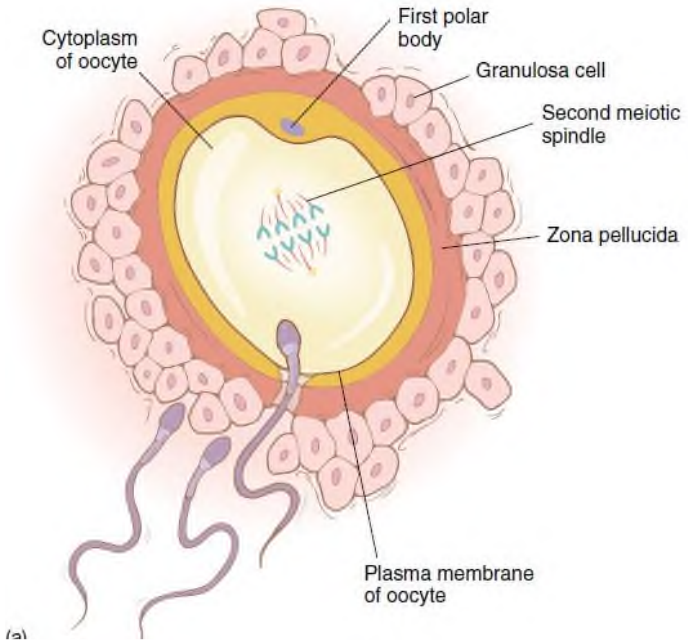


FIGURE 4.8. Fertilization. Sperm penetrated granulosa cells and zona pellucida before it reaches oocyte membrane.

This penetration is aided by digestive enzymes in the acrosome of the sperm.

Key Questions:

1. Meiosis. Features of the interphase preceding meiosis.
2. Meiosis I. Stages: pro-phase I (leptotena, zygotena, pakhitena, diplotena, diakinesis), metaphase I, anaphase I, telofaza I.
3. Interkinesis.
4. Meiosis II.
5. Principle difference of meiosis I from meiosis II.
6. The main difference of meiosis from a mitosis.
7. Biological importance of meiosis.
8. The main forms of sexual reproduction at one-cellular organisms (conjugation).

9. Spermatogenesis.
10. Oogenesis.
11. Concept about a menstrual cycle.
12. Morphology of gametes (sperm, egg).
13. Fertilization stages.

Examples of Review questions:

NN	Questions	Right answers
1	IN MEIOSIS ___ . 1) a single nucleus gives rise to two daughter nuclei 2) the daughter nuclei are genetically identical to the parent nucleus 3) the centromeres separate at the onset of anaphase I 4) homologous chromosomes synapse in prophase I 5) no spindle forms	**4**
2	CROSSING OVER OCCURS IN ___ 1) metaphase of mitosis 2) prophase I of meiosis 3) anaphase of mitosis 4) telophase II of meiosis	**2**
3	THE NUMBER OF DAUGHTER CHROMOSOMES IN A HUMAN SPERM CELL IS ___ . 1) 2 2) 23 3) 46 4) 69 5) 92	**2**
4	FERTILIZATION IS ___ . 1) the fusion of gametes nucleus 2) the restoration of diploid set of chromosomes 3) the fusion of heredity information of parents 4) the realization of connection between generations 5) a + b 6) all answers are correct	**6**
5	DURING OOGENESIS IN MAMMALS, THE SECOND MEIOTIC DIVISION COMPLIT ___ . 1) in the formation of the primary oocyte 2) in the formation of the secondary oocyte 3) before ovulation 4) after fertilization 5) after implantation	**4**

CHAPTER 5. THE NUCLEIC ACIDS (DNA AND RNA). PROKARYOTIC AND EUKARYOTIC GENES STRUCTURE. THE STEPS OF PROTEIN SYNTHESIS

THE NUCLEIC ACIDS STRUCTURE AND FUNCTIONS

Learning informally of Franklin's results before they were published in 1953, James Watson and Francis Crick, two young investigators at Cambridge University, quickly worked out a likely structure for the DNA molecule (Fig. 5.1), which we now know was substantially correct.

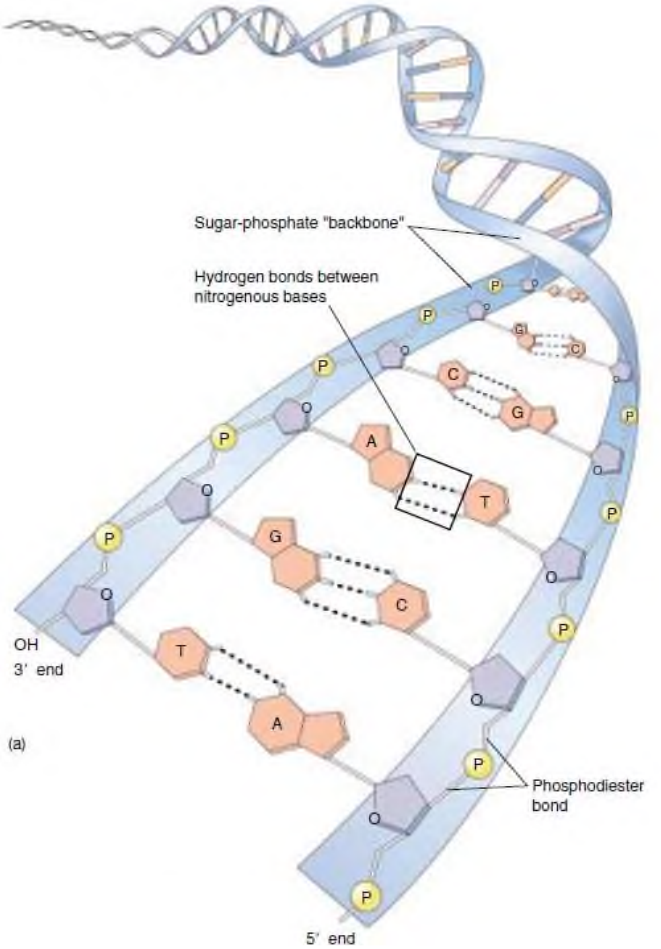


FIGURE 5.1. DNA is a double helix. In a DNA duplex molecule, only two base-pairs are possible: adenine (A) can pair with thymine (T), and guanine (G) can pair with cytosine (C). An A-T base-pair has two hydrogen bonds, while a G-C base-pair has three.

Nucleotide subunits of DNA and RNA. The nucleotide subunits of DNA and RNA are composed of three elements: a five-carbon sugar (deoxyribose in DNA and ribose in RNA), a phosphate group, and a nitrogenous base (either a purine or a pyrimidine).

Chargaff's rules: A single strand of DNA or RNA consists of a series of nucleotides joined together in a long chain. In all natural double-stranded DNA

molecules, the proportion of A equals that of T, and the proportion of G equals that of C. It follows that there is always an equal proportion of purines (A and G) and pyrimidines (C and T).

DNA is a double helix. In a DNA duplex molecule, only two base-pairs are possible: adenine (A) can pair with thymine (T), and guanine (G) can pair with cytosine (C). An A-T base-pair has two hydrogen bonds, while a G-C base-pair has three.

First function of DNA – *storage of hereditary information* by Gene code.

In 1964, Nirenberg and Philip Leder developed a powerful **triplet binding assay** in which a specific triplet was tested to see which radioactive amino acid (complexed to tRNA) it would bind. Some 47 of the 64 possible triplets gave unambiguous results. Har Gobind Khorana decoded the remaining 17 triplets by constructing artificial mRNA molecules of defined sequence and examining what polypeptides they directed. In these ways, all 64 possible three-nucleotide sequences were tested, and the full genetic code was determined (Table 5.1).

Table 5.1

Genetic Code

First Letter	Second Letter								Third Letter
	U		C		A		G		
U	UUU	Phenylalanine	UCU	Serine	UAU	Tyrosine	UGU	Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	Leucine	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Tryptophan	G
C	CUU	Leucine	CCU	Proline	CAU	Histidine	CGU	Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Isoleucine	ACU	Threonine	AAU	Asparagine	AGU	Serine	U
	AUC		ACC		AAC		AGC		C
	AUA	Methionine	ACA		AAA	Lysine	AGA	Arginine	A
	AUG	Start	ACG		AAG		AGG		G
G	GUU	Valine	GCU	Alanine	GAU	Aspartate	GGU	Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glutamate	GGA		A
	GUG		GCG		GAG		GGG		G

Within genes that encode proteins, the nucleotide sequence of DNA is read in blocks of three consecutive nucleotides, without punctuation between

the blocks. Each block, or codon, codes for one amino acid. A codon consists of three nucleotides read in the sequence shown. For example, ACU codes for threonine. The first letter, A, is in the First Letter column; the second letter, C, is in the Second Letter column; and the third letter, U, is in the Third Letter column. Each of the mRNA codons is recognized by a corresponding anticodon sequence on a tRNA molecule. Some tRNA molecules recognize more than one codon in mRNA, but they always code for the same amino acid. In fact, most amino acids are specified by more than one codon. For example, threonine is specified by four codons, which differ only in the third nucleotide (ACU, ACC, ACA, and ACG).

Second function of DNA – *transfer of hereditary information* by DNA-replication.

Origins of replication. At a site called the replication origin, the DNA duplex opens to create two separate strands, each of which can be used as a template for a new strand. Eukaryotic DNA has multiple origins of replication. DNA replication involves many different proteins (Table 5.2) that open and unwind the DNA double helix, stabilize the single strands, synthesize RNA primers, assemble new complementary strands on each exposed parental strand—one of them discontinuously—remove the RNA primer, and join new discontinuous segments on the lagging strand.

Table 5.2

DNA Replication Proteins of *E. coli*

Protein	Role	Size (kd)	Molecules per Cell
Helicase	Unwinds the double helix	300	20
Primase	Synthesizes RNA primers	60	50
Single-strand binding protein	Stabilizes single-stranded regions	74	300
DNA gyrase	Relieves torque	400	250
DNA polymerase III	Synthesizes DNA	≈900	20
DNA polymerase I	Erases primer and fills gaps	103	300
DNA ligase	Joins the ends of DNA segments	74	300

The Replication Process. The replication of the DNA double helix is a complex process that has taken decades of research to understand (Fig. 5.2). It takes place in five interlocking steps:

1. Opening up the DNA double helix. The very stable DNA double helix must be opened up and its strands separated from each other for semiconservative replication to occur.

Stage one: Initiating replication. The binding of **initiator proteins** to the replication origin starts an intricate series of interactions that opens the helix.

Stage two: Unwinding the duplex. After initiation, "unwinding" enzymes called **helicases** bind to and move along one strand, shouldering aside the other strand as they go.

Stage three: Stabilizing the single strands. The unwound portion of the DNA double helix is stabilized by **single-strand binding protein,** which binds to the exposed single strands, protecting them from cleavage and preventing them from rewinding.

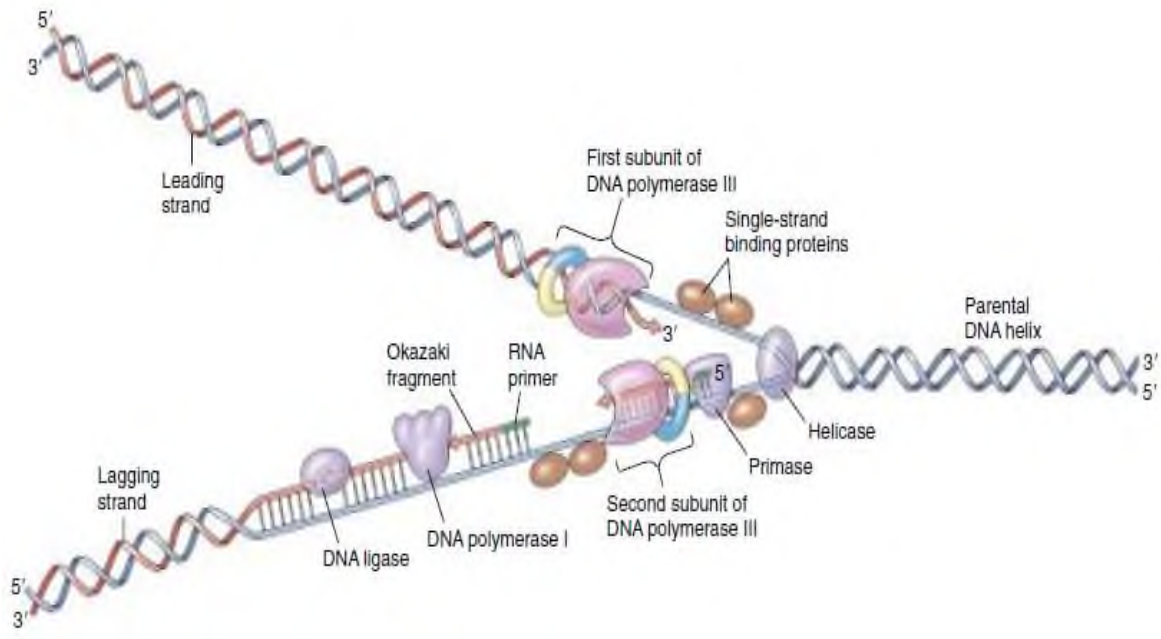


FIGURE 5.2. A DNA replication fork. Helicase enzymes separate the strands of the double helix, and single-strand binding proteins stabilize the single-stranded regions. Replication occurs by two mechanisms. (*1*) *Continuous synthesis:* After primase adds a short RNA primer, DNA polymerase III adds nucleotides to the 3′ end of the leading strand. DNA polymerase I then replaces the RNA primer with DNA nucleotides. (*2*) *Discontinuous synthesis:* Primase adds a short RNA primer (*green*) ahead of the 5′ end of the lagging strand. DNA polymerase III then adds nucleotides to the primer until the gap is filled in. DNA polymerase I replaces the primer with DNA nucleotides, and DNA ligase attaches the short segment of nucleotides to the lagging strand.

Stage four: Relieving the torque generated by unwinding. For replication to proceed at 1000 nucleotides per second, the parental helix ahead of the replication fork must rotate 100 revolutions per second!To relieve the resulting twisting, called torque, enzymes known as topisomerases—or, more informally, **gyrases**— cleave a strand of the helix, allow it to swivel around the intact strand, and then reseal the broken strand.

2. Building a primer. New DNA cannot be synthesized on the exposed templates until a primer is constructed, as DNA polymerases require 3′ primers to initiate replication. The necessary primer is a short stretch of RNA, added by a specialized RNA polymerase called *primase* in a multisubunit complex informally called a *primosome.* Why an RNA primer, rather than DNA? Starting chains on exposed templates introduces many errors; RNA marks this initial stretch as "temporary," making this error-prone stretch easy to excise later.

3. Assembling complementary strands. Next, the dimeric DNA polymerase III then binds to the replication fork. While the leading strand complexes with one half of the polymerase dimer, the lagging strand is thought to loop around and complex with the other half of the polymerase dimer. Moving in concert down the parental double helix, DNA polymerase III catalyzes the formation of complementary sequences on each of the two single strands at the same time.

4. Removing the primer. The enzyme DNA polymerase I now removes the RNA primer and fills in the gap, as well as any gaps between Okazaki fragments.

5. Joining the Okazaki fragments. After any gaps between Okazaki fragments are filled in, the enzyme DNA ligase joins the fragments to the lagging strand.

Third function of DNA – *Gene expression*

The Central Dogma of gene expression. DNA is transcribed to make mRNA, which is translated to make a protein (Fig. 5.3).

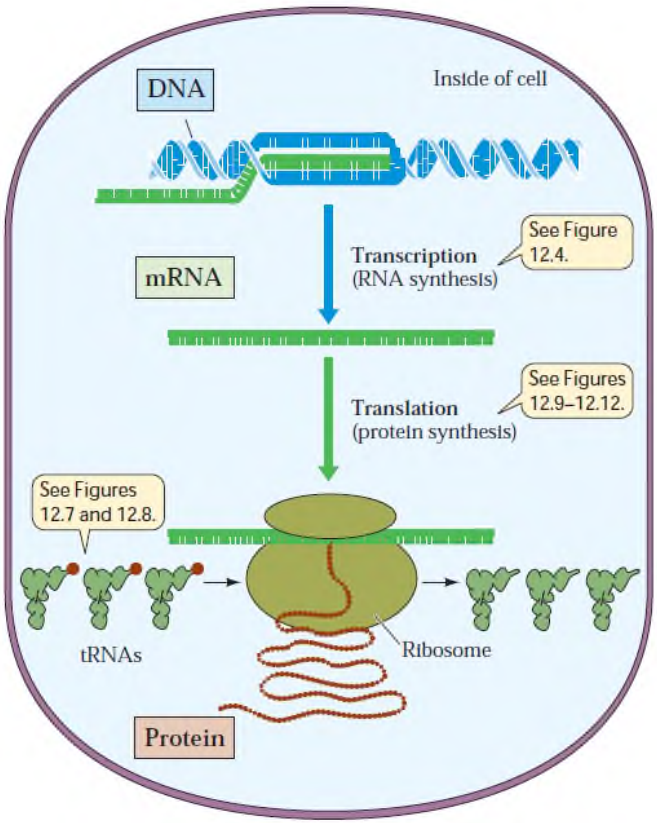


FIGURE 5.3. From Gene to Protein. This diagram summarizes the processes of gene expression in prokaryotes. In eukaryotes, the processes are somewhat more complex.

The information encoded in genes is expressed in two phases: transcription, in which an RNA polymerase enzyme assembles an mRNA molecule whose nucleotide sequence is complementary to the DNA nucleotide sequence of the gene; and translation, in which a ribosome assembles a polypeptide, whose amino acid sequence is specified by the nucleotide sequence in the mRNA.

The structure of prokaryotic and eukaryotic genes is present at Figures 5.4 and 5.5.

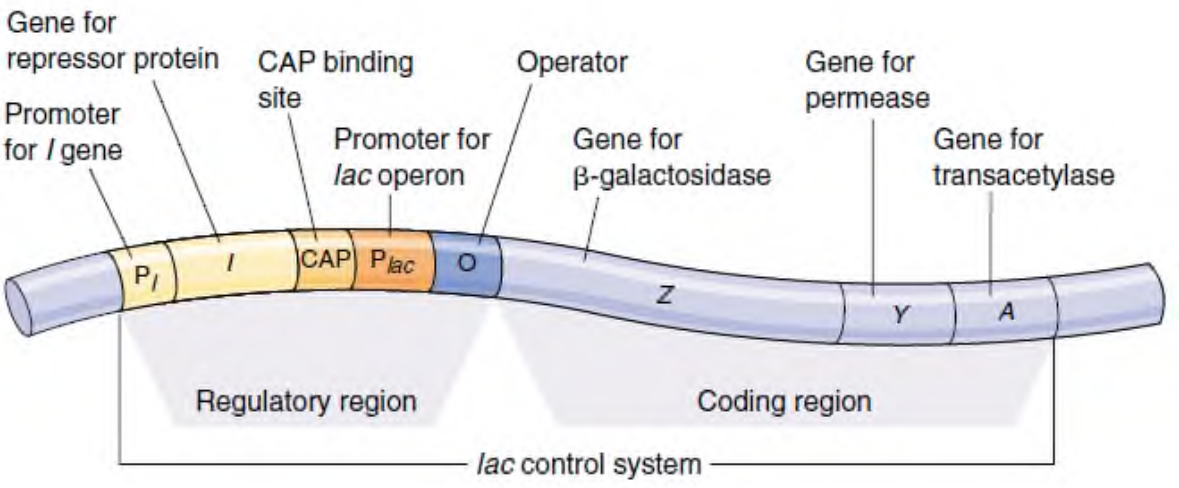


FIGURE 5.4. The *lac* region of the *Escherichia coli*. The *lac* operon consists of a promoter, an operator, and three genes that code for proteins required for the metabolism of lactose. In addition, there is a binding site for the catabolite activator protein (CAP), which affects whether or not RNA polymerase will bind to the promoter. Gene *I* codes for a repressor protein, which will bind to the operator and block transcription of the *lac* genes. The genes *Z*, *Y*, and *A* encode the two enzymes and the permease involved in the metabolism of lactose.

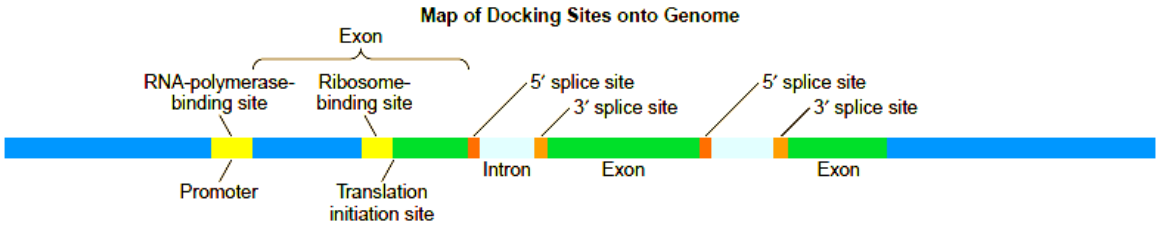


FIGURE 5.5. Eukaryotic gene structure.

TRANSCRIPTION

The first step in gene expression is the production of an RNA copy of the DNA sequence encoding the gene, a process called **transcription.** To understand the mechanism behind the transcription process, it is useful to focus first on RNA polymerase, the remarkable enzyme responsible for carrying it out.

Initiation. The binding of RNA polymerase to the promoter is the first step in gene transcription. In bacteria, a subunit of RNA polymerase called σ **(sigma)** recognizes the –10 sequence in the promoter and binds RNA polymerase there. Importantly, this subunit can detect the –10 sequence without unwinding the DNA double helix. In eukaryotes, the –25 sequence plays a similar

role in initiating transcription, as it is the binding site for a key protein factor. Other eukaryotic factors then bind one after another, assembling a large and complicated **transcription complex.** Once bound to the promoter, the RNA polymerase begins to unwind the DNA helix. Measurements indicate that bacterial RNA polymerase unwinds a segment approximately 17 base-pairs long, nearly two turns of the DNA double helix. This sets the stage for the assembly of the RNA chain.

Elongation. The transcription of the RNA chain usually starts with ATP or GTP. One of these forms the 5′ end of the chain, which grows in the 5′ → 3′ direction as ribonucleotides are added. Unlike DNA synthesis, a primer is not required. The region containing the RNA polymerase, DNA, and growing RNA transcript is called the **transcription bubble** because it contains a locally unwound "bubble" of DNA (figure 15.8). Within the bubble, the first 12 bases of the newly synthesized RNA strand temporarily form a helix with the template DNA strand. Corresponding to not quite one turn of the helix, this stabilizes the positioning of the 3′ end of the RNA so it can interact with an incoming ribonucleotide. The RNA-DNA hybrid helix rotates each time a nucleotide is added so that the 3′ end of the RNA stays at the catalytic site. The transcription bubble moves down the DNA at a constant rate, about 50 nucleotides per second, leaving the growing RNA strand protruding from the bubble. After the transcription bubble passes, the now transcribed DNA is rewound as it leaves the bubble. Unlike DNA polymerase, RNA polymerase has no proofreading capability. Transcription thus produces many more copying errors than replication. These mistakes, however, are not transmitted to progeny. Most genes are transcribed many times, so a few faulty copies are not harmful.

Termination. At the end of a gene are "stop" sequences that cause the formation of phosphodiester bonds to cease, the RNADNA hybrid within the transcription bubble to dissociate, the RNA polymerase to release the DNA, and the DNA within the transcription bubble to rewind. The simplest stop signal is a series of GC base-pairs followed by a series of AT base-pairs. The

RNA transcript of this stop region forms a GC hairpin, followed by four or more U ribonucleotides. How does this structure terminate transcription? The hairpin causes the RNA polymerase to pause immediately after the polymerase has synthesized it, placing the polymerase directly over the run of four uracils. The pairing of U with DNA's A is the weakest of the four hybrid base-pairs and is not strong enough to hold the hybrid strands together during the long pause. Instead, the RNA strand dissociates from the DNA within the transcription bubble, and transcription stops. A variety of protein factors aid hairpin loops in terminating transcription of particular genes.

Posttranscriptional Modifications. In eukaryotes, every mRNA transcript must travel a long journey out from the nucleus into the cytoplasm before it can be translated. Eukaryotic mRNA transcripts are modified in several ways to aid this journey: **5' caps.** Transcripts usually begin with A or G, and, in eukaryotes, the terminal phosphate of the 5' A or G is removed, and then a very unusual 5'-5' linkage forms with GTP. Called a **5 ' cap,** this structure protects the 5' end of the RNA template from nucleases and phosphatases during its long journey through the cytoplasm. Without these caps, RNA transcripts are rapidly degraded. **3' poly-A tails.** The 3' end of eukaryotic transcript is cleaved off at a specific site, often containing the sequence AAUAAA. A special poly-A polymerase enzyme then adds about 250 A ribonucleotides to the 3' end of the transcript. Called a 3' **poly-A tail,** this long string of Adenines protects the transcript from degradation by nucleases. It also appears to make the transcript a better template for protein synthesis.

RNA Splicing. When a gene is transcribed, the primary RNA transcript (that is, the gene copy as it is made by RNA polymerase, before any modification occurs) contains sequences complementary to the entire gene, including introns as well as exons (Fig. 5.6).

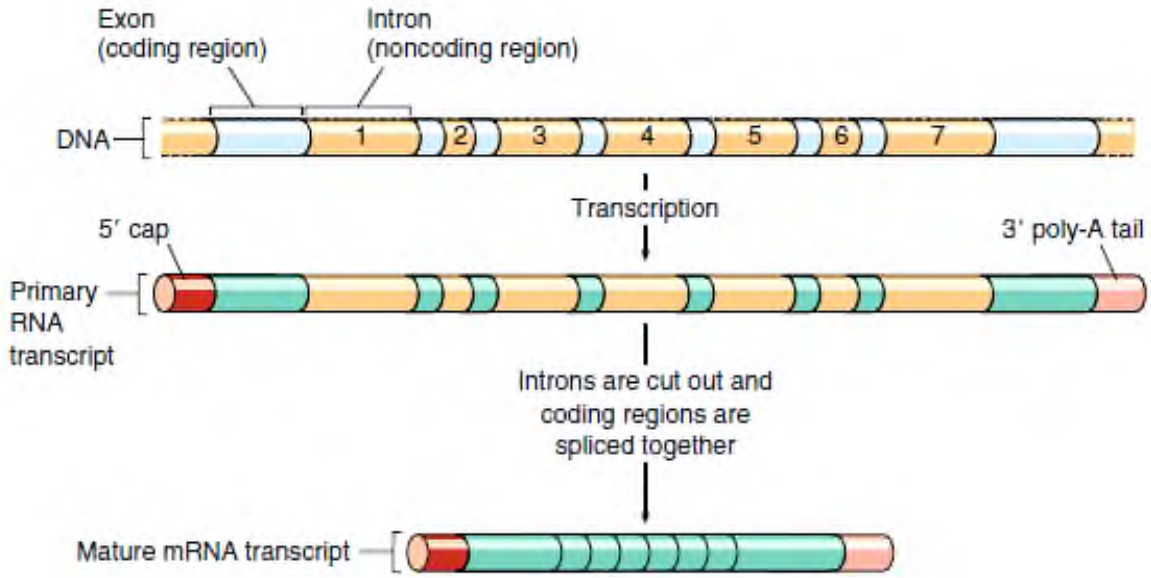


FIGURE 5.6. The eukaryotic gene structure, processing and splaicing. The ovalbumin gene and its primary RNA transcript contain seven segments not present in the mRNA the ribosomes use to direct protein synthesis. Enzymes cut these segments (introns) out and splice together the remaining segments (exons).

However, in a process called **RNA processing,** or **splicing,** the intron sequences are cut out of the primary transcript before it is used in polypeptide synthesis; therefore, those sequences are not translated.

The remaining sequences, which correspond to the exons, are spliced together to form the final, "processed" mRNA molecule that is translated. In a typical human gene, the introns can be 10 to 30 times larger than the exons. For example, even though only 432 nucleotides are required to encode the 144 amino acids of hemoglobin, there are actually 1356 nucleotides in the primary mRNA transcript of the hemoglobin gene.

Particles called *small nuclear ribonucleoproteins,*or *snRNPs (*more informally, **snurps**), are thought to play a role in RNA splicing (Fig. 5.7).

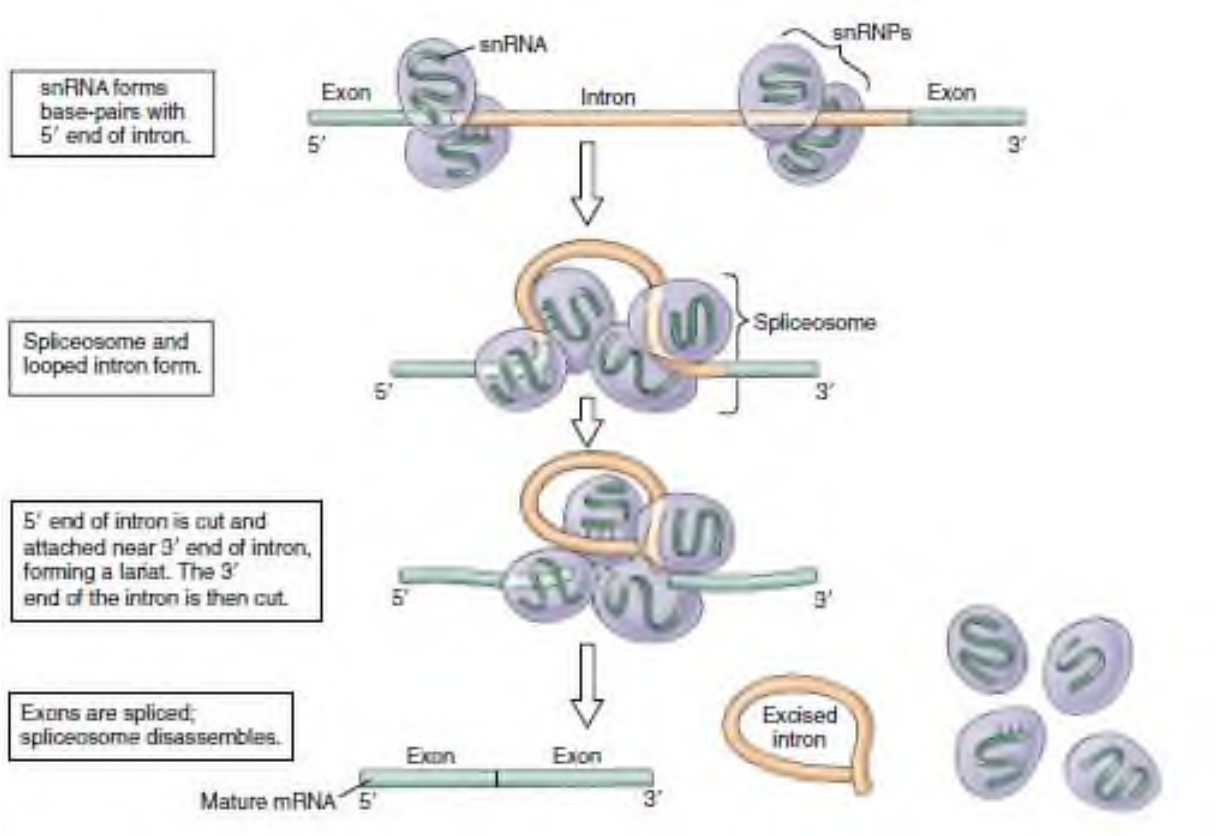


FIGURE 5.7. How spliceosomes process RNA. Particles called snRNPs contain snRNA that interacts with the 5r end of an intron. Several snRNPs come together and form a spliceosome. As the intron forms a loop, the 5r end is cut and linked to a site near the 3r end of the intron. The intron forms a lariat that is excised, and the exons are spliced together. The spliceosome then disassembles and releases the mature mRNA.

These particles reside in the nucleus of a cell and are composed of proteins and a special type of RNA called *small nuclear RNA,* or *snRNA.* One kind of snRNP contains snRNA that can bind to the 5r end of an intron by forming base-pairs with complementary sequences on the intron. When multiple snRNPs combine to form a larger complex called a **spliceosome,** the intron loops out and is excised RNA splicing provides a potential point where the expression of a gene can be controlled, because exons can be spliced together in different ways, allowing a variety of different polypeptides to be assembled from the same gene!

Alternative splicing is common in insects and vertebrates, with two or three different proteins produced from one gene (Fig. 5.8).

In many cases, gene expression is regulated by changing which splicing event occurs during different stages of development or in different tissues.

An excellent example of alternative splicing in action is found in two different human organs, the thyroid and the hypothalamus. The thyroid gland is responsible for producing hormones that control processes such as metabolic rate.

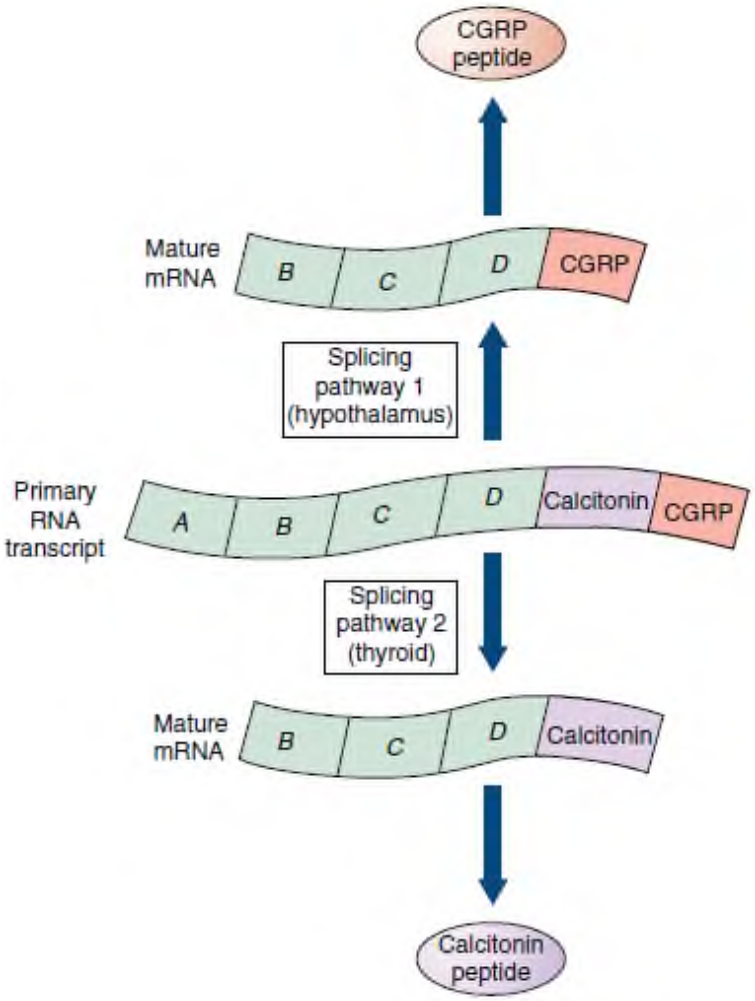


FIGURE 5.8. Alternative splicing products. The same transcript made from one gene can be spliced differently to give rise to two very distinct protein products, calcitonin and CGRP. The appearance of one product versus another is determined by tissue-specific factors that regulate the processing of the primary transcript. This ability offers another powerful way to control the expression of gene products, ranging from proteins with subtle differences to totally unrelated proteins.

The hypothalamus, located in the brain, collects information from the body (for example, salt balance) and releases hormones that in turn regulate the release of hormones from other glands, such as the pituitary gland. The two organs produce two distinct hormones, calcitonin and CGRP (calcitonin generelated peptide) as part of their function. Calcitonin is responsible for controlling the amount of calcium we take up from our food and the balance of calcium in tissues like bone and teeth. CGRP is involved in a number of neural and endo-

crine functions. Although these two hormones are used for very different physiological purposes, the hormones are made using the same transcript.

TRANSLATION

The second step in gene expression called **translation.**

Initiation. In prokaryotes, polypeptide synthesis begins with the formation of an **initiation complex** (Fig. 5.9).

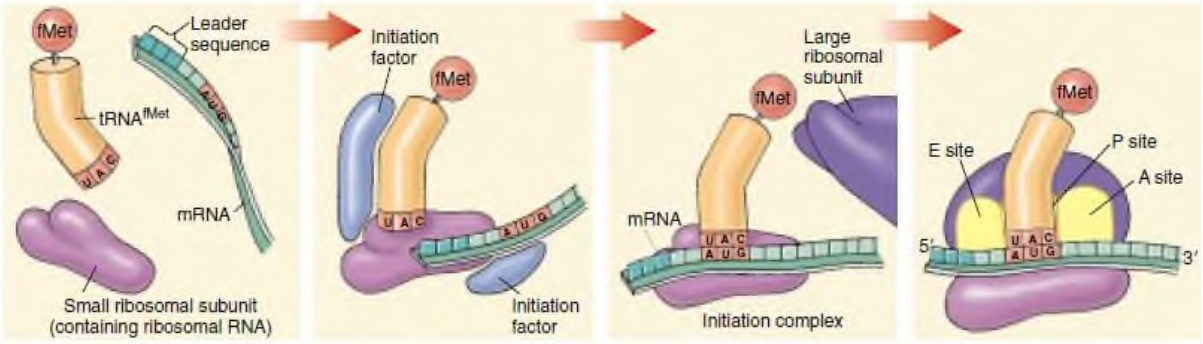


FIGURE 5.9. Formation of the initiation complex. In prokaryotes, proteins called initiation factors play key roles in positioning the small ribosomal subunit and the *N*-formylmethionine, or tRNAfMet, molecule at the beginning of the mRNA. When the tRNAfMet is positioned over the first AUG codon of the mRNA, the large ribosomal subunit binds, forming the P, A, and E sites where successive tRNA molecules bind to the ribosomes, and polypeptide synthesis begins.

First, a tRNA molecule carrying a chemically modified methionine (tRNAfMet) binds to the small ribosomal subunit. Proteins called **initiation factors** position the tRNAfMet on the ribosomal surface at the *P site* (for peptidyl), where peptide bonds will form.

Nearby, two other sites will form: the *A site* (for aminoacyl), where successive amino acid-bearing tRNAs will bind, and the *E site* (for exit), where empty tRNAs will exit the ribosome. This initiation complex, guided by another initiation factor, then binds to the anticodon AUG on the mRNA. Proper positioning of the mRNA is critical because it determines the reading frame—that is, which groups of three nucleotides will be read as codons.

Moreover, the complex must bind to the beginning of the mRNA molecule, so that all of the transcribed gene will be translated. In bacteria, the beginning of each mRNA molecule is marked by a *leader sequence* complemen-

tary to one of the rRNA molecules on the ribosome. This complementarity ensures that the mRNA is read from the beginning. Bacteria often include several genes within a single mRNA transcript (polycistronic mRNA), while each eukaryotic gene is transcribed on a separate mRNA (monocistronic mRNA).

Elongation. After the initiation complex has formed, the large ribosome subunit binds, exposing the mRNA codon adjacent to the initiating AUG codon, and so positioning it for interaction with another amino acid-bearing tRNA molecule. When a tRNA molecule with the appropriate anticodon appears, proteins called elongation factors assist in binding it to the exposed mRNA codon at the A site. When the second tRNA binds to the ribosome, it places its amino acid directly adjacent to the initial methionine, which is still attached to its tRNA molecule, which in turn is still bound to the ribosome. The two amino acids undergo a chemical reaction, catalyzed by *peptidyl transferase*, which releases the initial methionine from its tRNA and attaches it instead by a peptide bond to the second amino acid.

Translocation. In a process called translocation (Fig. 5.10), the ribosome now moves (translocates) three more nucleotides along the mRNA molecule in the 5′ → 3′ direction, guided by other elongation factors.

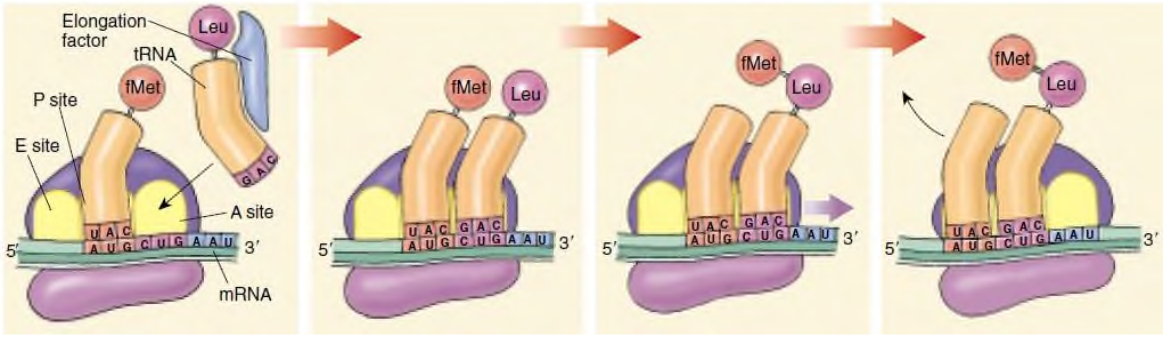


FIGURE 5.10. Translocation. The initiating tRNAfMet in prokaryotes (tRNAfMet in eukaryotes) occupies the P site, and a tRNA molecule with an anticodon complementary to the exposed mRNA codon binds at the A site. fMet is transferred to the incoming amino acid (Leu), as the ribosome moves three nucleotides to the right along the mRNA. The empty tRNAfMet moves to the E site to exit the ribosome, the growing polypeptide chain moves to the P site, and the A site is again exposed and ready to bind the next amino acid–laden tRNA.

This movement relocates the initial tRNA to the E site and ejects it from the ribosome, repositions the growing polypeptide chain (at this point containing two amino acids) to the P site, and exposes the next codon on the mRNA at the A site. When a tRNA molecule recognizing that codon appears, it binds to the codon at the A site, placing its amino acid adjacent to the growing chain. The chain then transfers to the new amino acid, and the entire process is repeated.

***Termination* of protein synthesis.** There is no tRNA with an anticodon complementary to any of the three termination signal codons, such as the UAA nonsense codon illustrated here Figure 5.11.

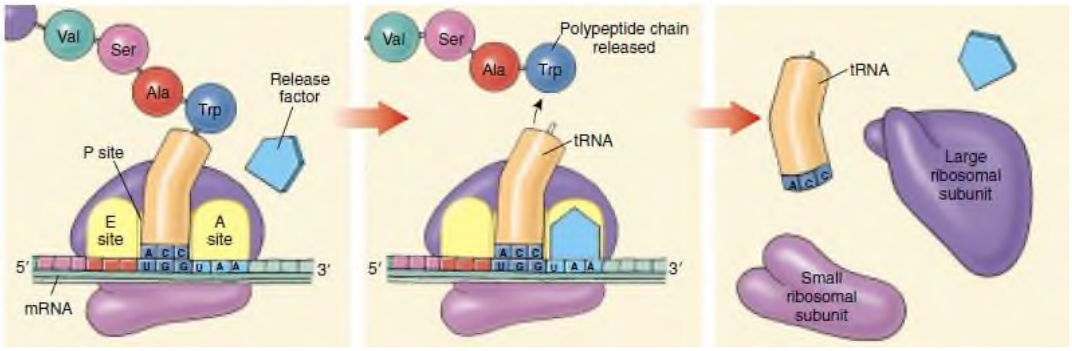


FIGURE 5.11. Termination of protein synthesis. There is no tRNA with an anticodon complementary to any of the three termination signal codons, such as the UAA nonsense codon illustrated here. When a ribosome encounters a termination codon, it therefore stops translocating. A specific release factor facilitates the release of the polypeptide chain by breaking the covalent bond that links the polypeptide to the P-site tRNA.

When a ribosome encounters a termination codon, it therefore stops translocating. A specific release factor facilitates the release of the polypeptide chain by breaking the covalent bond that links the polypeptide to the P-site tRNA.

The first step of translation is the formation of an initiation complex. Each step of the ribosome's progress exposes a codon, to which a tRNA molecule with thecomplementary anticodon binds. The amino acid carried by each tRNA molecule is added to the end of the growing polypeptide chain.

Figure 5.12 summarizes eukaryotic protein synthesis.

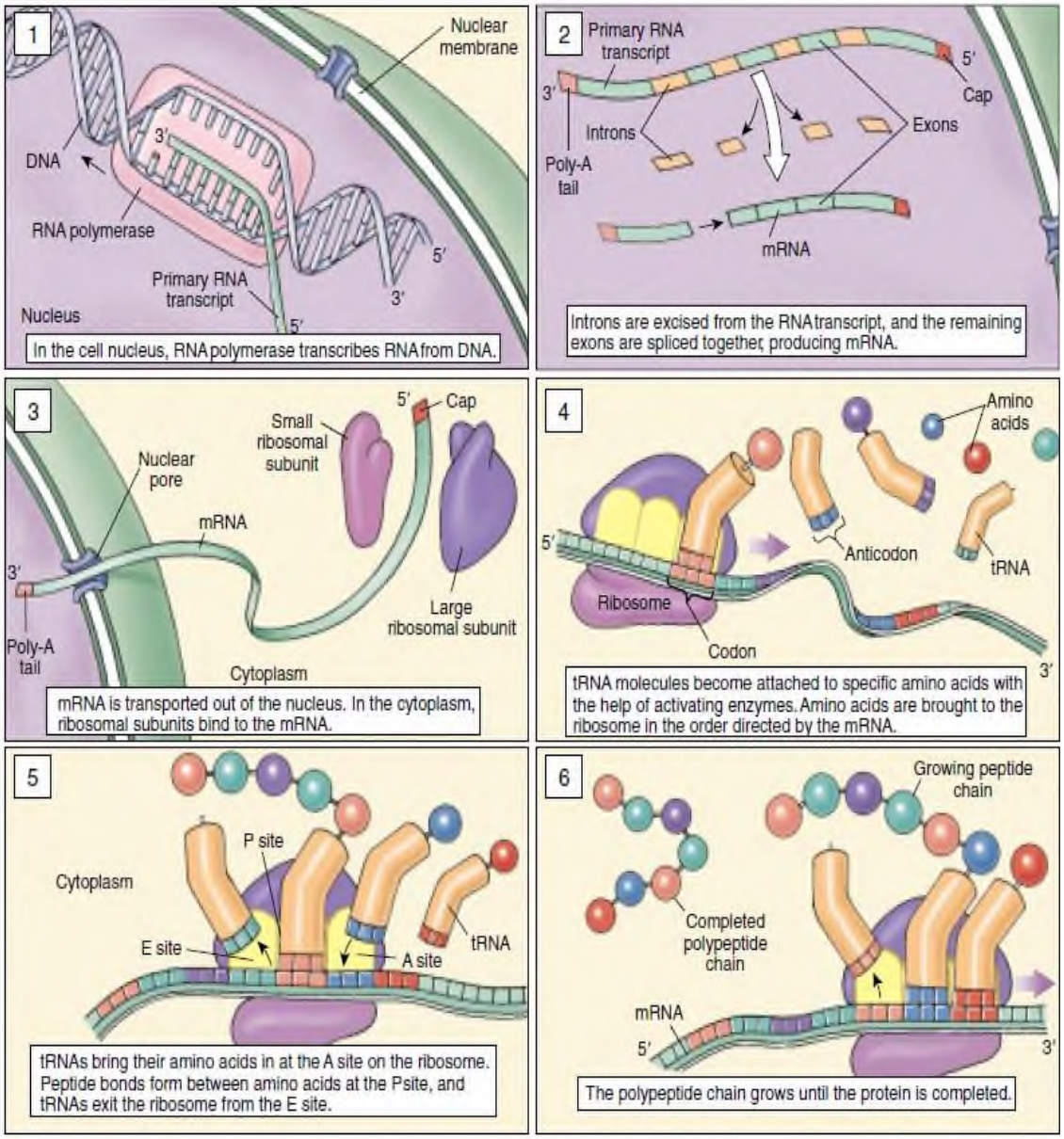


FIGURE 5.12. An overview of gene expression in eukaryotes.

CONTROL OF GENE EXPRESSION

Gene expression is controlled at the transcriptional and posttranscriptional levels. Transcriptional control, more common, is effected by the binding of proteins to regulatory sequences within the DNA (Fig. 5.13, 5.14).

The tryptophan repressor cannot bind the operator (which is located *within* the promoter) unless tryptophan first binds to the repressor. Therefore, in the absence of tryptophan, the promoter is free to function and RNA polymerase transcribes the operon. In the presence of tryptophan, the tryptophan-repressor

complex binds tightly to the operator, preventing RNA polymerase from initiating transcription.

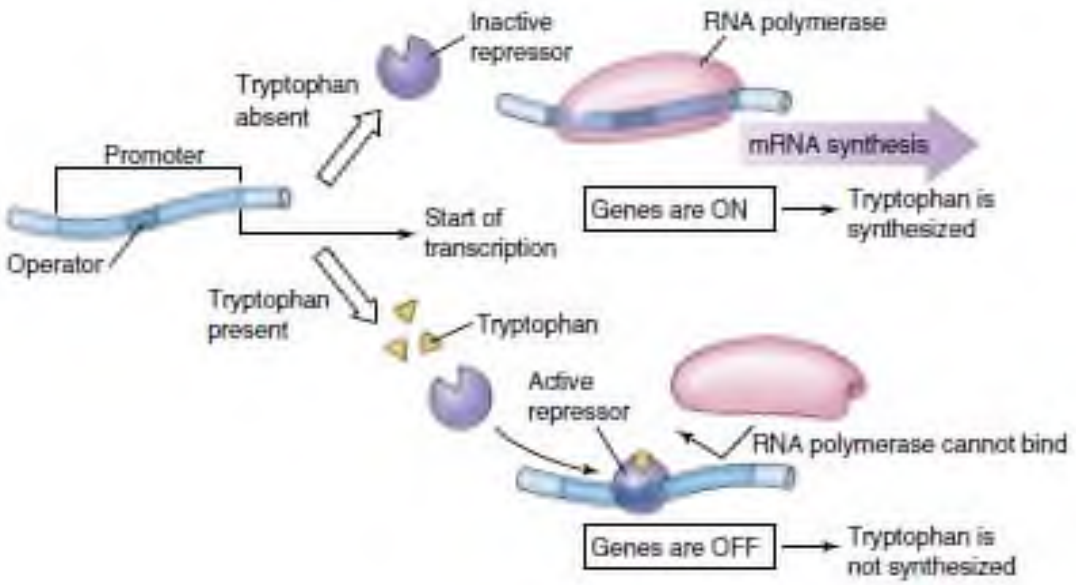


FIGURE 5.13. How the *trp* operon is controlled.

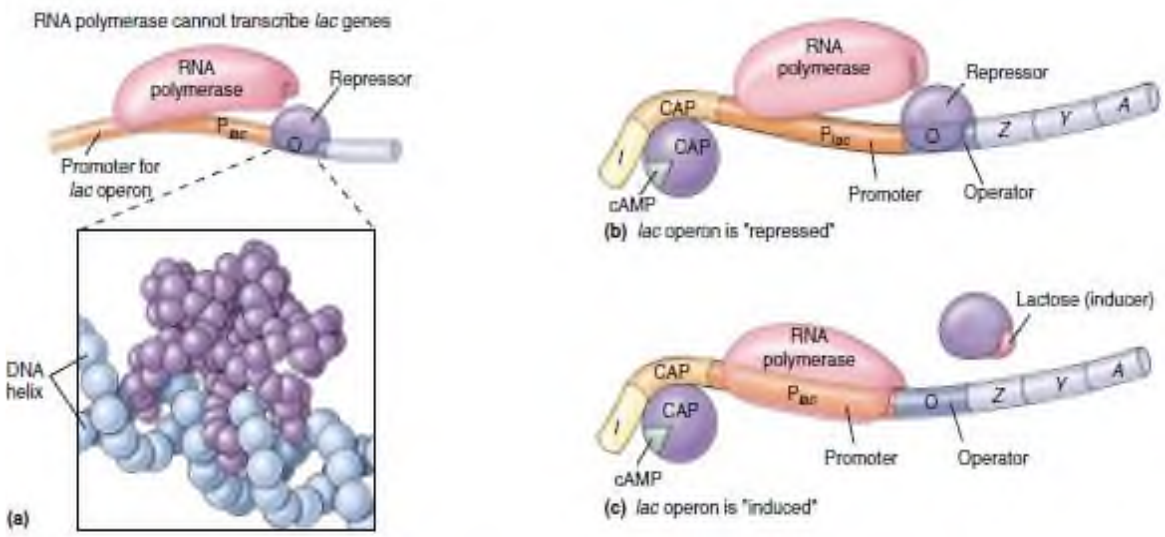


FIGURE 5.14. How the *lac* repressor works.

The *lac* repressor (*a*). Because the repressor fills the major groove of the DNA helix, RNA polymerase cannot fully attach to the promoter, and transcription is blocked. (*b*) The *lac* operon is shut down ("repressed") when the repressor protein is bound to the operator site. Because promoter and operator sites overlap, RNA polymerase and the repressor cannot functionally bind at the same time, any more than two people can sit in the same chair at once. (*c*)

The *lac* operon is transcribed (“induced”) when CAP is bound and when lactose binding to the repressor changes its shape so that it can no longer sit on the operator site and block RNA polymerase activity.

Transcriptional control in eukaryotes operates at a distance.

1.Transcription factors and enhancers confer great flexibility on the control of gene expression in eukaryotes.

Eukaryotic Transcription Factors. For RNA polymerase to successfully bind to a eukaryotic promoter and initiate transcription, a set of proteins called **transcription factors** must first assemble on the promoter, forming a complex that guides and stabilizes the binding of the polymerase. The assembly process begins some 25 nucleotides upstream from the transcription start site, where a transcription factor composed of many subunits binds to a short TATA sequence. Other transcription factors then bind, eventually forming a full transcription factor complex able to capture RNA polymerase. In many instances, the transcription factor complex then phosphorylates the bound polymerase, disengaging it from the complex so that it is free to begin transcription. The binding of several different transcription factors provides numerous points where control over transcription may be exerted. Anything that reduces the availability of a particular factor (for example, by regulating the promoter that governs the expression and synthesis of that factor) or limits its ease of assembly into the transcription factor complex will inhibit transcription.

The structure of a human transcription complex. The transcription complex that positions RNA polymerase at the beginning of a human gene consists of four kinds of proteins (Fig. 5.15).

Basal factors (the green shapes at bottom of complex with letter names) are transcription factors that are essential for transcription but cannot by themselves increase or decrease its rate. They include the TATA-binding protein, the first of the basal factors to bind to the core promoter sequence. Coactivators (the tan shapes that form the bulk of the transcription complex, named according to their molecular weights) are transcription factors that link the basal fac-

tors with regulatory proteins called activators (the red shapes). The activators bind to enhancer sequences at other locations on the DNA. The interaction of individual basal factors with particular activator proteins is necessary for proper positioning of the polymerase, and the rate of transcription is regulated by the availability of these activators. When a second kind of regulatory protein called a repressor (the purple shape) binds to a so-called "silencer" sequence located adjacent to or overlapping an enhancer sequence, the corresponding activator that would normally have bound that enhancer is no longer able to do so. The activator is thus unavailable to interact with the transcription complex and initiate transcription.

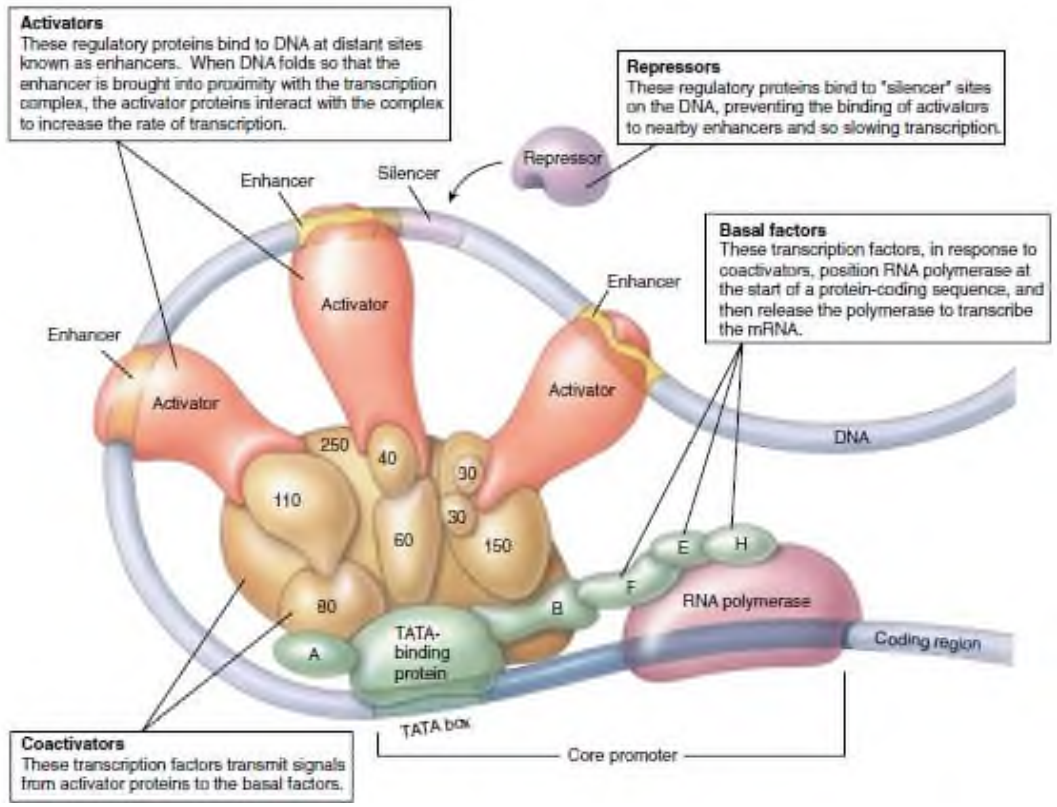


FIGURE 5.15. A human transcription complex.

2.The Effect of Chromosome Structure on Gene Regulation. Transcriptional control of gene expression occurs in eukaryotes despite the tight packaging of DNA into ***nucleosomes.*** The way DNA is packaged into chromosomes can have a profound effect on gene expression. As we saw in chapter 11, the DNA of eukaryotes is packaged in a highly compact form that enables it to fit into the cell nucleus. DNA is wrapped tightly around histone proteins to

form nucleosomes and then the strand of nucleosomes (Fig. 5.16.) and then the strand of nucleosomes is twisted into 30-nm filaments.

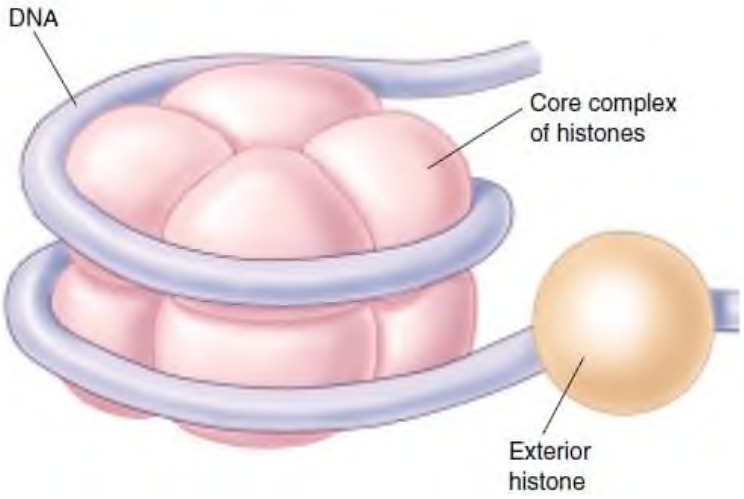


FIGURE 5.16. Nucleosomes. In the diagram of a nucleosome, the DNA double helix is wound around a core complex of eight histones; one additional histone binds to the outside of the nucleosome, exterior to the DNA.

3. DNA Methylation. Chemical **methylation** of the DNA was once thought to play a major role in gene regulation in vertebrate cells. The addition of a methyl group to cytosine creates 5-methylcytosine but has no effect on base-pairing with guanine (Fig. 5.17), just as the addition of a methyl group to uracil produces thymine without affecting basepairing with adenine.

Many inactive mammalian genes are methylated, and it was tempting to conclude that methylation caused the inactivation. However, methylation is now viewed as having a less direct role, blocking accidental transcription of "turned-off" genes. Vertebrate cells apparently possess a protein that binds to clusters of 5-methylcytosine, preventing transcriptional activators from gaining access to the DNA. DNA methylation in vertebrates thus ensures that once a gene is turned off, it stays off.

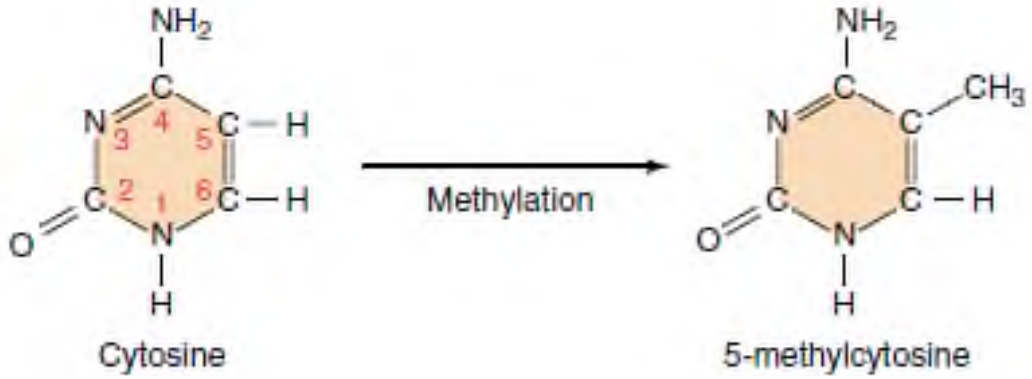


FIGURE 5.17. DNA methylation. Cytosine is methylated, creating 5-methylcytosine. Because the methyl group is positioned to the side, it does not interfere with the hydrogen bonds of a GC basepair.

Key Questions:

1. What is the threedimensional shape of DNA, and how does this shape fit with
2. Chargaff's observations on the proportions of purines and pyrimidines in DNA?
3. How is the leading strand of a DNA duplex replicated? How is the lagging strand replicated? What is the basis for the requirement that the leading and lagging strands be replicated by different mechanisms?
4. What are the three major classes of RNA? What is the function of each type?
5. What is the function of RNA polymerase in transcription? What determines where RNA polymerase begins and ends its function?
6. How did Crick and his colleagues determine how many nucleotides are used to specify each amino acid? What is an anticodon?
7. During protein synthesis, what mechanism ensures that only one amino acid is added to the growing polypeptide at a time? What mechanism ensures the correct amino acid is added at each position in the polypeptide?
8. How does an mRNA molecule specify where the polypeptide it encodes should begin? How does it specify where the polypeptide should end?
9. How does the *primary* RNA transcript of a eukaryotic gene differ from the mRNA transcript of that gene as it is translated in the cytoplasm?
10. What roles do elongation factors play in translation? What is an intron? What is an exon? How is each involved in the mRNA molecule that is ultimately translated?
11. Describe the mechanism by which the transcription of *trp* genes is regulated in *Escherichia coli* when tryptophan is present in the environment.
12. Describe the mechanism by which the transcription of *lac* genes is regulated in *E. coli* when glucose is absent but lactose is present in the environment.
13. How do transcription factors promote transcription in eukaryotic cells? How do the enhancers of eukaryotic cells differ from most regulatory sites on bacterial DNA?

14. What role does the methylation of DNA likely play in transcriptional control?

Examples of Review questions:

NN	Questions	Right answers
1	ALL NUCLEIC ACIDS ___ . 1) are polymers of nucleotides. 2) are polymers of amino acids. 3) are double-stranded. 4) are double-helical. 5) contain deoxyribose.	**1**
2	THE PYRIMIDINES ARE: 1) adenin, thymine, cytosine; 2) guanine, uracil, thymine; 3) thymine, cytosine, uracil; 4) adenin, cytosine, uracil.	**3**
3	THE ROLE OF DNA LIGASE IN DNA REPLICATION IS TO ___ . 1) add more nucleotides to the growing strand one at a time. 2) open up the two DNA strands to expose template strands. 3) ligate base to sugar to phosphate in a nucleotide. 4) bond Okazaki fragments to one another. 5) remove incorrectly paired bases.	**4**
4	TRANSFER RNA (TRNA) _____. 1) carries monosaccharides 2) is made of messenger RNA 3) has an anticodon region, which is complementary to the mRNAcodon 4) is the site of protein synthesis	**3**
5	MRNA HAS THE SEQUENCE 5'-AUGAAAUCCUAG-3'. WHAT IS THE TEMPLATE DNA STRAND FOR THIS SEQUENCE? 1) 3'-TACTTTAGGATC-5' 2) 3'-ATGAAATCCTAG-5' 3) 3'-GATCCTAAAGTA-5' 4) 3'-TACAAATCCTAG-5'	**1**

CHAPTER 6. THE INDEPENDENT INHERITANCE OF GENES AND TRAITS. MENDEL'S LAWS OF HEREDITY

MENDEL'S MODEL OF HEREDITY

Gregor Johann Mendel. Cultivating his plants in the garden of a monastery in Brunn, Austria (now Brno, Czech republic), Mendel studied how differences among varieties of peas were inherited when the varieties were crossed. Similar experiments had been done before, but Mendel was the first to quantify the results and appreciate their significance. GregorMendel did his key scientific experiments in this small garden in a monastery.

When Mendel crossed two contrasting varieties, he found all of the offspring in the first generation exhibited one (dominant) trait, and none exhibited the other (recessive) trait. In the following generation, 25% were pure-breeding for the dominant trait, 50% were hybrid for the two traits and exhibited the dominant trait, and 25% were pure-breeding for the recessive trait.

From his experiments, Mendel was able to understand four things about the nature of heredity. *First,* the plants he crossed did not produce progeny of intermediate appearance, as a theory of blending inheritance would have predicted. Instead, different plants inherited each alternative intact, as a discrete characteristic that either was or was not visible in a particular generation. *Second,* Mendel learned that for each pair of alternative forms of a character, one alternative was not expressed in the F1 hybrids, although it reappeared in some F2 individuals. ***The trait that "disappeared" must therefore be latent*** (***present but not expressed) in the F***1 ***individuals.*** *Third,* the pairs of alternative traits examined segregated among the progeny of a particular cross, some individuals exhibiting one trait, some the other. *Fourth,* these alternative traits were expressed in the F2 generation in the ratio of 3⁄4 dominant to 1⁄4 recessive. This characteristic 3:1 segregation is often referred to as the **Mendelian ratio.**

To explain these results, Mendel proposed a simple model. It has become one of the most famous models in the history of science, containing simple assumptions and making clear predictions. The model has five elements:

1. Parents do not transmit physiological traits directly to their offspring. Rather, they transmit discrete information about the traits, what Mendel called "factors." These factors later act in the offspring to produce the trait. In modern terms, we would say that information about the alternative forms of characters that an individual expresses is *encoded* by the factors that it receives from its parents.

2. Each individual receives two factors that may code for the same trait or for two alternative traits for a character. We now know that there are two factors for each character present in each individual because these factors are carried on chromosomes, and each adult individual is *diploid.* When the individual forms gametes (eggs or sperm), they contain only one of each kind of chromosome; the gametes are *haploid.* Therefore, only one factor for each character of the adult organism is contained in the gamete. Which of the two factors ends up in a particular gamete is randomly determined.

3. Not all copies of a factor are identical. In modern terms, the alternative forms of a factor, leading to alternative forms of a character, are called **alleles.** When two haploid gametes containing exactly the same allele of a factor fuse during fertilization to form a zygote, the offspring that develops from that zygote is said to be **homozygous;** when the two haploid gametes contain different alleles, the individual offspring is **heterozygous.** In modern terminology, Mendel's factors are called **genes.** We now know that each gene is composed of a particular DNA nucleotide sequence. The particular location of a gene on a chromosome is referred to as the gene's **locus** (plural, loci).

4. The two alleles, one contributed by the male gamete and one by the female, do not influence each other in any way. In the cells that develop within the new individual, these alleles remain discrete. They neither blend with nor alter each other. (Mendel referred to them as "uncontaminated.") Thus, when the individual matures and produces its own gametes, the alleles for each gene segregate randomly into these gametes, as described in element 2.

5. The presence of a particular allele does not ensure that the trait encoded by it will be expressed in an individual carrying that allele. In heterozygous individuals, only one allele (the dominant one) is expressed, while the other

(recessive) allele is present but unexpressed. To distinguish between the presence of an allele and its expression, modern geneticists refer to the totality of alleles that an individual contains as the individual's **genotype** and to the physical appearance of that individual as its **phenotype.** The phenotype of an individual is the observable outward manifestation of its genotype, the result of the functioning of the enzymes and proteins encoded by the genes it carries.In other words, the genotype is the blueprint, and the phenotype is the visible outcome.

These five elements, taken together, constitute Mendel's model of the hereditary process. Many traits in humans also exhibit dominant or recessive inheritance, similar to the traits Mendel studied in peas (Table 6.1).

Table 6.1

Some Dominant and Recessive Traits in Humans

Recessive Traits	Phenotypes	Dominant Traits	Phenotypes
Albinism	Lack of melanin pigmentation	Middigital hair	Presence of hair on middle segment of fingers
Alkaptonuria	Inability to metabolize homogenistic acid	Brachydactyly	Shaft fingers
Red-green color blindness	Inability to distinguish red or green wavelengths of light	Huntington's disease	Degeneration of nervous system, starting in middle age
Cystic fibrosis	Abnormal gland secretion, leading to liver degeneration and lung failure	Phenylthiocarttamide (PTC) sensitivity	Ability to taste PTC as bitter
Duchenne muscular dystrophy	Wasting away of muscles during childhood	Camptodactyly	Inability to straighten the little finger
Hemophilia	Inability to form blood clots	Hypercholesterolemia (the most common human Mendelian disorder – 1 in 500)	Elevated levels of blood cholesterol and risk of heart attack
Sickle cell anemia	Defective hemoglobin that causes red blood cells to curve and stick together	Polydactyly	Extra fingers and toes

THE TESTCROSS

To determine whether an individual exhibiting a dominant phenotype, such as purple flowers, is homozygous or heterozygous for the dominant allele, Mendel crossed the individual in question with a plant that he knew to be homozygous recessive, in this case a plant with white flowers (Fig. 6.1).

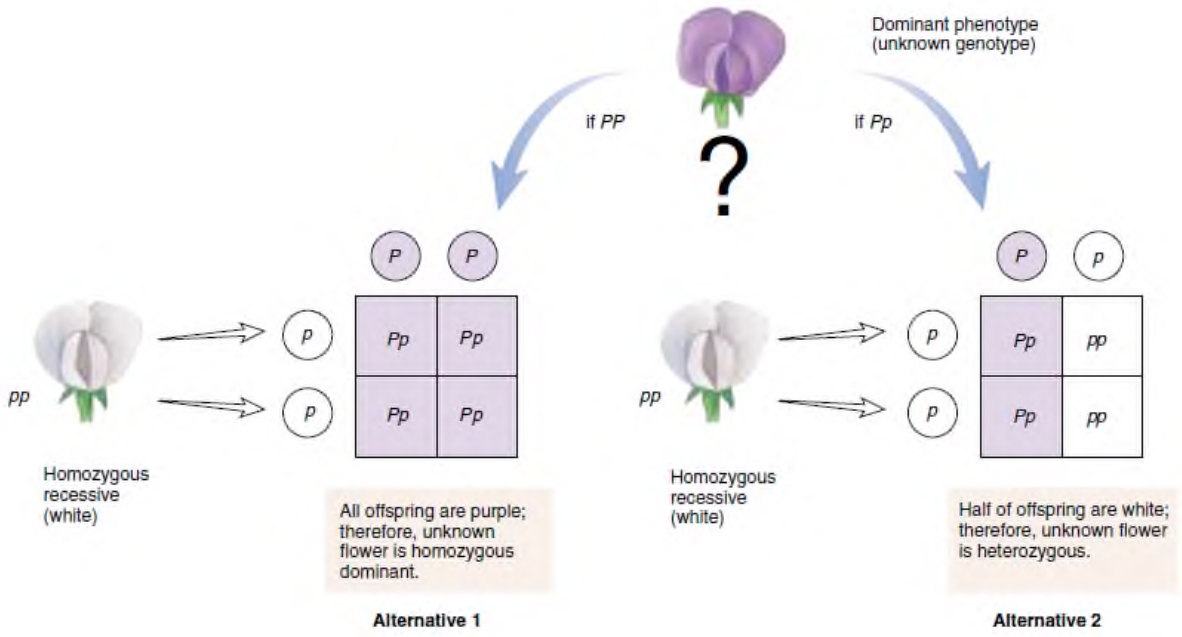


FIGURE 6.1. A testcross.

Alternative 1: unknown individual homozygous dominant (*PP*). *PP* × *pp:* all offspring have purple flowers (*Pp*).

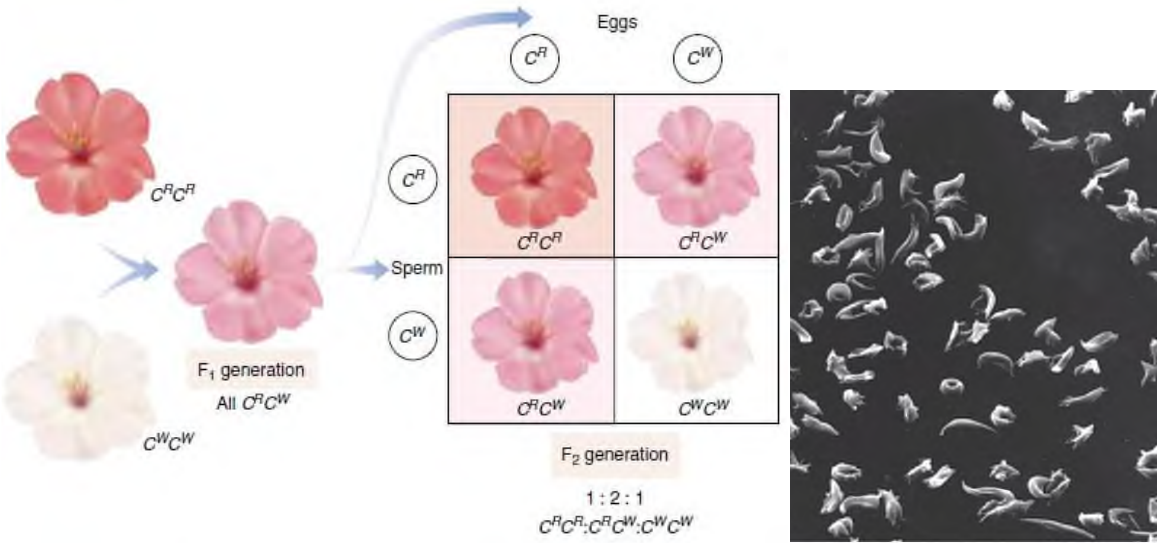
Alternative 2: unknown individual heterozygous (*Pp*).*Pp* × *pp:* 1⁄2 of offspring have white flowers (*pp*) and 1⁄2 have purple flowers (*Pp*).

MENDEL'S FIRST LAW OF HEREDITY: SEGREGATION

Mendel's model thus accounts in a neat and satisfying wayfor the segregation ratios he observed. Its central assumption— that alternative alleles of a character segregate from each other in heterozygous individuals and remain distinct—has since been verified in many other organisms. It is commonly referred to as **Mendel's First Law of Heredity,** or the **Law of Segregation.** The segregational behavior of alternative alleles has a simple physical basis, the alignment of chromosomes at random on the metaphase plate during meiosis I.

It is a tribute to the intellect of Mendel's analysis that he arrived at the correct scheme with no knowledge of the cellular mechanisms of inheritance; neither chromosomes nor meiosis had yet been described.

Incomplete dominance. Not all alternative alleles are fully dominant or fully recessive in heterozygotes. Some pairs of alleles instead produce a heterozygous phenotype that is either intermediate between those of the parents (incomplete dominance), or representative of both parental phenotypes (codominance). For example, in the cross of red and white flowering Japanese four o'-clocks described in Figure 6.2, all the F1 offspring had pink flowers—indicating that neither red nor white flower color was dominant. Does this example of incomplete dominance argue that Mendel was wrong? Not at all. When two of the F1 pink flowers were crossed, they produced red-, pink-, and white-flowered plants in a 1:2:1 ratio. Heterozygotes are simply intermediate in color.



(a) (b)

FIGURE 6.2. Incomplete dominance. (a) In a cross between a red-flowered Japanese four o'clock, genotype *CRCR*, and a white-flowered one (*CWCW*), neither allele is dominant (Fig. 8.2, 8.3). The heterozygous progeny have pink flowers and the genotype *CRCW*. If two of these heterozygotes are crossed, the phenotypes of their progeny occur in a ratio of 1:2:1 (red:pink:white). **(b)** Sickle-shaped red blood cells from a person with sickle-cell anemia. Red blood cells are about 7 to 8 _m in diameter. (Courtesy of Dr. Patricia N. Farnsworth.)

Multiple alleles control the ABO blood groups. Different combinations of the three *I* gene alleles result in four different blood type phenotypes: type A (either *I*A*I*A homozygotes or *I*A*i* heterozygotes), type B (either *I*B*I*B homozygotes or *I*B*i* heterozygotes), type AB (*I*A*I*B heterozygotes), and type O (*ii* homozygotes) (Fig. 6.3).

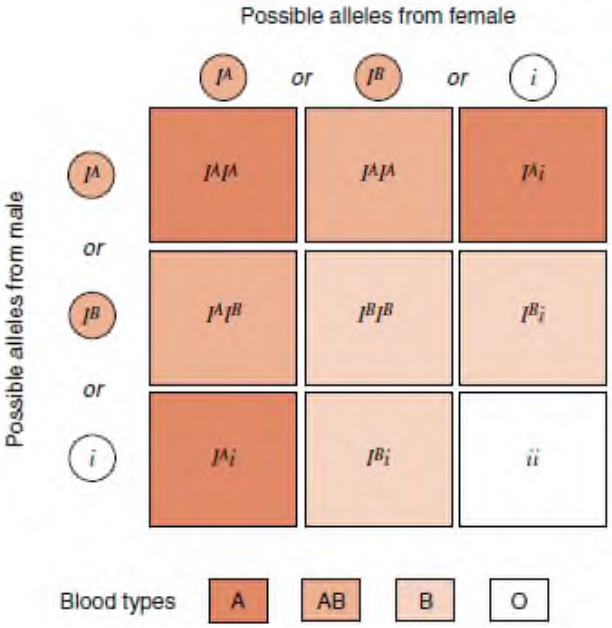


FIGURE 6.3. Multiple alleles control the ABO blood groups.

Gene Disorders Can Be Due to Simple Alterations of Proteins: some of the most common and serious gene defects result from single recessive mutations, including many of the defects listed in Table 6.2.

Table 6.2

Some Important Genetic Disorders

Disorder	Symptom	Defect	Dominant/Recessive	Frequency among Human Births
Cystic fibrosis	Mucus clogs lungs. liver, and pancreas	Failure of chloride ion transport mechanism	Recessive	1/2500 (Caucasians)
Sickle cel anemia	Poor blood circulation	Abnormal hemoglobin molecules	Recessive	1/625 (African Americans)
Tay-Sachs disease	Deterioration of central nervous system in infancy	Defective enzyme (hexosaminidase A)	Recessive	1/3500 (Ashkenazy Jews)
Phenylketonuria	Brain fails to develop in infancy	Defective enzyme (phenylalanine hydroxylase)	Recessive	1/12000
Hemophilia	Blood fails to clot	Defective blood clotting factor VIII	Sex-linked recessive	1/10000 (Caucasian males)
Huntington's disease	Brain tissue gradually deteriorates in middle age	Production of an inhibitor of brain cell metabolism	Dominant	1/24000
Muscular dystrophv (Duchenne)	Muscles wave away	Degradation of myelin coating of nerves stimulating muscles	Set-linked recessive	1/3700 (males)
Hypercholesterolemia	Excessive cholesterol levels in blood, leading to heart disease	Abnormal form of cholesterol cell surface receptor	Dominant	1/500

Sickle cell anemia (SCA) is caused by a single-nucleotide change in the gene for hemoglobin, producing a protein with a nonpolar amino acid on its surface that tends to make the molecules clump together.

Cystic fibrosis results from a defect in a single gene,called *cf*, that is passed down from parent to child.

MENDEL'S SECOND LAW OF HEREDITY: INDEPENDENT ASSORTMENT

Mendel's discovery is often referred to as **Mendel's Second Law of Heredity,** or the **Law of Independent Assortment.** Genes that assort independently of one another, like the seven genes Mendel studied, usually do so because they are located on different chromosomes, which segregate independently during the meiotic process of gamete formation (Fig. 6.4).

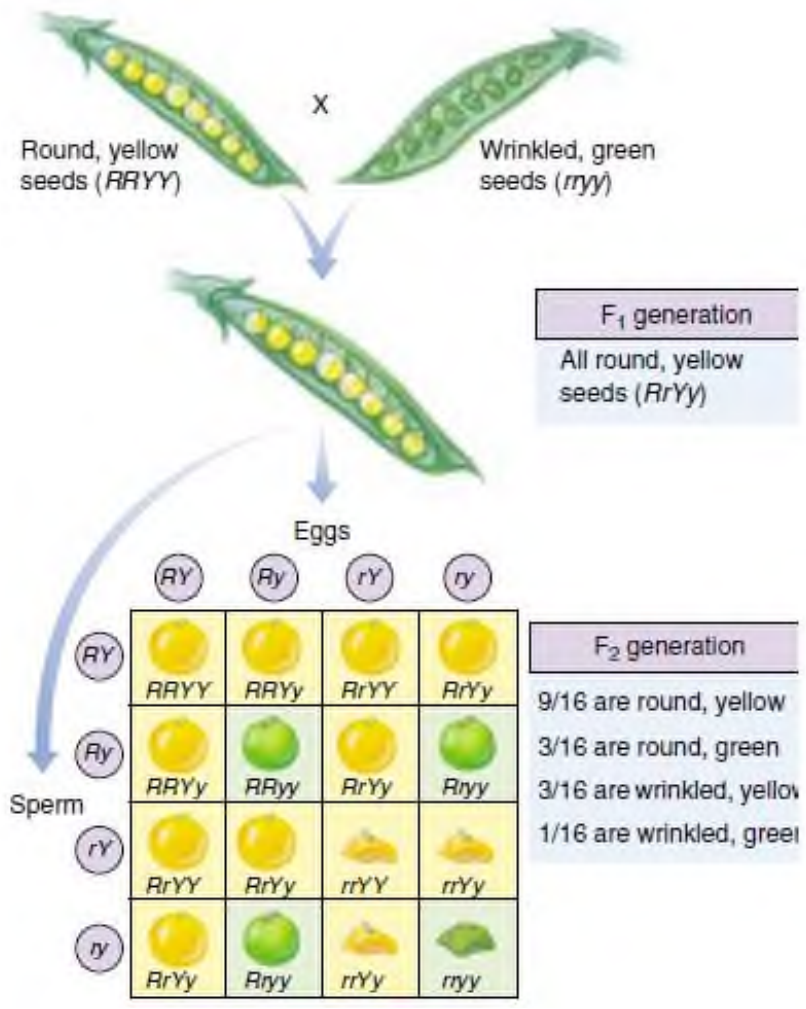


FIGURE 6.4. Analyzing a dihybrid cross. This Punnett square shows the results of Mendel's dihybrid cross between plants with round yellow seeds and plants with wrinkled green seeds. The ratio of the four possible combinations of phenotypes is predicted to be 9:3:3:1, the ratio that Mendel found.

Mendelian traits assort independently because they are determined by genes located on chromosomes that assort independently in meiosis.

The existence of genes was originally inferred by observing precise mathematical ratios in the descendants of two parental individuals that show contrasting phenotypes. These standard inheritance patterns are still used to deduce the existence of specific genes affecting specific characters.

The cellular and molecular basis of Mendelian genetics. Having examined the way that Mendel identified the existence of genes in peas, we can now translate his notion of the gene into a modern context. To Mendel the gene was an invented entity needed to explain a pattern of inheritance. However, today the gene is very much a reality, as a result of a great volume of research carried out for the very purpose of deducing its nature.We will examine such research throughout this book, but for the present let us summarize the modern view of the gene. Mendel proposed that genes come in different forms we now call *alleles*. What is the molecular nature of alleles? When alleles such as *A* and *a* are examined at the DNA level by using modern technology, they are generally found to be identical in most of their sequences and differ only at one or a few nucleotides of the thousands of nucleotides that make up the gene. Therefore, we see that the alleles are truly different versions of the same basic gene. Looked at another way, *gene* is the generic term and allele is specific. (The peacolor gene has two alleles coding for yellow and green.) The following diagram represents the DNA of two alleles of one gene; the letter "x" represents a difference in the nucleotide sequence:

Allele A: ____C_____

Allele a: ____T_____.

The chromosomal locations of some genes show at Figure 6.5.

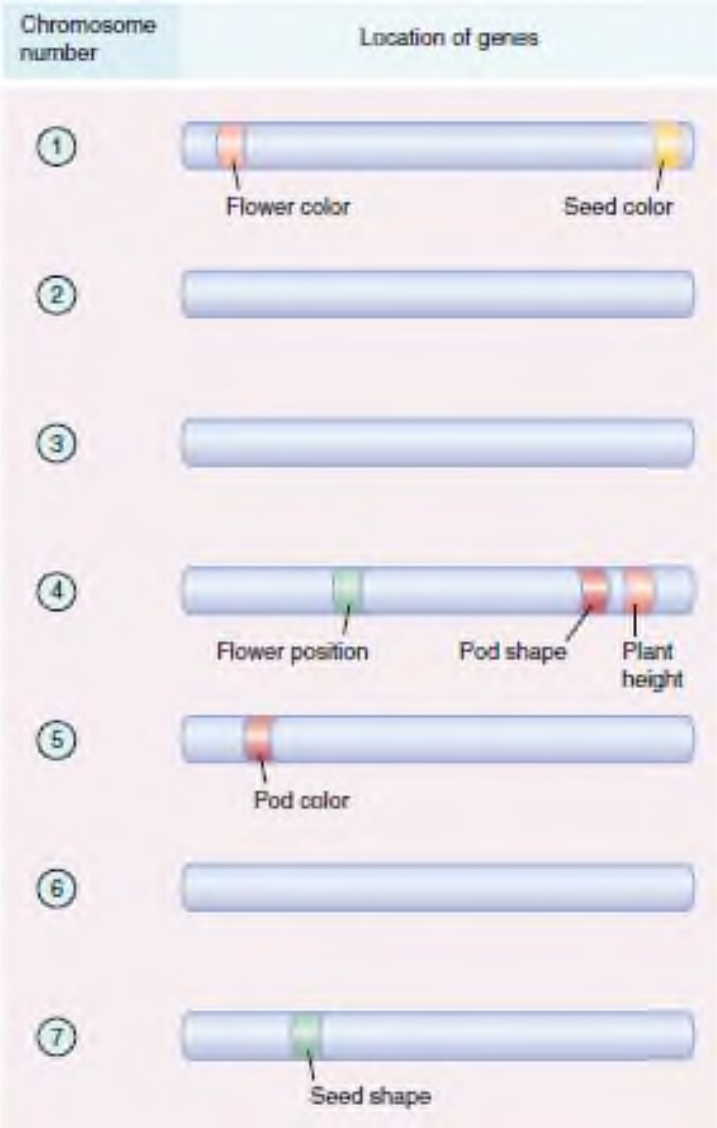


FIGURE 6.5. The chromosomal locations of the seven genes studied by Mendel in the garden pea.

To produce pigment, a plant must possess at least one functional copy of each enzyme gene (Fig. 6.6).

The dominant alleles encode functional enzymes, but the recessive alleles encode nonfunctional enzymes. Of the 16 genotypes predicted by random assortment, 9 contain at least one dominant allele of both genes; they produce purple progeny. The remaining 7 genotypes lack dominant alleles at either or both loci (3 + 3 + 1 = 7) and so are phenotypically the same (nonpigmented), giving the phenotypic ratio of 9:7 that Emerson observed. The inability to see the effect of enzyme 2 when enzyme 1 is nonfunctional is an example of epistasis.

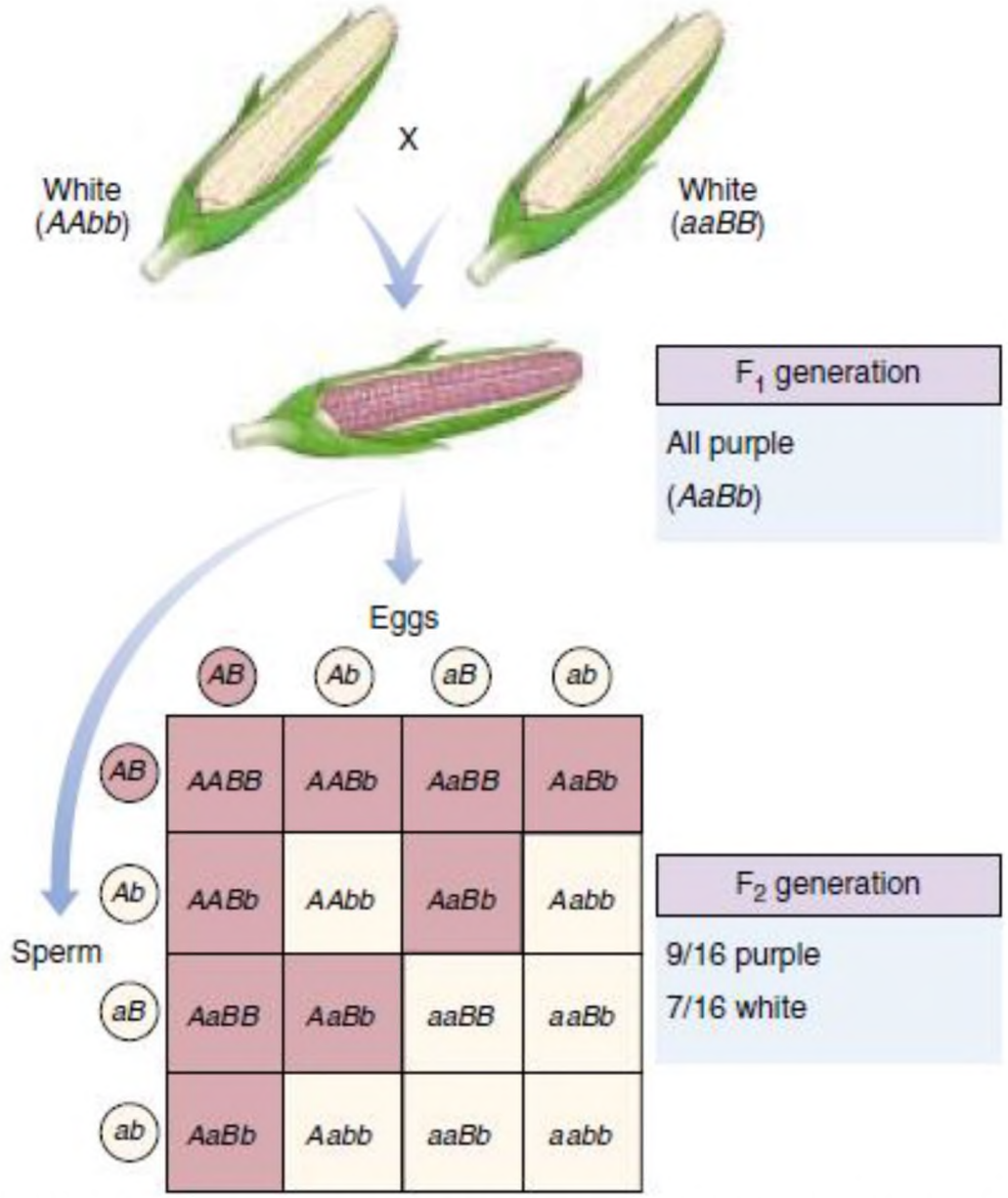


FIGURE 6.6. How epistasis affects grain color. The purple pigment found in some varieties of corn is the product of a two-step biochemical pathway. Unless both enzymes are active (the plant has a dominant allele for each of the two genes, *A* and *B*), no pigment is expressed.

In many animals, coat color is the result of epistatic interactions among genes. Coat color in Labrador retrievers, a breed of dog, is due primarily to the interaction of two genes. The *E* gene determines if dark pigment (eumelanin) will be deposited in the fur or not. If a dog has the genotype *ee,* no pigment will be deposited in the fur, and it will be yellow. If a dog has the genotype *EE* or *Ee* (*E_*), pigment will be deposited in the fur.

A second gene, the *B* gene, determines how dark the pigment will be. This gene controls the distribution of melanosomes in a hair. Dogs with the

genotype *E_bb* will have brown fur and are called chocolate labs. Dogs with the genotype *E_B_* will have black fur. But, even in yellow dogs, the *B* gene does have some effect. Yellow dogs with the genotype *eebb* will have brown pigment on their nose, lips, and eye rims, while yellow dogs with the genotype *eeB_* will have black pigment in these areas. The interaction among these alleles is illustrated in Figure 6.7.

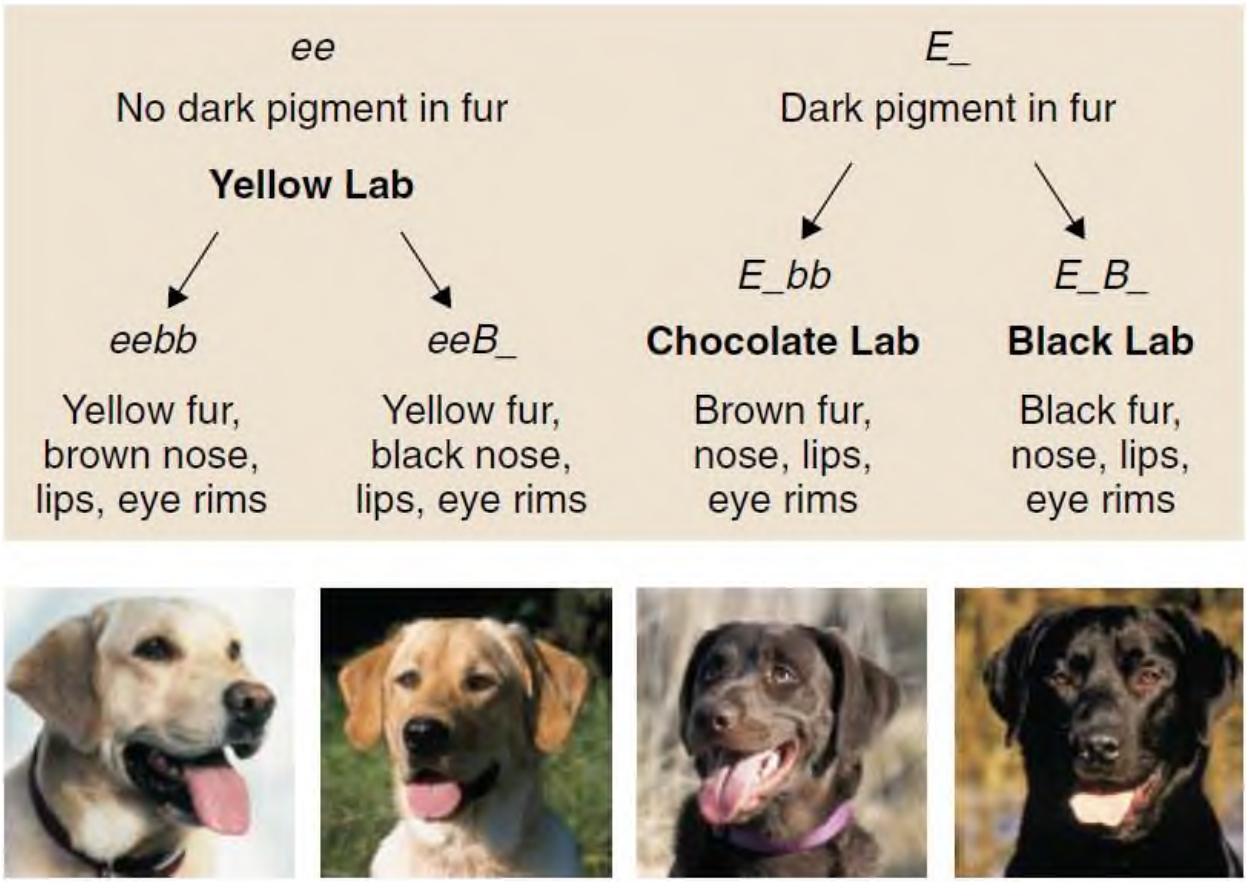


FIGURE 6.7. The effect of epistatic interactions on coat color in dogs. The coat color seen in Labrador retrievers is an example of the interaction of two genes, each with two alleles. The *E* gene determines if the pigment will be deposited in the fur, and the *B* gene determines how dark the pigment will be.

The genes for coat color in this breed have been found, and a genetic test is available to determine the coat colors in a litter of puppies.

Polygenic inheritance occurs when one trait is governed by two or more sets of alleles, and the individual has a copy of all allelic pairs, possibly located on many different pairs of chromosomes (Fig. 6.8.).

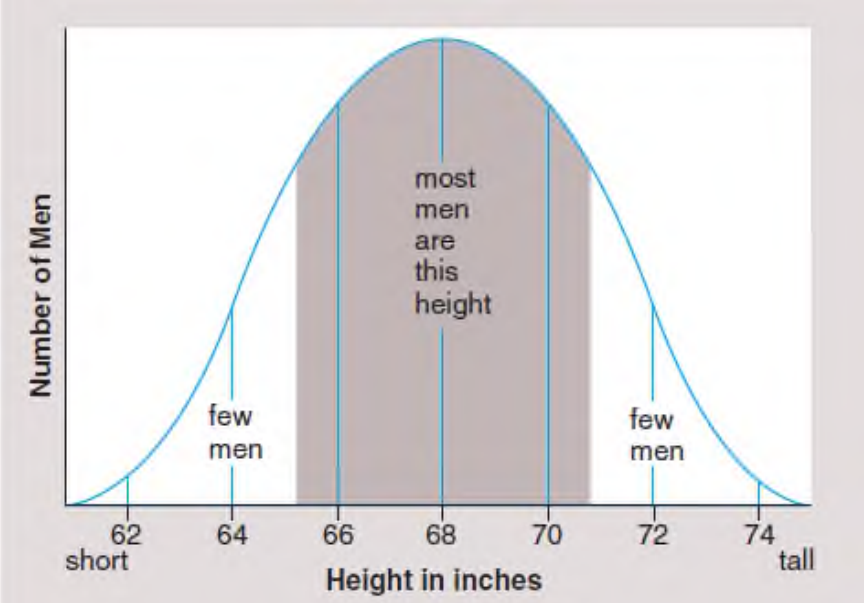


FIGURE 6.8 Polygenic inheritance. When you record the heights of a large group of young men, the values follow a bell-shaped curve. Such a continuous distribution is due to control of a trait by several sets of alleles. Environmental effects are also involved.

Each dominant allele has a quantitative effect on the phenotype, and these effects are additive. The result is a continuous variation of phenotypes, resulting in a distribution of these phenotypes that resembles a bell-shaped curve. The more genes involved, the more continuous the variation and the distribution of the phenotypes. Also, environmental effects cause many intervening phenotypes; in the case of height, differences in nutrition assure a bell-shaped curve.

Skin Color inheritance. Just how many pairs of alleles control skin color is not known, but a range in colors can be explained on the basis of two pairs. When a very dark person reproduces with a very light person, the children have medium-brown skin; when two people with medium-brown skin reproduce with one another, the children may range in skin color from very dark to very light. This can be explained by assuming that skin color is controlled by two pairs of alleles and that *each capital letter contributes pigment to the skin:*

Genotypes	**Phenotypes**
AABB	Very dark
AABb or *AaBB*	Dark
AaBb or *AAbb* or *aaBB*	Medium brown
Aabb or *aaBb*	Light
aabb	Very light

Notice again that there is a range in phenotypes and that there are several possible phenotypes in between the two extremes. Therefore, the distribution of these phenotypes is expected to follow a bell-shaped curve—few people have the extreme phenotypes, and most people have the phenotype that lies in the middle between the extremes.

Key Questions:

1. 1-st Mendel's law – the law of uniformity or the rule of domination.
2. The 2-nd Mendel's law – the law of segregation.
3. The testcross.
4. Reasons for rejection from Mendel's laws.
5. Types of interaction of allelic genes.
6. Complete dominance (fenilketonuriya).
7. Incomplete domination (crescent and cellular anemia).
8. Overdominance (heterosis).
9. Kodomination (the AB blood type formation).
10. Multiple alleles. Features of inheritance of ABO blood types in humans.
11. Di-hybrid and poly-hybrid crossing.
12. Inheritance of the genes and signs located in different chromosomes.
13. III Mendel's law.
14. Statistical regularities at poly-hybrid crossing. Formula of calculation of number of gametes and splitting.
15. Types of non-allelic genes interactions.
16. Complementarity.
17. Epistasis.
18. Polygenic inheritance.

Examples of Review questions:

NN	Questions	Right answers
1	ALLELIC GENES ARE___ . 1) control manifestation of the same indication in the organisms of different species 2) located in the homological chromosomes 3) located in the different pairs of chromosomes 4) disposed in the identical locus of homological chromosomes and determinated the alternative development of the same indication	**2**
2	WHEN THE EFFECTS OF AN ALLELE ARE SEEN ONLY WHEN AN INDIVIDUAL CARRIES TWO COPIES OF THE ALLELE, THE ALLELE IS TERMED _____. 1) dominant 2) incompletely dominant 3) recessive 4) codominant 5) genotypic	**3**
3	A NEWBORN INFANT IS TYPE A. THE MOTHER IS TYPE O. POSSIBLE GENOTYPES OF THE FATHER ARE___ . 1) A, B or AB 2) A, B or O 3) O only 4) A or AB	**4**
4	IN EPISTASIS ___ . 1) nothing changes from generation to generation 2) one gene alters the effect of another 3) a portion of a chromosome is deleted 4) a portion of a chromosome is inverted 5) the behavior of two genes is entirely independent	**2**
5	PHENOMENON OF THE INFLUENCE BY ONE GENE FOR SEVERAL TRATES IS CALLED: 1) polymery 2) pleiotropy 3) duplication 4) codominanting	**1**

CHAPTER 7. THE LINKED INHERITANCE OF GENES AND TRAITS. SEX LINKAGE INHERITANCE

THE CHROMOSOMAL THEORY OF INHERITANCE

A central role for chromosomes in heredity was first suggested in 1900 by the German geneticist Karl Correns, in one of the papers announcing the rediscovery of Mendel's work. Soon after, observations that similar chromosomes paired with one another during meiosis led directly to the **chromosomal theory of inheritance,** first formulated by the American Walter Sutton in 1902. Several pieces of evidence supported Sutton's theory. One was that reproduction involves the initial union of only two cells, egg and sperm. If Mendel's model were correct, then these two gametes must make equal hereditary contributions. Sperm, however, contain little cytoplasm, suggesting that the hereditary material must reside within the nuclei of the gametes. Furthermore, while diploid individuals have two copies of each pair of homologous chromosomes, gametes have only one. This observation was consistent with Mendel's model, in which diploid individuals have two copies of each heritable gene and gametes have one. Finally, chromosomes segregate during meiosis, and each pair of homologues orients on the metaphase plate independently of every other pair. Segregation and independent assortment were two characteristics of the genes in Mendel's model.

MORGAN'S EXPERIMENT

Morgan's experiment demonstrating the chromosomal basis of sex linkage in *Drosophila.* The white-eyed mutant male fly was crossed with a normal female. The F1 generation flies all exhibited red eyes, as expected for flies heterozygous for a recessive white-eye allele. In the F2 generation, all of the white-eyed flies were male.

Sex Linkage. The solution to this puzzle involve sex. In *Drosophila,* the sex of an individual is determined by the number of copies of a particular

chromosome, the **X chromosome,** that an individual possesses. A fly with two X chromosomes is a female, and a fly with only one X chromosome is a male. In males, the single X chromosome pairs in meiosis with a dissimilar partner called the **Y chromosome.** The female thus produces only X gametes, while the male produces both X and Y gametes. When fertilization involves an X sperm, the result is an XX zygote, which develops into a female; when fertilization involves a Y sperm, the result is an XY zygote, which develops into a male. The solution to Morgan's puzzle is that the gene causing the white-eye trait in *Drosophila* resides only on the X chromosome—it is absent from the Y chromosome. (We now know that the Y chromosome in flies carries almost no functional genes.) A trait determined by a gene on the X chromosome is said to be **sex-linked.** Knowing the white-eye trait is recessive to the red-eye trait, we can now see that Morgan's result was a natural consequence of the Mendelian assortment of chromosomes (Fig. 7.1).

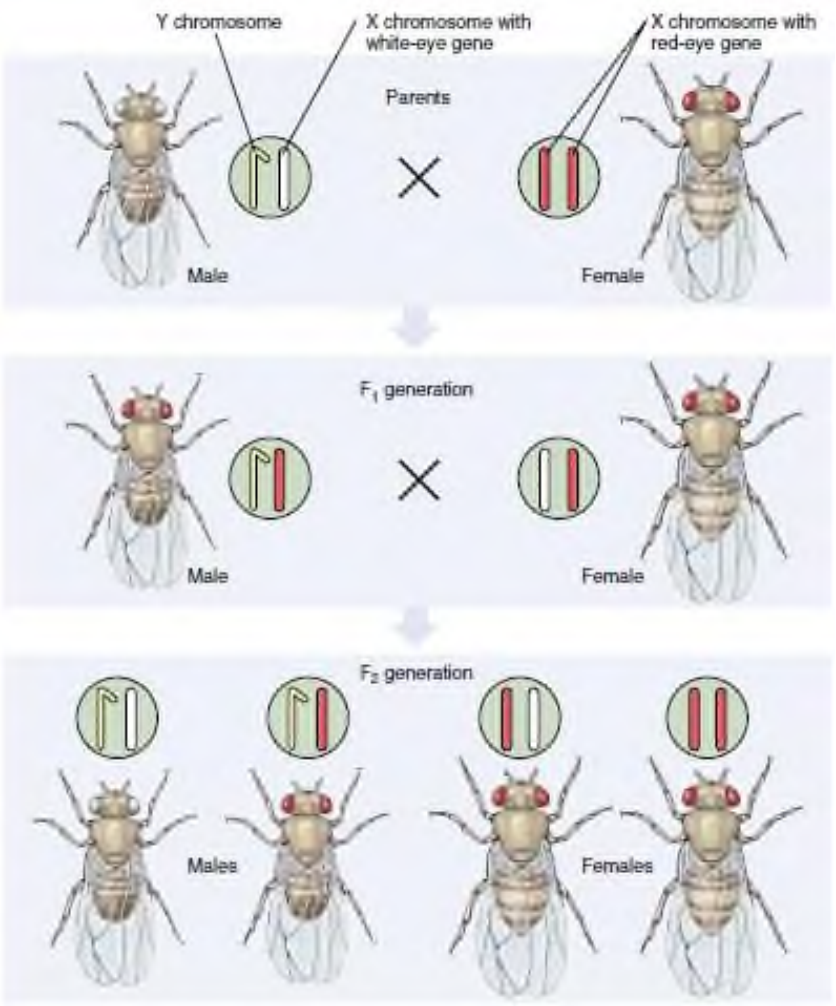


FIGURE 7.1. Morgan's experiment demonstrating the chromosomal basis of sex linkage in *Drosophila.* The white-eyed mutant male fly was crossed with a normal female. The F1 generation flies all exhibited red eyes, as expected for flies heterozygous for a recessive white-eye allele. In the F2 generation, all of the white-eyed flies were male.

Morgan's experiment was one of the most important in the history of genetics because it presented the first clear evidence that the genes determining Mendelian traits do indeed reside on the chromosomes, as Sutton had proposed. The segregation of the white-eye trait has a one-toone correspondence with the segregation of the X chromosome. In other words, Mendelian traits such as eye color in *Drosophila* assort independently because chromosomes do. When Mendel observed the segregation of alternative traits in pea plants, he was observing a reflection of the meiotic segregation of chromosomes.

GENETIC RECOMBINATION

Morgan's experiments led to the general acceptance of Sutton's chromosomal theory of inheritance. Scientists then attempted to resolve the paradox that there are many more independently assorting Mendelian genes than chromosomes. In 1903 the Dutch geneticist Hugo de Vries suggested that this paradox could be resolved only by assuming that homologous chromosomes exchange elements during meiosis. In 1909, French cytologist F. A. Janssens provided evidence to support this suggestion. Investigating chiasmata produced during amphibian meiosis, Janssens noticed that of the four chromatids involved in each chiasma, two crossed each other and two did not. He suggested that this crossing of chromatids reflected a switch in chromosomal arms between the paternal and maternal homologues, involving one chromatid in each homologue. His suggestion was not accepted widely, primarily because it was difficult to see how two chromatids could break and rejoin at exactly the same position.

Crossing Over. Later experiments clearly established that Janssens was indeed correct. One of these experiments, performed in 1931 by American geneticist Curt Stern, is described in Figure 7.2.

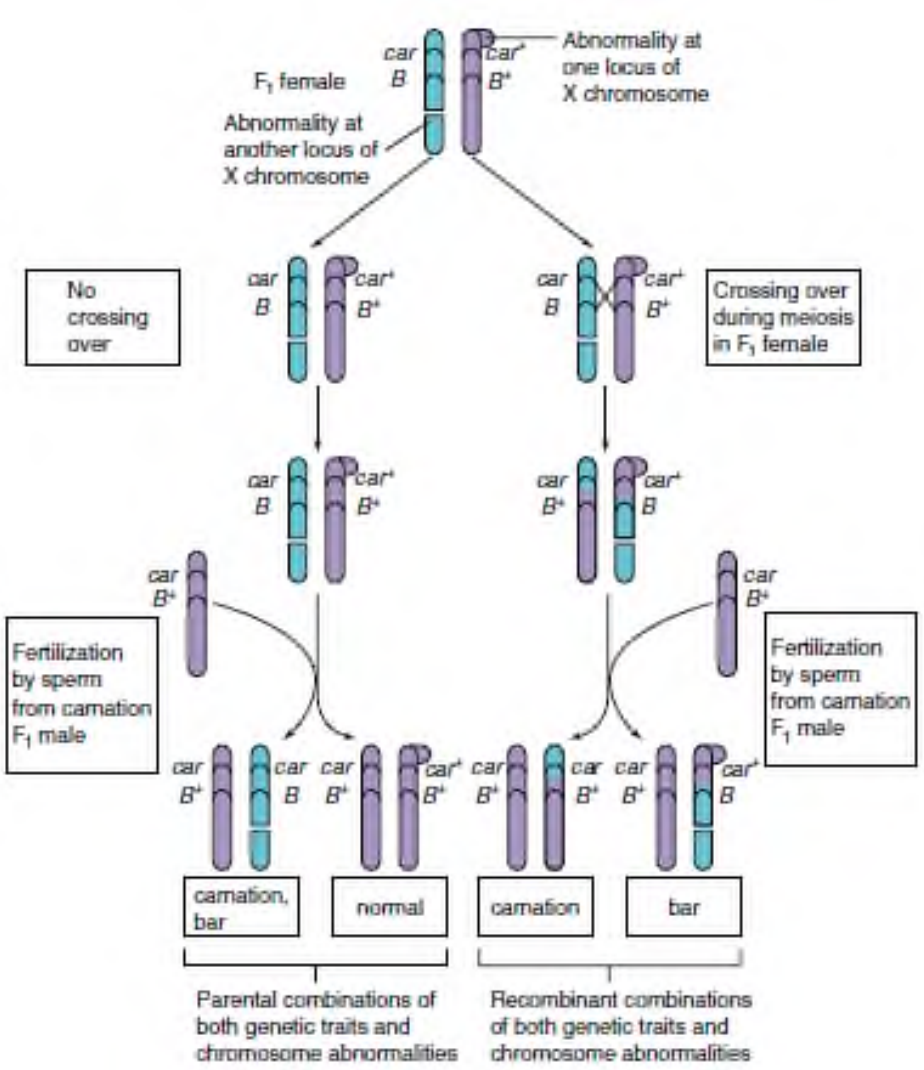


FIGURE 7.2. Stern's experiment demonstrating the physical exchange of chromosomal arms during crossing over. Stern monitored crossing over between two genes, the recessive carnation eye color (*car*) and the dominant bar-shaped eye (*B*), on chromosomes with physical peculiarities visible under a microscope. Whenever these genes recombined through crossing over, the chromosomes recombined as well. Therefore, the recombination of genes reflects a physical exchange of chromosome arms. The "+" notation on the alleles refers to the wild-type allele, the most common allele at a particular gene.

Stern studied two sex-linked eye characters in *Drosophila* strains whose X chromosomes were visibly abnormal at both ends. He first examined many flies and identified those in which an exchange had occurred with respect to the two eye characters. He then studied the chromosomes of those flies to see if their X c hromosomes had exchanged arms. Stern found that all of the individuals that had exchanged eye traits also possessed chromosomes that had exchanged abnormal ends. The conclusion was inescapable: genetic exchanges of

characters such as eye color involve the physical exchange of chromosome arms, a phenomenon called **crossing over.** Crossing over creates new combinations of genes, and is thus a form of **genetic recombination.** The chromosomal exchanges Stern demonstrated provide the solution to the paradox, because crossing over can occur between homologues anywhere along the length of the chromosome, in locations that seem to be randomly determined. Thus, if two different genes are located relatively far apart on a chromosome, crossing over is more likely to occur somewhere between them than if they are located close together. Two genes can be on the same chromosome and still show independent assortment if they are located so far apart on the chromosome that crossing over occurs regularly between them.

Using Recombination to Make Genetic Maps. Because crossing over is more frequent between two genes that are relatively far apart than between two that are close together, the frequency of crossing over can be used to map the relative positions of genes on chromosomes. In a cross, the proportion of progeny exhibiting an exchange between two genes is a measure of the frequency of crossover events between them, and thus indicates the relative distance separating them. The results of such crosses can be used to construct a **genetic map** that measures distance between genes in terms of the frequency of recombination. One "map unit" is defined as the distance within which a crossover event is expected to occur in an average of 1% of gametes. A map unit is now called a **centimorgan,** after Thomas Hunt Morgan. In recent times new technologies have allowed geneticists to create gene maps based on the relative positions of specific gene sequences called *restriction sites* because they are recognized by DNA-cleaving enzymes called restriction endonucleases. Restriction maps have largely supplanted genetic recombination maps for detailed gene analysis because they are far easier to produce. Recombination maps remain the method of choice for genes widely separated on a chromosome.

The Three-Point Cross. In constructing a genetic map, one simultaneously monitors recombination among three or more genes located on the same

chromosome, referred to as **syntenic** genes. When genes are close enough together on a chromosome that they do not assort independently, they are said to be **linked** to one another. A cross involving three linked genes is called a **three-point cross.** Data obtained by Morgan on traits encoded by genes on the X chromosome of *Drosophila* were used by his student A. H. Sturtevant, to draw the first genetic map (Fig. 7.3). By convention, the most common allele of a gene is often denoted with the symbol "+" and is designated as **wild type.** All other alleles are denoted with just the specific letters.

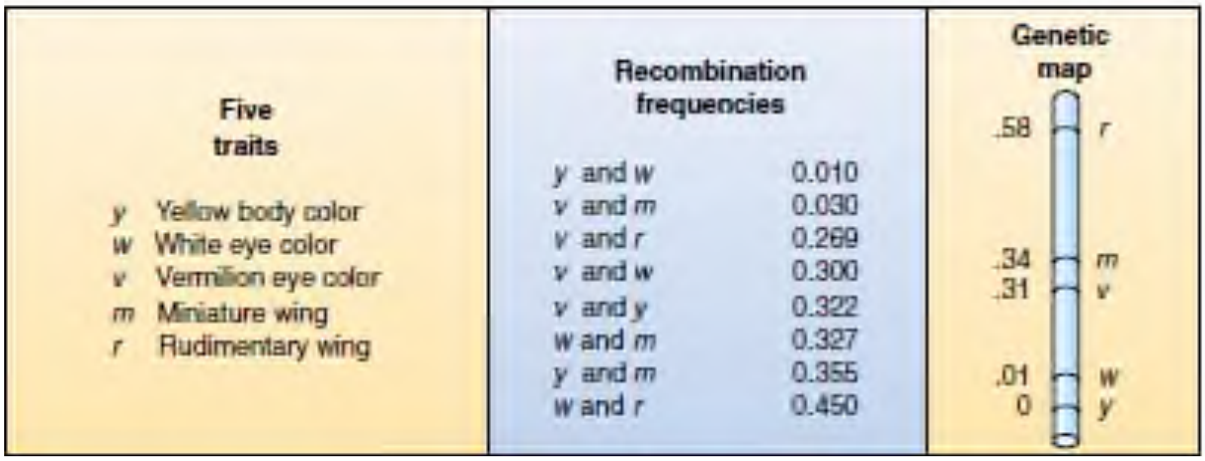


FIGURE 7.3. The first genetic map. This map of the X chromosome of *Drosophila* was prepared in 1913 by A. H. Sturtevant, a student of Morgan. On it he located the relative positions of five recessive traits that exhibited sex linkage by estimating their relative recombination frequencies in genetic crosses. Sturtevant arbitrarily chose the position of the *yellow* gene as zero on his map to provide a frame of reference. The higher the recombination frequency, the farther apart the two genes.

Gene maps locate the relative positions of different genes on the chromosomes of an organism. Traditionally produced by analyzing the relative a mounts of recombination in genetic crosses, gene maps are increasingly being made by analyzing the sizes of fragments made by restriction enzymes.

THE HUMAN GENETIC MAP

Genetic maps of human chromosomes (Fig. 7.4) are of great importance. Knowing where particular genes are located on human chromosomes can often be used to tell whether a fetus at risk of inheriting a genetic disorder actually has the disorder.

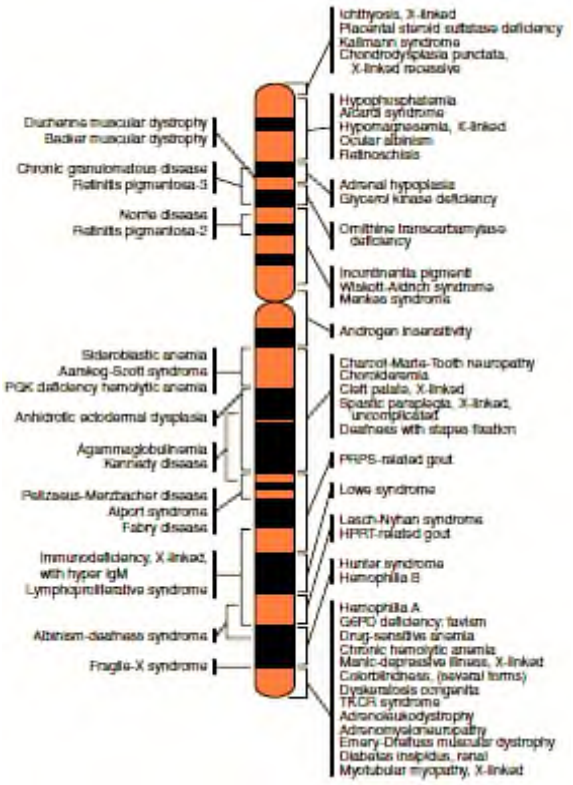


FIGURE 7.4. The human X chromosome gene map. Over 59 diseases have been traced to specific segments of the X chromosome. Many of these disorders are also influenced by genes on other chromosomes.

Human Sex Chromosomes. Of the 23 pairs of human chromosomes, 22 are perfectly matched in both males and females and are called **autosomes.** The remaining pair, the **sex chromosomes,** consist of two similar chromosomes in females and two dissimilar chromosomes in males. In humans, females are designated XX and males XY. One of the sex chromosomes in the male (the Y chromosome) is highly condensed and bears few functional genes.

The **Y chromosome** is considerably shorter than the X. At meiosis in females, the two X chromosomes pair and segregate like autosomes so that each egg receives one X chromosome. Hence with regard to sex chromosomes the gametes are of only one type, and the female is said to be the **homogametic sex.** At meiosis in males, the X and the Y chromosomes pair over a short region, which ensures that the X and Y separate to opposite ends of the meiotic cell, creating two types of sperm, half with an X and the other half with a Y. Therefore the male is called the **heterogametic sex.**

Cytogenetics divide the X and Y chromosomes into homologous and differential regions. Again, let's use humans as an example (Fig. 7.5).

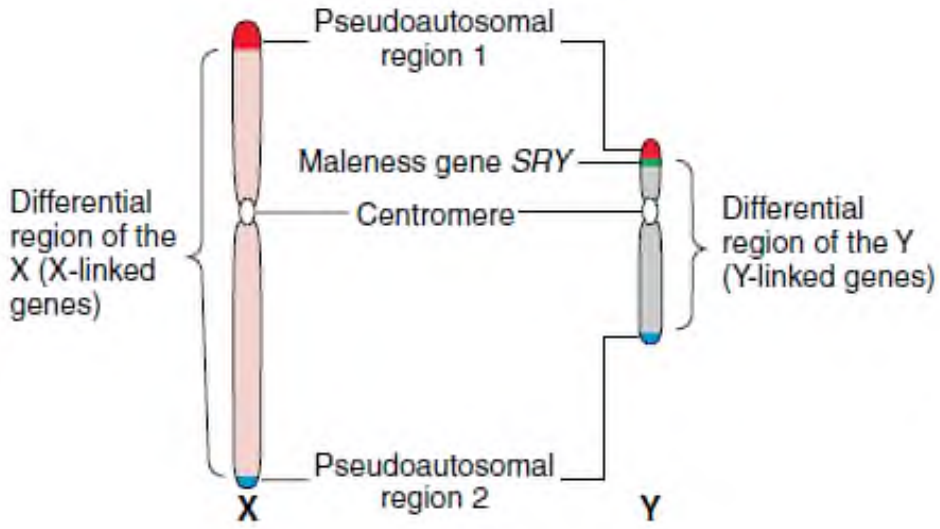


FIGURE 7.5. Differential and pairing regions of human sex chromosomes. The regions were located by observing where the chromosomes paired up in meiosis and where they did not.

In being homologous these regions are autosomal-like, so they are called *pseudoautosomal regions 1* and *2*. One or both of these regions pair during meiosis, and undergo crossing over. In this way, the X and the Y can act as a pair and segregate into equal numbers of sperm. The *homologous* regions contain DNA sequences that are substantially similar on both sex chromosomes. The *differential* regions contain genes that have no counterparts on the other sex chromosome. Hence in males these genes in the differential regions are thus said to be **hemizygous** ("half zygous"). The X chromosome contains many hundreds of genes; most of these genes are not involved in sexual function, and most have no counterparts on the Y.

The Y chromosome contains only a few dozen genes (Fig. 7.6). Some of these genes have counterparts on the X, but some do not. Most of the latter type are involved in male sexual function. One of these genes, SRY, determines maleness itself. Several other genes are specific for sperm production in males.

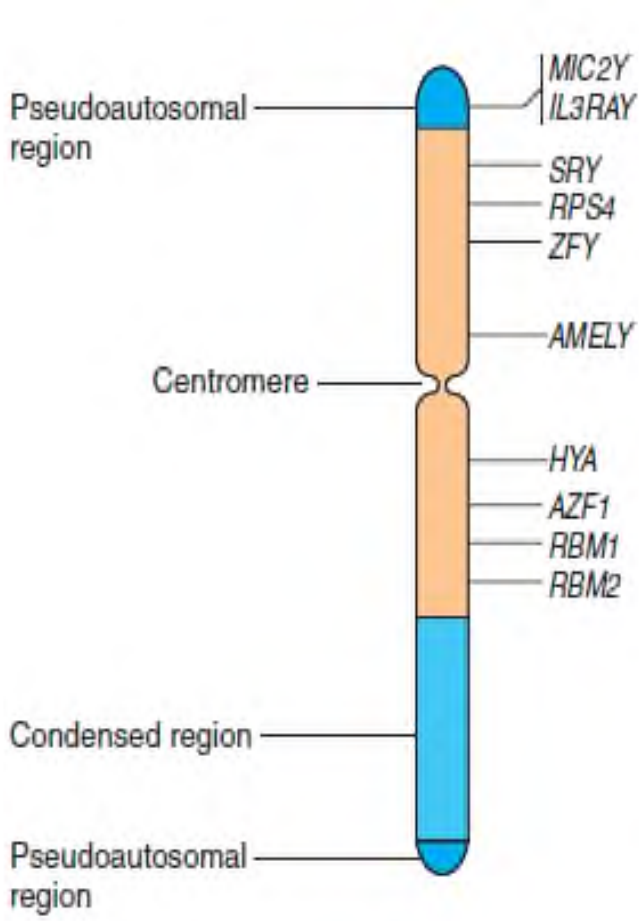


FIGURE 7.6. The human Y chromosome. In addition to the genes shown, the Y chromosome carries other genes, homologous to X chromosome genes, that do not function because of accumulated mutations. Some of these are in multiple copies. Note the two pseudoautosomal regions that allow synapsis between the Y and X chromosomes. The gene symbols shown include *MIC2Y*, T cell adhesion antigen; *IL3RAY*, interleukin-3 receptor; *RPS4*, a ribosomal protein; *AMELY*, amelogenin; *HYA*, histocompatibility Y antigen; *AZF1*, azoospermia factor 1 (mutants result in tailless sperm); and *RBM1*, *RBM2*, RNA binding proteins 1 and 2. (Adapted from http://www3.ncbi.nlm.nih.gov/omim).

Genes in the differential region of the X show an inheritance pattern called **X linkage;** those in the differential region of the Y show **Y linkage.** In general, genes on the differential regions are said to show **sex linkage.** A gene that is sex-linked shows patterns of inheritance related to sex. This pattern contrasts with the inheritance patterns of genes on the autosomes, which are not connected to sex. In autosomal inheritance, male and female progeny show inherited phenotypes in exactly the same proportions, as typified by Mendel's results (for example, both sexes of an F2 might show a 3:1 ratio). In contrast, crosses performed to track the inheritance of genes on the sex chromosomes often produce male and female progeny that show different phenotypic ratios. In fact, for studies of genes of unknown chromosomal location, this pattern is a diagnostic of location on the sex chromosomes.

Barr bodies. In the developing female embryo, one of the X chromosomes (determined randomly) condenses and becomes inactivated. These con-

densed X chromosomes, called Barr bodies, then attach to the nuclear membrane (Fig. 7.7).

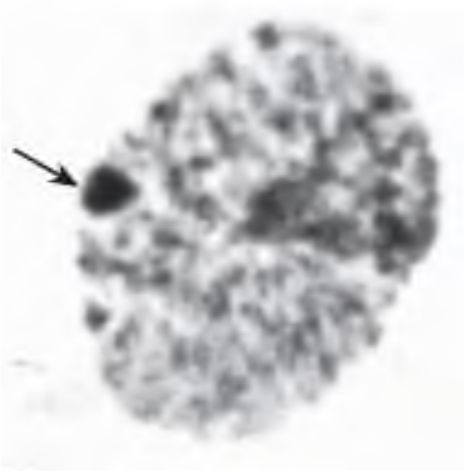


FIGURE 7.7. Barr body (*arrow*) in the nucleus of a cheek mucosal cell of a normal woman. This visible mass of heterochromatin is an inactivated X chromosome. (Thomas G. Brewster and Park S. Gerald, "Chromosome disorders associated with mental retardation," *Pediatric Annals,* 7, no. 2, 1978. Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

Sex determination in mammals is made by a region of the Y chromosome designated *SRY.* Testes are formed when the Y chromosome and *SRY* are present; ovaries are formed when they are absent. Sexual reproduction is most common among animals, but many reproduce asexually by fission, budding, or parthenogenesis. Sexual reproduction generally involves the fusion of gametes derived from different individuals of a species, but some species are hermaphroditic.

Key Questions:

1. Features of inheritance of the genes located in one chromosome. The linked inheritance at the drosophila (Morgan's experiences).
2. Full and incomplete linkage genes.
3. Crossing over and gene recombination.
4. The chromosomal theory of inheritance.
5. Gene map of X-chromosomes.
6. Sex linkage genes.
7. Morphology of human sex-chromosomes. Genes linked to the X and Y-chromosomes.
8. Ways of sex determination at animals.
9. Primary and secondary sexual signs.

Examples of Review questions:

NN	Questions	Right answers
1	LINKED GENES ___ . 1) must be immediately adjacent to one another on a chromosome 2) have alleles that assort independently of one another 3) never show crossing over 4) are on the same chromosome 5) always have multiple alleles	**4**
2	THE SEX OF A HUMAN IS DETERMINED BY ___ . 1) ploidy, the male being haploid 2) the Y chromosome 3) X and Y chromosomes, the male being XY 4) the number of X chromosomes, the male being XO 5) Z and W chromosomes, the male being ZZ	**3**
3	THE MALE SEX IS: 1) 44 autosomes and XX 2) 44 autosomes and XY 3) 44 autosomes and XO 4) XX 5) XY	**2**
4	THE FEMALE SEX IS: 1) 44 autosomes and XX 2) 44 autosomes and XY 3) 44 autosomes and XO 4) XX 5) XY	**4**
5	IN HUMANS, SPOTTED TEETH IS CAUSED BY A DOMINANT SEXLINKED GENE. A MAN WITH SPOTTED TEETH WHOSE MOTHER HAD NORMAL TEETH MARRIES A WOMAN WITH NORMAL TEETH. THEREFORE ___ . 1) all of their daughters will have normal teeth. 2) all of their daughters will have spotted teeth. 3) all of their children will have spotted teeth. 4) half of their sons will have spotted teeth. 5) none of their sons will have spotted teeth.	**2**

CHAPTER 8. VARIABILITY

PHENOTYPIC VARIABILITY

When the Role of Genes Is Unclear. The single-gene traits discussed in the previous section have a distinct "off or on" character; people either have one phenotype (the disease is present), or the other (the disease is absent). Conditions like this, such as cystic fibrosis, are known as **qualitative traits**. However, many of the traits that interest women who are choosing a sperm donor do not have this off or on character. Traits such as height, weight, eye color, musical ability, susceptibility to cancer, and intelligence, all of which have many possible values, are called **quantitative traits**. Quantitative traits show **continuous variation**—that is, we can see a *range* of phenotypes in a population, for instance from very short people to very tall people. Wide variation in quantitative traits leads to the great diversity we see in the human population.

The distribution of phenotypes of a quantitative trait in a population can be displayed on a graph, and typically takes the form of a bell-shaped curve called a *normal distribution.* Figure 8.1(a) graphs a normal distribution that represents the height of men in an Amherst College class in 1884. Each column on the graph shows the number of men measured at the height indicated along the bottom of the column. The curved line on the figure is an idealized bell-shaped curve that summarizes this data. A trait that is normally distributed in a population may be described in a number of ways. We are used to thinking about the average, or *mean*, value for data. This is calculated by adding all of the values for a trait in a population, and dividing by the number of individuals in that population. Figure 8.1(a) shows that the mean height of men in this population is 1.73 meters. However, the average value of a continuously variable trait does not tell you very much about the population. Examine Figure 4.10a closely: Does an average height of 1.73 meters in this particular population imply that most men were this height? Were most men in this population close to the mean, or was there a wide range of heights? In addition to knowing the

mean value for a trait, we also must understand how much *variability* exists in the population for the trait. The amount of variation in a population is described with a mathematical term called *variance*. A low variance for a trait indicates a small amount of variability in the population, while a high variance indicates a large amount of variability (Fig. 8.1(b)). Scientists who study the inheritance of quantitative traits are interested in determining the genetic basis for the variance in human traits.

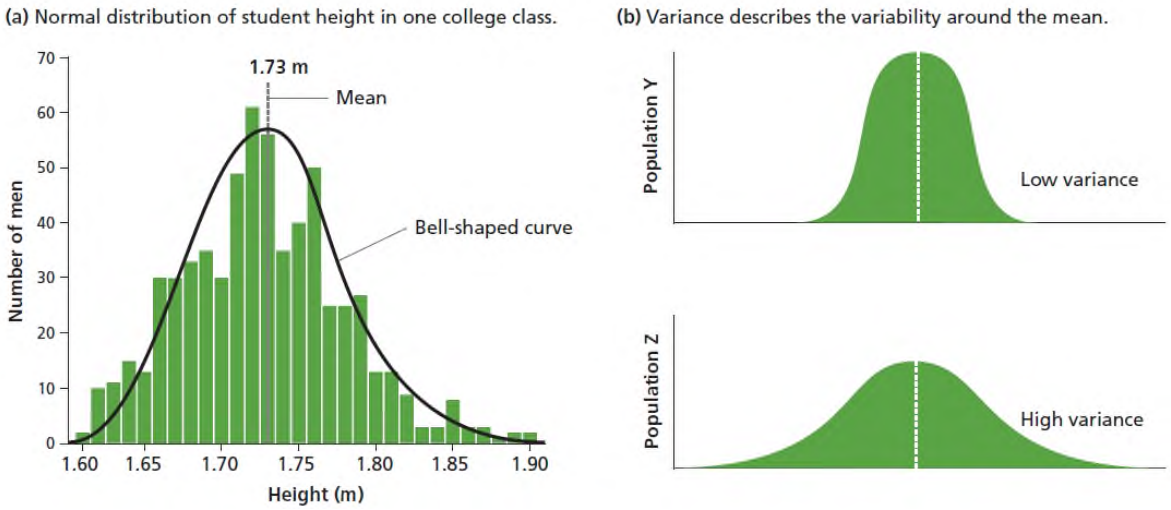


FIGURE 8.1. A quantitative trait. (a) This graph of the number of men in each category of height is a normal distribution with a center around the mean height of 1.73 m. (b) Though both of these populations have the same mean value, there is much less variation among individuals in population Y than in population Z.

Why Traits Are Quantitative One reason we may see a range of phenotypes in a human population is because numerous genotypes exist among the individuals in the population. This happens when a trait is influenced by more than one gene; traits influenced by many genes are called *polygenic traits*. As we saw above, when a single gene with two alleles determines a trait, three possible genotypes are present: AA, Aa, and aa. However, when two genes each with two alleles influence a trait, nine genotypes are possible. This is because each egg and sperm contains one of four possible allele combinations. For example, eye color in humans is a polygenic trait influenced by at least three genes. These genes help produce and distribute the pigment melanin to

the iris-people with very dark eyes have a lot of melanin in each iris, people with brown eyes have a little less melanin, and blue eyes result when there is very little melanin present. When the genes for eye-pigment production and distribution interact, a range of eye colors, from dark brown to pale blue, is produced in humans. The continuous variation in eye color among people is a result of more than one gene influencing the phenotype.

Another reason that continuous variation may occur in a quantitative trait is due to the influence of environmental factors. In this case, each genotype is capable of producing a range of phenotypes depending upon outside influences. This phenomenon is called *phenotypic plasticity* because the phenotype is plastic, or flexible. Thus, even if all individuals have the same genotype, many different phenotypes can result if they are raised in a variety of environments. A clear example of phenotypic plasticity: identical twins have the exact same genotype, but are quite different in appearance due to environmental factors. Identical twins share 100% of their genes, but are quite different in appearance. This is due to variations in their environment—the twin on the bottom smoked and had much greater sun exposure than the twin on top. Quantitative variation in life expectancy among people of different cultures and economic groups is also largely a result of environmental factors. People born in industrialized countries generally have longer life expectancies than people born in the developing world (Fig. 8.2).

Norm of reaction. How can we quantify the relation between the genotype, the environment, and the phenotype? For a particular genotype, we could prepare a table showing the phenotype that would result from the development of that genotype in each possible environment. Such a set of environment-phenotype relations for a given genotype is called the **norm of reaction** of the genotype. In practice, we can make such a tabulation only for a partial genotype, a partial phenotype, and some particular aspects of the environment. For example, we might specify the eye sizes that fruit flies would have after devel-

oping at various constant temperatures; we could do this for several different eye-size genotypes to get the norms of reaction of the species.

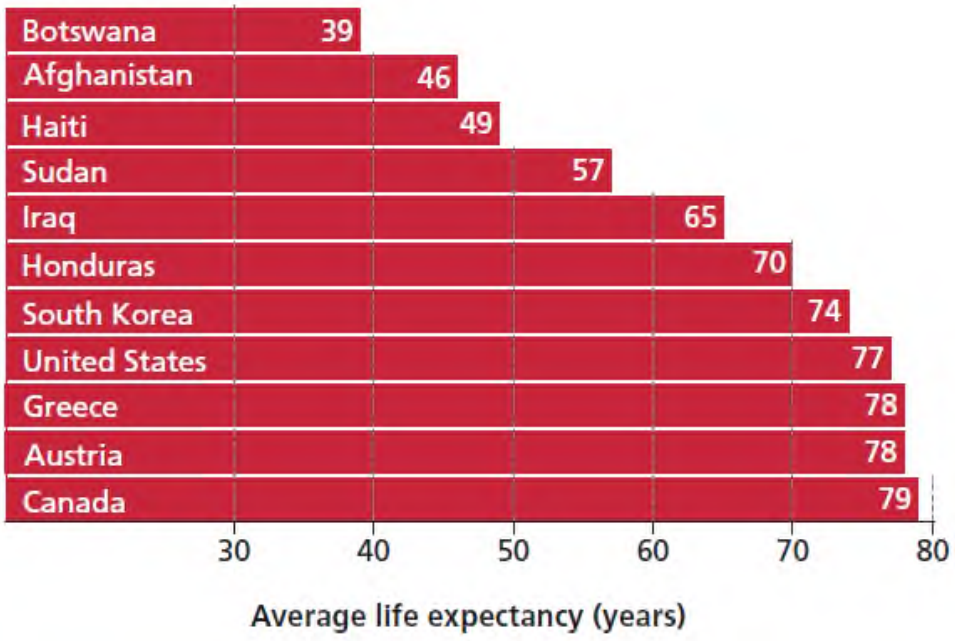


FIGURE 8.2. Life expectancy in selected countries, 2000. This graph shows a 40-year difference in average life expectancy. Differences in the life expectancy of human populations in different countries are entirely due to differences in their environments, particularly income levels.

Environmental Effects. The degree to which an allele is expressed may depend on the environment. Some alleles are heat-sensitive, for example. Traits influenced by such alleles are more sensitive to temperature or light than are the products of other alleles. The arctic foxes, for example, make fur pigment only when the weather is warm. Similarly, the *ch* allele in Himalayan rabbits and Siamese cats encodes a heat-sensitive version of tyrosinase, one of the enzymes mediating the production of melanin, a dark pigment. The ch version of the enzyme is inactivated at temperatures above about 33°C. At the surface of the body and head, the temperature is above 33°C and the tyrosinase enzyme is inactive, while it is more active at body extremities such as the tips of the ears and tail, where the temperature is below 33°C. The dark melanin pigment this enzyme produces causes the ears, snout, feet, and tail of Himalayan rabbits and Siamese cats to be black.

A single genotype may produce different phenotypes, depending on the environment in which organisms develop. The same phenotype may be produced by different genotypes, depending on the environment.

GENETIC VARIABILITY.
GENE MUTATIONS

A mutation is a change in a gene's nucleotide base sequence that affects the phenotype. It is a type of polymorphism. A mutation can occur at the molecular level, substituting one DNA base for another or adding or deleting a few bases, or at the chromosome level. DNA mutates in many ways. Bases may be substituted, deleted, inserted, or moved. Mutations that disrupt the reading frame—that is, the sequence of DNA triplets—tend to be the most drastic in effect.

Types of Mutations. Mutations are classified by exactly how they alter DNA. Table 8.1 summarizes the types of genetic changes described in this section using an analogy to an English sentence.

Table 8.1

Types of Mutations

A sentence comprised of three-letter words can provide an analogy to the effect of mutations on a gene's sequence:

Normal	THE ONE BIG FLY HAD ONE RED EYE
Missense	THQ ONE BIG FLY HAD ONE RED EYE
Nonsense	THE ONE BIG
Frameshift	THE ONE QBI GFL YHA DON ERE DEY
Deletion	THE ONE BIG HAD ONE RED EYE
Insertion	THE ONE BIG WET FLY HAD ONE RED EYE
Duplication	THE ONE BIG FLY FLY HAD ONE RED EYE
Expanding mutation	
generation 1	THE ONE BIG FLY HAD ONE RED EYE
generation 2	THE ONE BIG FLY FLY FLY HAD ONE RED EYE
generation 3	THE ONE BIG FLY FLY FLY FLY FLY HAD ONE RED EYE

Point Mutations. A **point mutation** is a change in a single DNA base. It is a **transition** if a purine replaces a purine (A to G or G to A) or a pyrimidine replaces a pyrimidine (C to T or T to C). It is a **transversion** if a purine replaces a pyrimidine or vice versa (A or G to T or C). A point mutation can have any of several consequences—or it may have no obvious effect at all on the phenotype, acting as a silent mutation.

Missense Mutations. A point mutation that changes a codon that normally specifies a particular amino acid into one that codes for a different amino acid is called a **missense mutation.** If the substituted amino acid alters the protein's conformation sufficiently or occurs at a site critical to its function, signs or symptoms of disease or an observable variant of a trait may result. The point mutation that causes sickle cell disease (see below) is a missense mutation. The DNA sequence CTC encodes the mRNA codon GAG, which specifies glutamic acid. In sickle cell disease, the mutation

changes the DNA sequence to CAC, which encodes GUG in the mRNA, which specifies valine. This mutation changes the protein's shape, which alters its function.

Nonsense Mutations. A point mutation that changes a codon specifying an amino acid into a "stop" codon— UAA, UAG, or UGA in mRNA—is a **nonsense mutation.** A premature stop codon shortens the protein product, which can profoundly influence the phenotype. Nonsense mutations are predictable by considering which codons can mutate to a "stop" codon. The most common cause of factor XI deficiency, a blood clotting disorder, is a nonsense mutation that changes the GAA codon specifying glutamic acid to UAA, signifying "stop." The shortened clotting factor cannot halt the profuse bleeding that occurs during surgery or from injury. In the opposite situation, when a normal stop codon mutates into a codon that specifies an amino acid, the resulting protein is longer than normal, because translation proceeds through what is normally a stop codon.

A ***deletion mutation*** removes genetic material. A deletion that removes three or a multiple of three bases will not cause a frameshift, but can still alter the phenotype.

Deletions range from a single DNA nucleotide to thousands of bases to larger pieces of chromosomes. The next chapter considers large deletions. Many common inherited disorders result from deletions. About two-thirds of people with Duchenne muscular dystrophy, for example, are missing large sections of the very extensive gene that encodes dystrophin. Some cases of male infertility are caused by tiny deletions in the Y chromosome.

An ***insertion mutation*** adds genetic material and it, too, can offset a gene's reading frame. In one form of Gaucher disease, for example, an inserted single base prevents production of an enzyme that normally breaks down glycolipids in lysosomes. The resulting buildup of glycolipid enlarges the liver and spleen and causes easily fractured bones and neurological impairment. Gaucher disease is common among Jewish people of eastern European descent. Although most cases arise from a missense mutation, some families have the insertion mutation. Gaucher disease provides a good illustration of how different types of mutations in the same gene result in the same or a similar phenotype.

Pseudogenes. A pseudogene is a stretch of DNA with a sequence very similar to that of another gene. A pseudogene is not translated into protein, although it may be transcribed. The pseudogene may have descended from the original gene sequence, which was duplicated when DNA strands misaligned during meiosis. When this happens, a gene and its pseudogene end up right next to each other on the chromosome. The original gene or the copy then mutated to such an extent that it was no longer functional and became a pseudogene. Its duplicate, though, lived on as the functional gene. Although a pseudogene is not translated, its presence can interfere with the expression of the functional gene and cause a mutation. For example, some cases of Gaucher disease can result from a crossover between the working gene and its pseudogene, which has 95 percent of the same sequence located 16,000 bases away. The re-

sult is a fusion gene, which is a sequence containing part of the functional gene and part of the pseudogene. The fusion gene does not retain enough of the normal gene sequence to enable the enzyme to be synthesized. Gaucher disease results.

Transposons, or "jumping genes", can alter gene function in several ways. They can disrupt the site they jump from, shut off transcription of the gene they jump into, or alter the reading frame of their destination if they are not a multiple of three bases. For example, a boy with X-linked hemophilia A had a transposon in his factor VIII gene—a sequence that was also in his carrier mother's genome, but on her chromosome 22. Apparently, in the oocyte, the transposon jumped into the factor VIII gene on the X chromosome, causing the boy's hemophilia.

Mutation is a part of life. Naturally occurring errors in DNA replication may result in spontaneous mutation. Mutagens such as chemicals or radiation can cause mutation by adding, deleting, or replacing DNA bases. Induced mutation is a research tool, but it may also result from exposure to environmental agents.

The effects of mutation vary. A mutation can completely halt production of a protein, lower the amount of a protein synthesized, overproduce it, or impair the protein's function. A mutation may even offer protection. Research on inherited disease has traditionally begun with studies of mutations. Medical geneticists tried to identify precisely how a specific mutation alters the phenotype in a way that harms health.

Recessive Disorders

Of the many autosomal (non-sex-linked) recessive disorders, we will discuss only three.

The first genetic illness to be understood at the molecular level was ***sickle cell disease.*** Researchers knew in the 1940s that an inherited anemia (weakness and fatigue caused by too few red blood cells) was associated with sickle-

shaped red blood cells (Fig. 8.3). Sickle cell disease was the first inherited illness linked to a molecular abnormality, but it wasn't the first known condition that results from a mutation in the beta globin genes. Using then newly invented protein sequencing techniques, Ingram identified the tiny mutation responsible for sickle cell disease. It is a substitution of the amino acid valine for the glutamic acid that normally is the sixth amino acid in the beta globin polypeptide chain. At the DNA level, the change was even smaller - a CTC to a CAC, corresponding to RNA codons GAG and GUG, which was learned after researchers deciphered the genetic code.

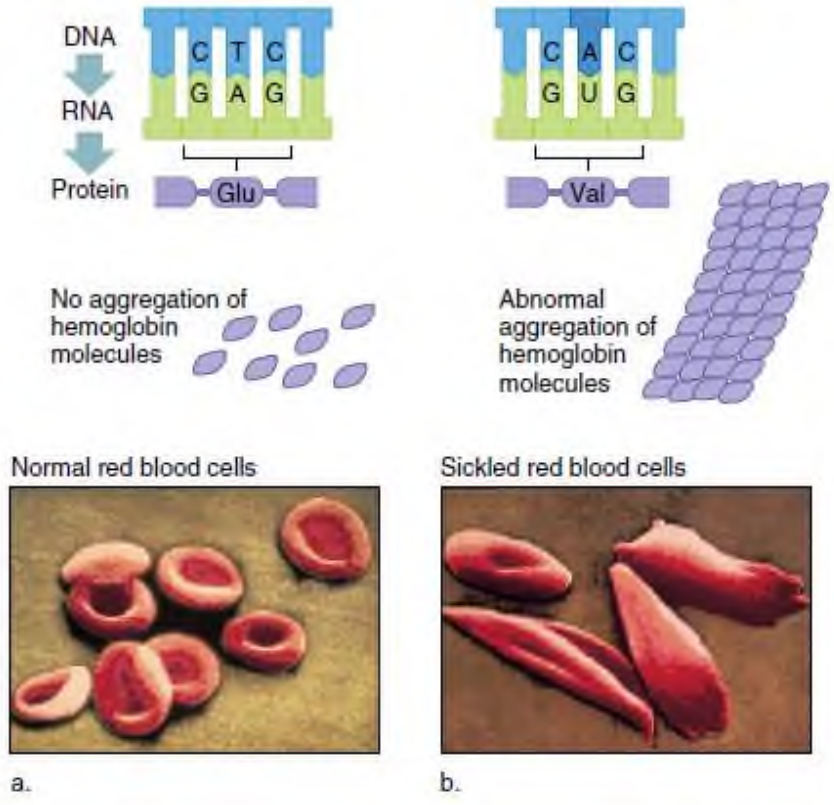


FIGURE 8.3. Sickle cell disease results from a single base change. Hemoglobin carries oxygen throughout the body. When normal (*a*), the globular molecules do not aggregate, enabling the cell to assume a rounded shape. In sickle cell disease (*b*), a single DNA base change replaces one amino acid in the protein with a different one (valine replaces glutamic acid). The result is a change in the surface of the molecule that causes hemoglobins to aggregate into long, curved rods that deform the red blood cell. Sets of 14 rods twist together.

Eventually, researchers found that this mutation changes the surfaces of hemoglobin molecules, which causes them to link when in low-oxygen conditions. This action makes red blood cells rigid and fragile, and they bend into

sickle shapes that cause anemia, joint pain, and organ damage when the cells lodge in narrow blood vessels, cutting off local blood supplies.

Cystic Fibrosis. Cystic fibrosis is the most common lethal genetic disease among Caucasians in the United States. About one in 20 Caucasians is a carrier, and about one in 2,500 newborns has the disorder. The mucus in the bronchial tubes and pancreatic ducts is particularly thick and viscous, interfering with the function of the lungs and pancreas. To ease breathing, the thick mucus in the lungs has to be manually loosened periodically, but still the lungs become infected frequently. The clogged pancreatic ducts prevent digestive enzymes from reaching the small intestine, and patients usually take digestive enzymes mixed with applesauce before every meal. Life expectancy has now increased to as much as 28 years of age. Research has demonstrated that chloride ions (Cl^-) fail to pass through plasma membrane channel proteins in cystic fibrosis patients. Ordinarily, after chloride ions have passed through the membrane, water follows. It is thought that lack of water is the cause of the abnormally thick mucus in the bronchial tubes and pancreatic ducts. The cystic fibrosis allele, which is located on chromosome 7, has been isolated, and attempts have been made to insert it into nasal epithelium, so far with little success. Genetic testing for the allele in adult carriers and In fetuses is possible; if present, couples have to decide whether to risk having a child with the condition or whether abortion is an option.

Phenylketonuria (PKU). Phenylketonuria (PKU) occurs once in 5,000 newborns, so it is not as frequent as the disorders previously discussed. However, it is the most commonly inherited metabolic disorder that affects nervous system development. First cousins who marry are more apt to have a PKU child. Affected individuals lack an enzyme that is needed for the normal metabolism of the amino acid phenylalanine, and an abnormal breakdown product, phenylketone, accumulates in the urine. The PKU allele is located on chromosome 12, and a prenatal DNA-test can determine the presence of this allele. Newborns are routinely tested in the hospital for elevated levels of phenylala-

nine in the blood. If elevated levels are detected, newborns are placed on a diet low in phenylalanine, which must be continued until the brain is fully developed, around age seven, or else severe mental retardation develops. Some doctors recommend that the diet continue for life, but in any case, a pregnant woman with phenylketonuria must be on the diet in order to protect her unborn child from harm.

Dominant Disorders

Of the many autosomal dominant disorders, we will discuss only neurofibromatosis and Huntington disease.

Huntington Disease. One in 20,000 persons in the United States has Huntington disease, a neurological disorder that leads to progressive degeneration of brain cells, which in turn causes severe muscle spasms and personality disorders. Most people appear normal until they are of middle age and have already had children who might also be stricken. Occasionally, the first signs of the disease are seen in these children when they are teenagers or even younger. There is no effective treatment, and death comes ten to fifteen years after the onset of symptoms. Several years ago, researchers found that the allele for Huntington disease was located on chromosome 4. A test was developed for the presence of the allele, but few people want to know if they have inherited the allele because as yet there is no treatment for Huntington disease. It appears that persons most at risk have inherited the disorder from their fathers. The latter observation is consistent with a new hypothesis called **genomic imprinting.** The genes are imprinted differently during formation of sperm and egg, and therefore, the sex of the parent passing on the disorder becomes important.

X-Linked Disorders

X-linked conditions can be dominant or recessive, but most of the X-linked conditions we know about are recessive. More males than females have the trait because recessive alleles on the X chromosome are always expressed in males since the Y chromosome does not have a corresponding allele. If a

male has an X-linked recessive condition, his daughters are often carriers; therefore, the condition passes from grandfather to grandson. Females who have the condition inherited the allele from both their mother and their father, and all the sons of such a female will have the condition. Three well-known X-linked recessive disorders are color blindness, muscular dystrophy, and hemophilia.

Hemophilia. About one in 10,000 males is a hemophiliac. There are two common types of hemophilia: hemophilia A is due to the absence or minimal presence of a clotting factor known as factor IX, and hemophilia B is due to the absence of clotting factor VIII. Hemophilia is called the bleeder's disease because the affected person's blood does not clot or clots very slowly. Although hemophiliacs bleed externally after an injury, they also bleed internally, particularly around joints. Hemorrhages can be stopped with transfusions of fresh blood (or plasma) or concentrates of the clotting protein. Also, factor VIII is now available as a biotechnology product. At the turn of the century, hemophilia was prevalent among the royal families of Europe, and all of the affected males could trace their ancestry to Queen Victoria of England. Figure 20.16 shows that of Queen Victoria's 26 grandchildren, four grandsons had hemophilia and four granddaughters were carriers. Because none of Queen Victoria's forebears or relatives were affected, it seems that the faulty allele she carried arose by mutation either in Victoria or in one of her parents. Her carrier daughters Alice and Beatrice introduced the allele into the ruling houses of Russia and Spain, respectively. Alexis, the last heir to the Russian throne before the Russian Revolution, was a hemophiliac. There are no hemophiliacs in the present British royal family because Victoria's eldest son, King Edward VII, did not receive the allele and therefore could not pass it on to any of his descendants.

Polygenic disorders

Many human disorders, such as cleft lip and/or palate, clubfoot, congenital dislocations of the hip, hypertension, diabetes, schizophrenia, and even allergies and cancers, are most likely controlled by polygenes and are subject to environmental influences. Therefore, many investigators are in the process of considering the nature versus nurture question; that is, what percentage of the trait is controlled by genes and what percentage is controlled by the environment? Thus far, it has not been possible to come to precise, generally accepted percentages for any particular trait. In recent years, reports have surfaced that all sorts of behavioral traits, such as alcoholism, phobias, and even suicide, can be associated with particular genes. No doubt behavioral traits are to a degree controlled by genes, but again, it is impossible at this time to determine to what degree. And very few scientists would support the idea that these behavioral traits are predetermined by our genes.

DNA REPAIR

Any manufacturing facility tests a product in several ways to see whether it has been built correctly. Mistakes in production are rectified before the item goes on the market—at least, most of the time. The same is true for a cell's manufacture of DNA.

DNA replication is incredibly accurate—only about 1 in 100 million bases is incorrectly incorporated. Repair enzymes oversee the fidelity of replication.

Since its beginning, the Earth has been periodically bathed in ultraviolet radiation. Volcanoes, comets, meteorites, and supernovas all depleted ozone in the atmosphere, which allowed ultraviolet wavelengths of light to reach organisms. The shorter wavelengths—UVA—are not dangerous, but the longer UVB wavelengths damage DNA by causing an extra covalent bond to form between adjacent (same strand) pyrimidines, particularly thymines (Fig. 8.4).

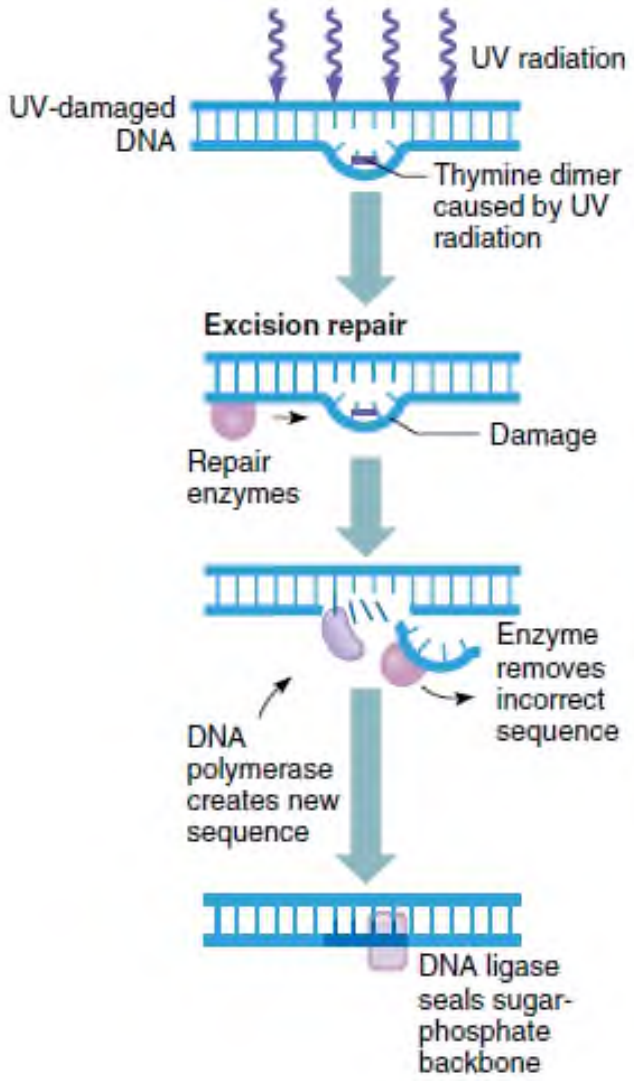


FIGURE 8.4. Excision repair. Human DNA damaged by UV light is repaired by excision repair, in which the pyrimidine dimer and a few surrounding bases are removed and replaced.

The linked thymines are called thymine dimers. Their extra bonds kink the double helix sufficiently to disrupt replication and lead to possible insertion of a noncomplementary base. For example, an A might be inserted opposite a G or C instead of a T.

Nucleotide excision repair replaces up to 30 nucleotides and removes errors that result from several types of insults, including exposure to chemical carcinogens, UVB in sunlight, and oxidative damage. The second type, called **base excision repair,** replaces one to five nucleotides at a time, but specifically corrects errors that result from oxidative damage. Oxygen free radicals are highly reactive forms of oxygen that arise during chemical reactions such as those of metabolism and transcription. Free radicals damage DNA. Genes that

are very actively transcribed face greater oxidative damage from free radicals. It is base excision repair that targets this type of damage.

ABNORMAL CHROMOSOME STRUCTURE

Structural chromosomal defects include missing, extra, or inverted genetic material within a chromosome or combined or exchanged parts of nonhomologs (translocations) (Fig. 8.5).

Deletions and Duplications. A **deletion** is missing genetic material. Deletions range greatly in size, and the larger ones tend to have greater effects because they remove more genes. Consider cri-du-chat syndrome (French for "cat's cry"), caused by deletion of part of the short arm of chromosome 5 (also called 5p– syndrome).

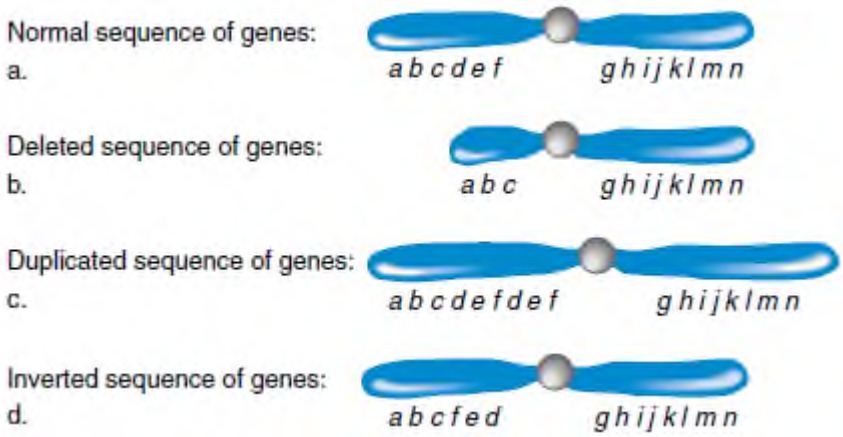


FIGURE 8.5. Chromosome abnormalities. If a hypothetical normal gene sequence appears as shown in (*a*), then (*b*) represents a deletion, (*c*) a duplication, and (*d*) an inversion.

Translocations. In a translocation, different (nonhomologous) chromosomes exchange or combine parts. Exposure to certain viruses, drugs, and radiation can cause translocations, but often they arise for no apparent reason. There are two major types of translocations. In a ***Robertsonian translocation,*** the short arms of two different acrocentric chromosomes break, leaving sticky ends that then cause the two long arms to adhere. A new, large chromosome forms from the long arms of the two different chromosomes. Because genes on the short arms of the involved chromosomes are repeated elsewhere, their ab-

sence in a Robertsonian translocation does not affect the phenotype. The person with the large, translocated chromosome, called a translocation carrier, has 45 chromosomes, but may not have symptoms if a crucial gene has not been deleted or damaged.

In a ***reciprocal translocation,*** two different chromosomes exchange parts. FISH can be used to highlight the involved chromosomes. If the chromosome exchange does not break any genes, then a person who has both translocated chromosomes is healthy and is also a translocation carrier.He or she has the normal amount of genetic material, but it is rearranged.

Cri-du-chat Syndrome. A chromosomal deletion is responsible for cri du chat (cat's cry) syndrome, which has a frequency of one in 50,000 live births. Affected children have a high-pitched cry similar to the mewing of a cat and pinched facial features, and are mentally retarded and developmentally delayed. The chromosome region responsible for the catlike cry is distinct from the region that causes mental retardation and developmental delay, suggesting that the deletion can remove more than one gene. A cytogeneticist can determine by examining a detailed karyotype whether a child will have only the catlike cry and perhaps poor weight gain, or will have all of the signs and symptoms, which include low birth weight, poor muscle tone, a small head, and impaired language skills. In Their Own Words on page 253 describes a child who had 5p– syndrome.

ANEUPLOIDS

Autosomal aneuploids. Most autosomal aneuploids are very rarely seen in live births, due to the lethality of a large imbalance of genetic material. Following are descriptions of the most common autosomal aneuploids among liveborns, summarized in table 8.2

Table 8.2

Comparing and Contrasting Trisomies 13, 18, and 21

Type of Trisomy	Incidence at Birth	Percent of Conceptions That Survive 1 Year After Birth
13 (Patau)	1/12,500 – 1/21,700	<5%
18 (Edward)	1/6,000 – 1/10,000	<5%
21 (Down)	1/800 – 1/826	85%

Down Syndrome. The major inherited autosomal abnormality is Down syndrome, in which the individual inherits three copies of chromosome 21. Down syndrome is easily recognized by these characteristics: short stature, an eyelid fold, stubby fingers, a wide gap between the first and second toes, a large, fissured tongue, a round head, a palm crease (the so-called simian line), and unfortunately, mental retardation, which can sometimes be severe. Down syndrome is also called trisomy 21 because the individual usually has three copies of chromosome 21. In most instances, the egg had two copies instead of one of this chromosome. (In 23% of the cases studied, however, the sperm had the extra chromosome 21.) The chance of a woman having a Down syndrome child increases rapidly with age, starting at about age 40. The frequency of Down syndrome is 1 in 800 births for mothers under 40 years of age and 1 in 80 for mothers over 40 years of age. Most Down syndrome babies are born to women younger than age forty, however, because this is the age group having the most babies. Amniocentesis (removing fluid and cells from the amniotic sac surrounding the fetus) followed by karyotyping can detect a Down syndrome child. It is known that the genes that cause Down syndrome are located on the bottom third of chromosome 21, and extensive investigative work has been directed toward discovering the specific genes responsible for the characteristics of the syndrome. One day it might be possible to control the expression of these genes even before birth, so that the symptoms of Down syndrome do not appear.

Trisomy 18—Edward Syndrome. The severe symptoms of trisomy 18 explain why few affected fetuses survive and also make the syndrome relative-

ly easy to diagnose prenatally using ultrasound—yet the symptoms are presumably milder than those associated with the majority of aneuploids, which are manifest solely as spontaneous abortions. The associated major abnormalities include heart defects, a displaced liver, growth retardation, and oddly clenched fists. After birth, additional anomalies are apparent. These include overlapping placement of fingers, a narrow and flat skull, abnormally shaped and low-set ears, a small mouth and face, unusual or absent fingerprints, short large toes with fused second and third toes, and "rocker-bottom" feet. Affected children have great physical and mental disabilities, with developmental skills stalled at the six-month level. Most cases of trisomy 18 are traced to nondisjunction in meiosis II during oocyte formation.

Trisomy 13—Patau Syndrome. Trisomy 13 is very rare, but, as is the case with trisomy 18, the number of newborns with the anomaly reflects only a small percentage of affected conceptions. Trisomy 13 has a different set of signs and symptoms than trisomy 18. Most striking, although rare, is a fusion of the developing eyes, so that a fetus has one large eyelike structure in the center of the face. More common is a small or absent eye.Major abnormalities affect the heart, kidneys, brain, face, and limbs. The nose is often malformed, and cleft lip and/or palate is present in a small head. Extra fingers and toes may occur. Appearance of a facial cleft and extra digits on an ultrasound exam are considered sufficient evidence to pursue chromosome analysis of the fetus to detect trisomy 13. Ultrasound examinations of affected newborns reveal more extensive anomalies, including an extra spleen, abnormal liver structure, rotated intestines, and an abnormal pancreas. A few individuals have survived until adulthood, but they do not progress developmentally beyond the sixmonth level.

Sex Chromosome Aneuploids. People with sex chromosome aneuploidy have extra or missing sex chromosomes. Note that some conditions can result from nondisjunction in meiosis in the male or female. Examples of abnormal sex chromosomal inheritance are a fragile X chromosome and abnormal

chromosomal numbers: Turner syndrome (XO), Klinefelter syndrome (XXY), poly-X syndrome (XXX and higher), and Jacob syndrome (XYY).

Turner Syndrome (45,X). In 1938, at a medical conference, an endocrinologist named Henry Turner described seven young women, aged 15 to 23, who were sexually undeveloped, short, had folds of skin on the back of the neck, and had malformed elbows. About 1 in every 1,000 females has an extra X chromosome in each of her cells, a condition called triplo-X. The only symptom seems to be tallness and menstrual irregularities. Although triplo-X females are rarely mentally retarded, they tend to be less intelligent than their siblings. The lack of symptoms associated with having extra X chromosomes reflects the protective effect of X inactivation—all but one of the X chromosomes is inactivated.

Klinefelter syndrome (XXY). About 1 in 1,000 males has an extra X chromosome, which causes Klinefelter syndrome (XXY). Physicians first described the signs and symptoms in 1942, and geneticists identified the underlying chromosomal anomaly in 1959. Men severely affected with Klinefelter syndrome are underdeveloped sexually,with rudimentary testes and prostate glands and sparse pubic and facial hair. They have very long arms and legs, large hands and feet, and may develop breast tissue. Klinefelter syndrome is the most common genetic or chromosomal cause of male infertility, accounting for 4 to 6 percent of infertile men. Testosterone injections during adolescence can limit limb lengthening and prompt development of secondary sexual characteristics. Boys and men with Klinefelter syndrome may be slow to learn, but they are usually not mentally retarded unless they have more than two X chromosomes,which happens rarely. Many textbooks include photographs of very extreme cases of Klinefelter syndrome, which may give the erroneous impression that the syndrome is always severe.

Actually, many men who have the condition discover it only when they have an infertility problem. Some affected men probably never learn that they have Klinefelter syndrome. The photograph of the young man who wrote “A Personal Look at Klinefelter Syndrome” (Fig. 8.6) shows that affected individuals can look quite like anyone else.

FIGURE 8.6. Stefan Schwarz (Klinefelter syndrome patient).

"I was diagnosed with Klinefelter syndrome (KS) at age 25, in February 1996. Being diagnosed has been . . . a big sigh of relief after a life of frustrations. Throughout my early childhood, teens, and even somewhat now, I was very shy, reserved, and had trouble making friends. I would fly into rages for no apparent reason. My parents knew when I was very young that there was something about me that wasn't right. I saw many psychologists, psychiatrists, therapists, and doctors, and their only diagnosis was "learning disabilities." In the seventh grade, I was told by a psychologist that I was stupid and lazy, and I would never amount to anything. After barely graduating high school, I started out at a local community college. I received an associate degree in business administration, and never once sought special help. I transferred to a small liberal arts college to finish up my bachelor of science degree, and spent an extra year to complete a second degree. Then I started a job as a software engineer for an Internet-based company. I have been usingcomputers for 20 years and have learned everything I needed to know on my own.

To find out my KS diagnosis, I had gone to my general physician for a physical. He noticed that my testes were smaller than they should be and sent me for blood work. The karyotype showed Klinefelter syndrome, 47,XXY. After seeing the symptoms of KS and what effects they might have, I found it described me perfectly. But, after getting over the initial shock and dealing with the denial, depression, and anger, I decided that there could be things much worse in life. I decided to take a positive approach. There are several types of treatments for KS. I give myself a testosterone injection in the thigh once every two weeks. My learning and thought processes have become stronger, and I am much more outgoing and have become more of a leader.

Granted, not all of this is due to the increased testosterone level, some of it is from a new confidence level and from maturing. I feel that parents who are finding out prior to the birth of their son (that he will have Klinefelter syndrome) or parents of affected infants or young children are very lucky. There is so much they can do to help their child have a great life. I have had most all of the symptoms at some time in my life, and I've gotten through and done well."

(Stefan Schwarz runs a Boston-area Support group for KS)

XYY Syndrome. One male in 1,000 has an extra Y chromosome. Awareness of this condition arose in 1961, when a tall, healthy, middle-aged man, known for his boisterous behavior, underwent a routine chromosome check after fathering a child with Down syndrome. The man had an extra Y chromosome. A few other cases were detected over the next several years. In 1965, researcher Patricia Jacobs published results of a survey among 197 inmates at Carstairs, a high-security prison in Scotland. Of 12 men with unusual chromosomes, seven had an extra Y.Might their violent or aggressive behavior be linked to their extra Y chromosome? Jacobs's findings were repeated in studies in English and Swedish mental institutions. Soon after, *Newsweek* magazine ran a cover story on "congenital criminals." In 1968, defense attorneys in France and Australia pleaded their violent clients' cases on the basis of an inherited flaw, the extra Y of what became known as Jacobs syndrome. Meanwhile, the National Institute of Mental Health, in Bethesda, Maryland, held a conference on the condition, lending legitimacy to the hypothesis that an extra Y predisposes to violent behavior.

In the early 1970s, newborn screens began in hospital nurseries in England, Canada, Denmark, and Boston. XYY babies were visited by social workers and psychologists who offered "anticipatory guidance" to the parents on how to deal with their toddling future criminals. By 1974, geneticists and others halted the program, pointing out that singling out these boys on the basis of a few statistical studies was inviting selffulfilling prophecy.

Today, we know that 96 percent of XYY males are apparently normal. The only symptoms attributable to the extra chromosome may be great height, acne, and perhaps speech and reading problems. An explanation of the continued prevalence of XYY among mental-penal institution populations may be more psychological than biological. Large body size may lead teachers, employers, parents, and others to expect more of these people, and a few of them may deal with this stress by becoming aggressive. ***Jacobs syndrome*** can arise from nondisjunction in the male, producing a sperm with two Y chromosomes that fertilizes an X-bearing oocyte. Geneticists have never observed a sex chromosome constitution of one Y and no X. Since the Y chromosome carries

little genetic material, and the gene-packed X chromosome would not be present, the absence of so many genes makes development beyond a few cell divisions in a YO embryo impossible.

Polyploids have extra sets of chromosomes, and do not survive for long. Aneuploids have extra or missing chromosomes. Nondisjunction during meiosis causes aneuploidy. Trisomics are more likely to survive than monosomics, and sex chromosome aneuploidy is less severe than autosomal aneuploidy. Mitotic nondisjunction produces chromosomal mosaics. Down syndrome (trisomy 21) is the most common autosomal aneuploid, followed by trisomies 18 and 13. Sex chromosome aneuploids include Turner syndrome (XO), triplo-X females, Klinefelter syndrome (XXY), and XYY syndrome males.

Key Questions:

1. What is the variability. Types of variability.
2. Not hereditary variability (definition, classification).
3. Modification variability. Main properties of modifications. Norm of reaction.
4. Expressivity. Penetrance.
5. Phenocopy and genocopy.
6. Hereditary variability (definition, classification).
7. Kombinative variability, emergence mechanisms.
8. Mutational variability.
9. Concept about mutations. Classification of mutations.
10. Mutagen factors (physical, chemical, biological), mechanisms of their action. DNA reparation (light, excision).
11. Classification of mutations.
12. Genomic mutations (definition, emergence mechanisms). The chromosomal diseases caused by genomic mutations.
13. Chromosomal mutations (definition, emergence mechanisms). The chromosomal diseases caused by chromosomal mutations.
14. Gene mutations (definition, emergence mechanisms). The hereditary monogenic diseases caused by gene mutations.

Examples of Review questions:

NN	Questions	Right answers
1	A QUANTITATIVE TRAIT _____. 1) may be one that is strongly influenced by the environment 2) varies continuously in a population 3) may be influenced by many genes 4) has more than a few values in a population 5) All of the above are correct.	**3**
2	WHEN A TRAIT IS HIGHLY HERITABLE _____. 1) it is influenced by genes 2) it is not influenced by the environment 3) the variance of the trait in a population can be explained primarily by variance in their genotypes	**1**
3	MOST GENETIC DISEASES ARE RARE BECAUSE ___ . 1) each person is unlikely to be a carrier for harmful alleles 2) genetic diseases are usually sex-linked and so uncommon in females 3) genetic diseases are always dominant 4) a married couple probably do not carry the same recessive alleles 5) mutation rates in human are low	**4**
4	MULTIFACTORIAL (COMPLEX) DISEASES ___ . 1) are less common than single-gene diseases 2) involve the interaction of many genes with the environment 3) affect less than 1 percent of humans 4) involve the interactions of several mRNAs 5) are exempified by sickle-cell anemia	**2**
5	MOST HUMAN CANCERS ___ . 1) are caused by viruses 2) are in blood cells or their precursors 3) involve mutations of somatic cells 4) spread through solid tissues rather than by the blood or lymphatic system 5) are inherited	**3**

CHAPTER 9. METHODS OF HUMAN GENETIC STUDY

PEDIGREE ANALYSIS

Human matings, like those of experimental organisms, show many examples of the inheritance patterns described above. Because controlled experimental crosses cannot be made with humans, geneticists must resort to scrutinizing records in the hope that informative matings have been made by chance. Such a scrutiny of records of matings is called **pedigree analysis.** A member of a family who first comes to the attention of a geneticist is called the **propositus.** Usually the phenotype of the propositus is exceptional in some way (for example, the propositus might suffer from some type of disorder). The investigator then traces the history of the phenotype in the propositus back through the history of the family and draws a family tree, or pedigree, by using the standard symbols given in Figure 9.1.

Many variant phenotypes of humans are determined by the alleles of single autosomal genes, in the same manner we encountered in peas. Human pedigrees often show inheritance patterns of this simple Mendelian type. However, the patterns in the pedigree have to be interpreted differently, depending on whether one of the contrasting phenotypes is a rare disorder or whether both phenotypes of a pair are common morphs of a polymorphism.

Most pedigrees are drawn up for medical reasons and hence inherently concern medical disorders that are almost by definition rare. Let’s look first at rare recessive disorders caused by recessive alleles of single autosomal genes.

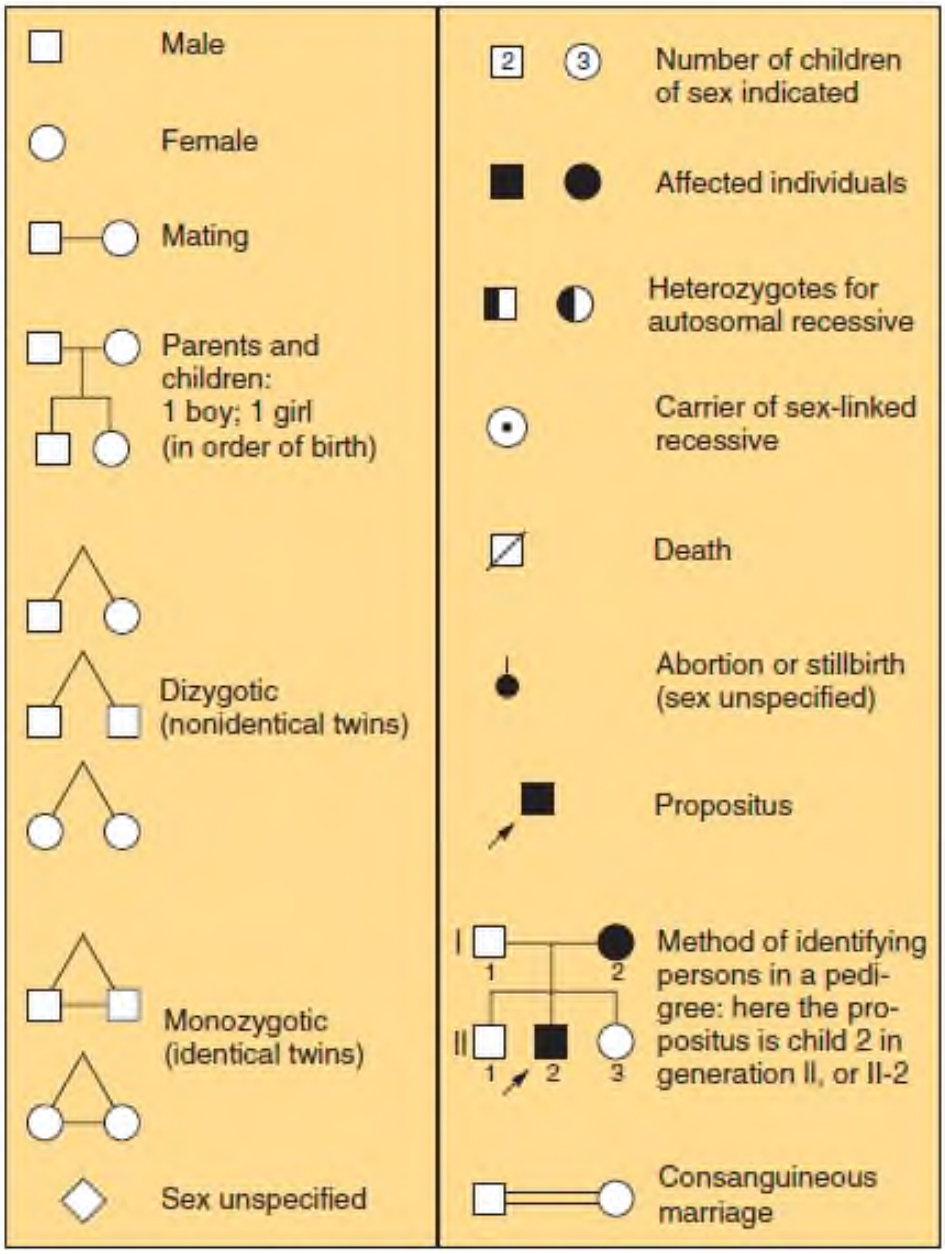


FIGURE 9.1. Symbols used in human pedigree analysis. [After W. F. Bodmer and L. L. Cavalli-Sforza, *Genetics, Evolution, and Man.* Copyright 1976 by W. H. Freeman and Company.]

Pedigree analysis of Autosomal Recessive Disorders

In human pedigrees, an autosomal recessive disorder is revealed by the appearance of the disorder in the male and female progeny of unaffected persons.

The affected phenotype of an autosomal recessive disorder is determined by a recessive allele, and hence the corresponding unaffected phenotype must be determined by the corresponding dominant allele. For example, the human

disease phenylketonuria (PKU) is inherited in a simple Mendelian manner as a recessive phenotype, with PKU determined by the allele *p* and the normal condition by *P*. Therefore, sufferers from this disease are of genotype *p*/*p*, and people who do not have the disease are either *P*/*P* or *P*/*p*.

What patterns in a pedigree would reveal such an inheritance? The two key points are that (1) generally the disease appears in the progeny of unaffected parents and (2) the affected progeny include both males and females. When we know that both male and female progeny are affected, we can assume that we are most likely dealing with simple Mendelian inheritance of a gene on an autosome, rather than a gene on a sex chromosome. The following typical pedigree illustrates the key point that affected children are born to unaffected parents (Fig. 9.2):

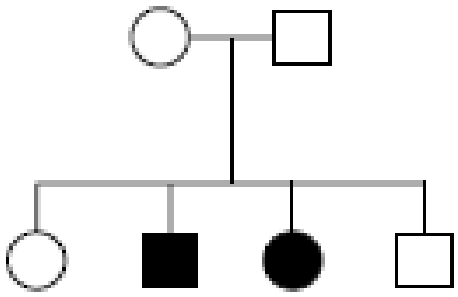
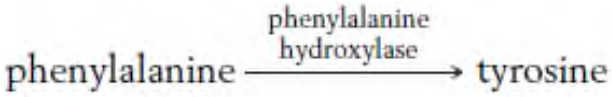


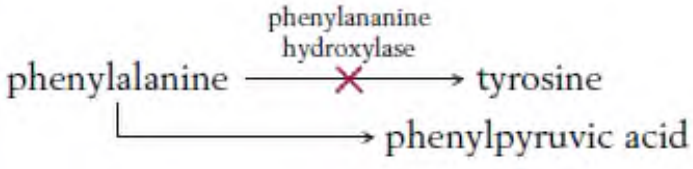
FIGURE 9.2. Pedigree of Autosomal Recessive Inheritance.

From this pattern, we can deduce simple Mendelian inheritance of the recessive allele responsible for the exceptional phenotype (indicated in black). Furthermore, we can deduce that the parents are both heterozygotes, say *A*/*a*; both must have an *a* allele because each contributed an *a* allele to each affected child, and both must have an *A* allele because they are phenotypically normal. We can identify the genotypes of the children (in the order shown) as *A*/_, *a*/*a*, *a*/*a*, and *A*/_. What are some examples of human recessive disorders?

Phenilketonuria (PKU) has already served as an example of pedigree analysis, but what kind of phenotype is it? PKU is a disease caused by abnormal processing of the amino acid phenylalanine, a component of all proteins in the food that we eat. Phenylalanine is normally converted into the amino acid tyrosine by the enzyme phenylalanine hydroxylase:



However, if a mutation in the gene encoding this enzyme alters the amino acid sequence in the vicinity of the enzyme's active site, the enzyme cannot bind phenylalanine (its substrate) or convert it to tyrosine. Therefore phenylalanine builds up in the body and is converted instead into phenylpyruvic acid. This compound interferes with the development of the nervous system, leading to mental retardation.



Babies are now routinely tested for this processing deficiency at birth. If the deficiency is detected, phenylalanine can be withheld by use of a special diet and the development

Cystic fibrosis is another disease inherited according to Mendelian rules as a recessive phenotype. Cystic fibrosis is a disease whose most important symptom is the secretion of large amounts of mucus into the lungs, resulting in death from a combination of effects but usually precipitated by infection of the respiratory tract. The mucus can be dislodged by mechanical chest thumpers, and pulmonary infection can be prevented by antibiotics; thus, with treatment, cystic fibrosis patients can live to adulthood. The allele that causes cystic fibrosis was isolated in 1989, and the sequence of its DNA was determined. This line of research eventually revealed that the disorder is caused by a defective protein that transports chloride ions across the cell membrane. The resultant alteration of the salt balance changes the constitution of the lung mucus. This new understanding of gene function in affected and unaffected persons has given hope for more effective treatment.

Albinism, which served as a model of how differing alleles determine contrasting phenotypes, also is inherited in the standard autosomal recessive manner.

The recessive allele *a* is caused by a base-pair change that introduces a stop codon into the middle of the gene, resulting in a truncated protein. The mutation, by chance, also introduces a new target site for a restriction enzyme. Hence, a probe for the gene detects two fragments in the case of *a* and only one in *A*. (Other types of mutations would produce different effects at the level detected by Southern, Northern, and Western analyses.) In all the examples considered so far, the disorder is caused by an allele that codes for a defective protein. In heterozygotes, the single functional allele provides enough active protein for the cell's needs. This situation is called *haplosufficiency*. Thus the amount of protein is insufficient only if the mutant allele is present in two copies, producing the recessive trait.

Pedigree analysis of autosomal Dominant Disorders

Pedigrees of Mendelian autosomal dominant disorders show affected males and females in each generation; they also show that affected men and women transmit the condition to equal proportions of their sons and daughters.

What pedigree patterns are expected from autosomal dominant disorders? Here the normal allele is recessive, and the abnormal allele is dominant. It may seem paradoxical that a rare disorder can be dominant, but remember that dominance and recessiveness are simply properties of how alleles act and are not defined in terms of how common they are in the population.

A good example of a rare dominant phenotype with Mendelian inheritance is ***pseudoachondroplasia***, a type of dwarfism. In regard to this gene, people with normal stature are genotypically *d*/*d*, and the dwarf phenotype in principle could be *D*/*d* or *D*/*D*. However, it is believed that the two "doses" of the *D* allele in the *D*/*D* genotype produce such a severe effect that this genotype

is lethal. If this is true, all dwarf individuals are heterozygotes. In pedigree analysis, the main clues for identifying an autosomal dominant disorder with Mendelian inheritance are that the phenotype tends to appear in every generation of the pedigree and that affected fathers and mothers transmit the phenotype to both sons and daughters. Again, the equal representation of both sexes among the affected offspring rules out inheritance via the sex chromosomes. The phenotype appears in every generation because generally the abnormal allele carried by a person must have come from a parent in the preceding generation. (Abnormal alleles can also arise de novo by the process of mutation. This event is relatively rare but must be kept in mind as a possibility.) Notice that Mendelian ratios are not necessarily observed in families. As with recessive disorders, persons bearing one copy of the rare *A* allele (*A*/*a*) are much more common than those bearing two copies (*A*/*A*), so most affected people are heterozygotes, and virtually all matings that produce progeny with dominant disorders are *A*/*a* _ *a*/*a*. Therefore, when the progeny of such matings are totaled, a 1 : 1 ratio is expected of unaffected (*a*/*a*) to affected (*A*/*a*) persons.

Huntington disease is example of a disease inherited as a dominant phenotype determined by an allele of a single gene. The phenotype is one of neural degeneration, leading to convulsions and premature death. However, it is a late-onset disease, the symptoms generally not appearing until after the person has begun to have children. Each child of a carrier of the abnormal allele stands a 50 percent chance of inheriting the allele and the associated disease. This tragic pattern has inspired a great effort to find ways of identifying people who carry the abnormal allele before they experience the onset of the disease. The application of molecular techniques has resulted in useful screening procedures. Some other rare dominant conditions are polydactyly (extra digits).

Pedigree analysis of X-linked Recessive disorders

In the pedigree analysis of rare X-linked recessives, a normal female of unknown genotype is assumed to be homozygous unless there is evidence to the contrary. Perhaps the most familiar example of X-linked recessive inheritance is red-green colorblindness. People with this condition are unable to distinguish red from green. The genes for color vision have been characterized at the molecular level. Color vision is based on three different kinds of cone cells in the retina, each sensitive to red, green, or blue wavelengths. The genetic determinants for the red and green cone cells are on the X chromosome. As with any X-linked recessive, there are many more males with the phenotype than females.

Familiar example is ***hemophilia***, the failure of blood to clot. Many proteins act in sequence to make blood clot. The most common type of hemophilia is caused by the absence or malfunction of one of these proteins, called *factor VIII.* The most well known cases of hemophilia are found in the pedigree of interrelated royal families in Europe. The original hemophilia allele in the pedigree arose spontaneously (as a mutation) in the reproductive cells of either Queen Victoria's parents or Queen Victoria herself. The son of the last czar of Russia, Alexis, inherited the allele ultimately from Queen Victoria, who was the grandmother of his mother Alexandra. Nowadays, hemophilia can be treated medically, but it was formerly a potentially fatal condition. It is interesting to note that in the Jewish Talmud there are rules about exemptions to male circumcision that show clearly that the mode of transmission of the disease through unaffected carrier females was well understood in ancient times. For example, one exemption was for the sons of women whose sisters' sons had bled profusely when they were circumcised.

Duchenne muscular dystrophy is a fatal X-linked recessive disease. The phenotype is a wasting and atrophy of muscles. Generally the onset is before the age of 6, with confinement to a wheelchair by 12, and death by 20. The gene for Duchenne muscular dystrophy has now been isolated and shown to

encode the muscle protein dystrophin. This discovery holds out hope for a better understanding of the physiology of this condition and, ultimately, a therapy.

A rare X-linked recessive phenotype that is interesting from the point of view of sexual differentiation is a condition called ***testicular feminization syndrome***, which has a frequency of about 1 in 65,000 male births. People afflicted with this syndrome are chromosomally males, having 44 autosomes plus an X and a Y, but they develop as females. They have female external genitalia, a blind vagina, and no uterus. Testes may be present either in the labia or in the abdomen. Although many such persons marry, they are sterile. The condition is not reversed by treatment with the male hormone androgen, so it is sometimes called *androgen insensitivity syndrome.* The reason for the insensitivity is that a mutation in the androgen receptor gene causes the receptor to malfunction, so the male hormone can have no effect on the target organs that contribute to maleness. In humans, femaleness results when the male-determining system is not functional.

Pedigree analysis of X-linked dominant disorders

These disorders have the following characteristics:

1. Affected males pass the condition to all their daughters but to none of their sons

2. Affected heterozygous females married to unaffected males pass the condition to half their sons and daughters.

There are few examples of X-linked dominant phenotypes in humans. One example is ***hypophosphatemia***, a type of vitamin D–resistant rickets.

Y-linked inheritance

Only males inherit genes on the differential region of the human Y chromosome, with fathers transmitting the genes to their sons. The gene that plays a primary role in maleness is the ***SRY* gene,** sometimes called the *testis-determining factor***.** The *SRY* gene has been located and mapped on the differ-

ential region of the Y chromosome. Hence maleness itself is Ylinked, and shows the expected pattern of exclusively male-to-male transmission. Some cases of male sterility have been shown to be caused by deletions of Y chromosome regions containing sperm-promoting genes. Male sterility is not heritable, but interestingly the fathers of these men have normal Y chromosomes, showing that the deletions are new. There have been no convincing cases of nonsexual phenotypic variants associated with the Y.

Hairy ear rims has been proposed as a possibility. The phenotype is extremely rare among the populations of most countries but more common among the populations of India. In some (but not all) families hairy ear rims are transmitted exclusively from father to son.

CYTOPLASMIC INHERITANCE

Variant phenotypes caused by mutations in cytoplasmic organelle DNA are generally inherited maternally.

Mitochondria and chloroplasts are specialized organelles located in the cytoplasm. They contain small circular chromosomes that carry a defined subset of the total cell genome. Mitochondrial genes are concerned with the mitochondrion's task of energy production, whereas chloroplast genes are needed for the chloroplast to carry out its function of photosynthesis. However, neither organelle is genetically independent, because each relies to some extent on nuclear genes for function. Why some of the necessary genes are in the organelles themselves while others are in the nucleus is still something of a mystery, which we will not address here. Organelle genes show their own special mode of inheritance called uniparental inheritance; that is, progeny inherit organelle genes exclusively from one parent. In most cases, that parent is the mother: ***maternal inheritance.*** Why only the mother? The answer lies in the fact that the organelle chromosomes are located in the cytoplasm rather than the nucleus and the fact that male and female gametes do not contribute cytoplasm equally to the zygote. In the case of nuclear genes, we have seen that both parents do

contribute equally to the zygote. However, the egg contributes the bulk of the cytoplasm and the sperm essentially none. Therefore, because organelles reside in the cytoplasm, the female parent contributes the organelles along with the cytoplasm and essentially none of the organelle DNA in the zygote is from the male parent.

MODEL ORGANISMS

The science of genetics discussed in this book is meant to provide an understanding of features of inheritance and development that are characteristic of organisms in general. Some of these features, especially at the molecular level, are true of all known living forms. For others there is some variation between large groups of organisms, for example, between bacteria and all multicellular species. Even for the features that vary, however, that variation is always between major groups of living forms, so that we do not have to investigate the basic phenomena of genetics over and over again for every species. In fact, all the phenomena of genetics have been investigated by experiments on a small number of species, **model organisms,** whose genetic mechanisms are common either to all species or to a large group of related organisms.

Multicellular organisms. For the genetic study of the differentiation of cells, tissues, and organs, as well as the development of body form, it is necessary to use more complex organisms. These organisms must be easy to culture under controlled conditions, have life cycles short enough to allow breeding experiments over many generations, and be small enough to make the production of large numbers of individuals practical. The main model organisms that fill these requirements are:

- ***Arabidopsis thaliana***, a small flowering plant that can be cultured in large numbers in the greenhouse or laboratory. It has a small genome contained in only five chromosomes. It is an ideal model for studying the development of higher plants and the comparison of animal and plant development and genome structure.

- ***Drosophila melanogaster***, a fruit fly with only four chromosomes. In the larval stage these chromosomes have a well-marked pattern of banding that makes it possible to observe physical changes such as deletions and duplications, which can then be correlated with genetic changes in morphology and biochemistry. The development of *Drosophila* produces body segments in an anterior-posterior order that is an example of the basic body plan common to invertebrates and vertebrates.
- ***Caenorhabditis elegans,*** a tiny roundworm with a total of only a few thousand adult cells. These form a nervous system; a digestive tract with a mouth, pharynx, and anus; and a reproductive system that can produce both eggs and sperm.
- ***Mus musculus***, the house mouse, the model organism for vertebrates. It has been studied to compare the genetic basis of vertebrate and invertebrate development as well as to explore the genetics of antigen-antibody systems, of maternal-fetal interactions in utero, and in understanding the genetics of cancer.
- The genomes of all the model organisms discussed above have been sequenced. Despite the great differences in biology there are many similarities in their genomes.

CYTOGENETICAL ANALYSIS

Chromosome structure and movement have long been an integral part of genetics, but new technologies have provided ways of labeling genes and gene products so that their locations can be easily visualized under the microscope.

Swelling, Squashing, and Untangling. The dilemma of how to untangle the spaghettilike mass of chromosomes in a human cell was solved by accident in 1951. A technician mistakenly washed white blood cells being prepared for chromosome analysis in a salt solution that was less concentrated than the interiors of the cells. Water rushed into the cells, swelling them and separating the chromosomes.

Two years later, cell biologists Albert Levan and Joe-Hin Tjio found that when they drew cell-rich fluid into a pipette and dropped it onto a microscope slide prepared with stain, the cells burst open and freed the mass of chromosomes. Adding a glass coverslip spread the chromosomes enough that they could be counted. Another researcher, a former student of Painter named John Biesele, suggested that Levan and Tjio use cells from tissue culture, and by 1956, they finally settled the matter of how many chromosomes occupy a diploid human cell—46.

In the same year, J. L. Hamerton and C. E. Ford identified the expected 23 chromosomes in human gametes. In 1960 came another advance in visualizing chromosomes— through the use of a kidney bean extract called phytohemagglutinin. Originally used to clump red blood cells to separate them from white blood cells, the substance also could stimulate division of white blood cells.

Until recently, a karyotype was constructed using a microscope to locate a cell in which the chromosomes were not touching, photographing the cell, developing a print, and cutting out the individual chromosomes and arranging them into a size-order chart. A computerized approach has largely replaced the cut-and-paste method. The device scans ruptured cells in a drop of stain and selects one in which the chromosomes are the most visible and well spread. Then image analysis software recognizes the band patterns of each stained chromosome pair, sorts the structures into a size-order chart, and prints the karyotype—in minutes. If the software recognizes an abnormal band pattern, a database pulls out identical or similar karyotypes from other patients, providing clinical information on the anomaly. Genome sequence information is also scanned. However, the expert eyes of a skilled technician are still needed to detect subtle abnormalities in chromosome structure.

Staining. In the earliest karyotypes, dyes stained the chromosomes a uniform color. Chromosomes were grouped into size classes, designated A through G, in decreasing size order. The first stains that were applied to chromosomes

could highlight large deletions and duplications, but more often than not, researchers only vaguely understood the nature of a chromosomal syndrome. In 1967, a mentally retarded child with material missing from chromosome 4 would have been diagnosed as having a "B-group chromosome" disorder. Today the exact genes that are missing can be identified.

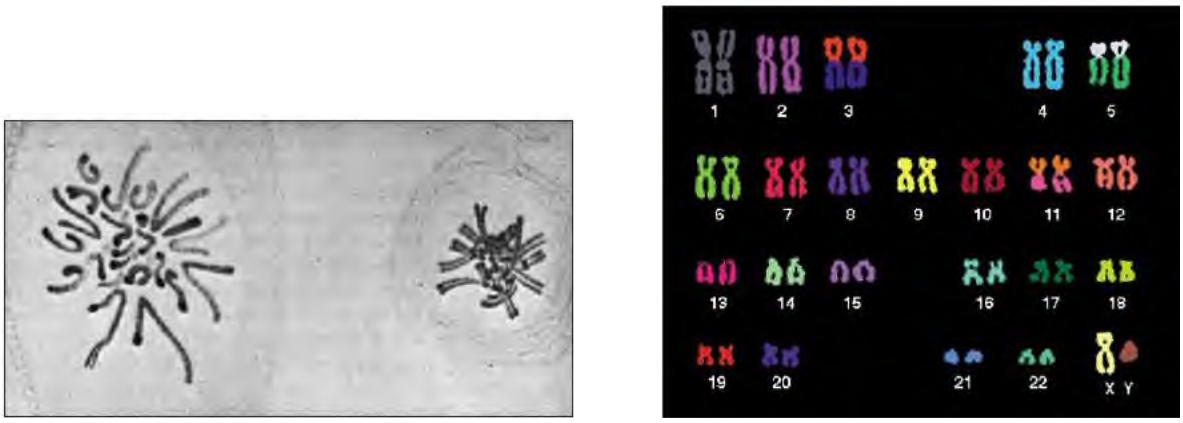
Describing smaller-scale chromosomal aberrations required better ways to distinguish among the chromosomes. In the 1970s, Swedish scientists developed more specific chromosome stains that create banding patterns unique to each chromosome. Combining stains reveals even more bands, making it easier to distinguish chromosomes.

Stains are specific for AT-rich or GC-rich stretches of DNA, or for heterochromatin, which stains darkly at the centromere and telomeres. The ability to detect missing, extra, inverted, or misplaced bands allowed researchers to link many more syndromes with specific chromosome aberrations. In the late 1970s, Jorge Yunis at the University of Minnesota improved chromosome staining further by developing a way to synchronize white blood cells in culture, arresting them in early mitosis. His approach, called high-resolution chromosome banding, revealed many more bands. Today, **fluorescence *in situ* hybridization,** or FISH, is eclipsing even high-resolution chromosome banding, enabling cytogeneticists to focus on individual genes.

FISHing. One drawback of conventional chromosome stains is that they are not specific to particular chromosomes. Rather, they generate different banding patterns among the 24 human chromosome types. FISH is much more specific because it uses DNA probes that are complementary to DNA sequences found only on one chromosome type. The probes are attached to molecules that fluoresce when illuminated, producing a flash of color precisely where the probe binds to a chromosome in a patient's sample. The technique can reveal a particular extra chromosome in a day or two. FISH is based on a technique, developed in 1970, called *in situ* hybridization, which originally used radioactive labels rather than fluorescent ones. *In situ* hybridization took weeks to work,

because it relied on exposing photographic film to reveal where DNA probes bound among the chromosomes. The danger of working with radioactivity, and the crudeness of the results, eventually prompted researchers to seek alternative ways of highlighting bound DNA probes. FISH is used to identify specific chromosomes and to "paint" entire karyotypes, providing a different color for each chromosome. In an application of FISH called spectral karyotyping, each chromosome is probed with several different fluorescent molecules. A computer integrates the images and creates a false color for each chromosome (Fig. 9.3).

A new approach to prenatal chromosome analysis called quantitative PCR amplifies certain repeated sequences on chromosomes 13, 18, 21, X, and Y. The technique distinguishes paternally derived from maternally derived repeats on each homolog for these five chromosomes. An abnormal ratio of maternal to paternal repeats indicates a numerical problem, such as two copies of one parent's chromosome 21.



(a) (b)

FIGURE 9.3. Karyotypes old and new. (*a*) The earliest drawings of chromosomes, by German biologist Walter Flemming, date from 1882. (*b*) This karyotype was constructed using a technique called FISH to "paint" the individual chromosomes.

Figure 9.4 shows a normal karyotype with the chromosomes distinguished by stained bands.

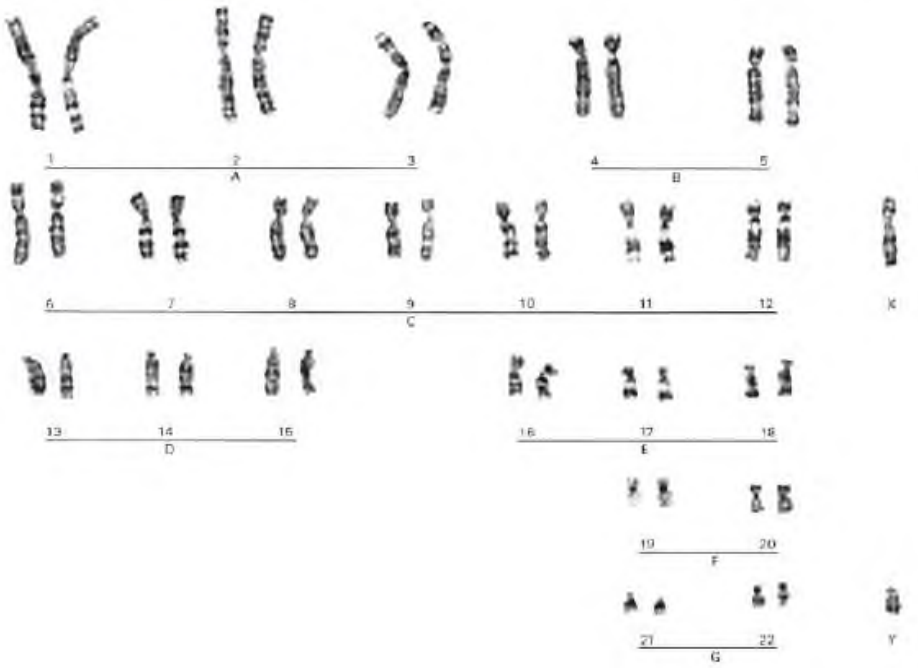


FIGURE 9.4. A human male karyotype. Note the X and Y chromosomes. A female would have a second X chromosome in place of the Y. The chromosomes are grouped into categories (A–G, X, Y) by length and centromere position. Similar chromosomes are often distinguished by their chromomeres. (Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

POPULATION-GENETICS ANALYSIS

Gene Variation in Nature. Evolution within a species may result from any process that causes a change in the genetic composition of a population. In considering this theory of population genetics, it is best to start by looking at the genetic variation present among individuals within a species. This is the raw material available for the selective process.

Population genetics is the study of the properties of genes in populations. Genetic variation within natural populations was a puzzle to Darwin and his contemporaries. The way in which meiosis produces genetic segregation among the progeny of a hybrid had not yet been discovered. Selection, scientists then thought, should always favor an optimal form, and so tend to eliminate variation. Moreover, the theory of **blending inheritance**—in which offspring were expected to be phenotypically intermediate relative to their parents—was widely accepted. If blending inheritance were correct, then the effect of any new genetic variant would quickly be diluted to the point of disappearance in subsequent generations.

The Hardy–Weinberg Principle. Following the rediscovery of Mendel's research, two people in 1908 independently solved the puzzle of why genetic variation persists—G. H. Hardy, an English mathematician, and G. Weinberg, a German physician. They pointed out that the original proportions of the genotypes in a population will remain constant from generation to generation, as long as the following assumptions are met:

1. The population size is very large.

2. Random mating is occurring.

3. No mutation takes place.

4. No genes are input from other sources (no immigration takes place).

5. No selection occurs.

Dominant alleles do not, in fact, replace recessive ones. Because their proportions do not change, the genotypes are said to be in **Hardy–Weinberg equilibrium.** In algebraic terms, the Hardy–Weinberg principle is written as an equation. Consider a population of 100 cats, with 84 black and 16 white cats (Fig. 9.5). In statistics, **frequency** is defined as the proportion of individuals falling within a certain category in relation to the total number of individuals under consideration. In this case, the respective frequencies would be 0.84 (or 84%) and 0.16 (or 16%). Based on these phenotypic frequencies, can we deduce the underlying frequency of genotypes? If we assume that the white cats are homozygous recessive for an allele we designate *b*, and the black cats are therefore either homozygous dominant *BB* or heterozygous *Bb*, we can calculate the **allele frequencies** of the two alleles in the population from the proportion of black and white individuals. Let the letter *p* designate the frequency of one allele and the letter *q* the frequency of the alternative allele. Because there are only two alleles, *p* plus *q* must always equal 1.

The Hardy-Weinberg equation can now be expressed in the form of what is known as a binomial expansion:

$$(p + q)2 = p2 + 2pq + q2$$

If $q2 = 0.16$ (the frequency of white cats), then $q = 0.4$. Therefore, *p*, the frequency of allele *B*, would be 0.6 (1.0 – 0.4 = 0.6). We can now easily calculate the **genotype frequencies:** there are $p2 = (0.6)2$ _ 100 (the number of cats in

the total population), or 36 homozygous dominant *BB* individuals. The heterozygous individuals have the *Bb* genotype, and there would be 2*pq*, or (2 _ 0.6 _ 0.4) _ 100, or 48 heterozygous *Bb* individuals. Figure 9.5 allows you to trace genetic reassortment during sexual reproduction and see how it affects the frequencies of the *B* and *b* alleles during the next generation.

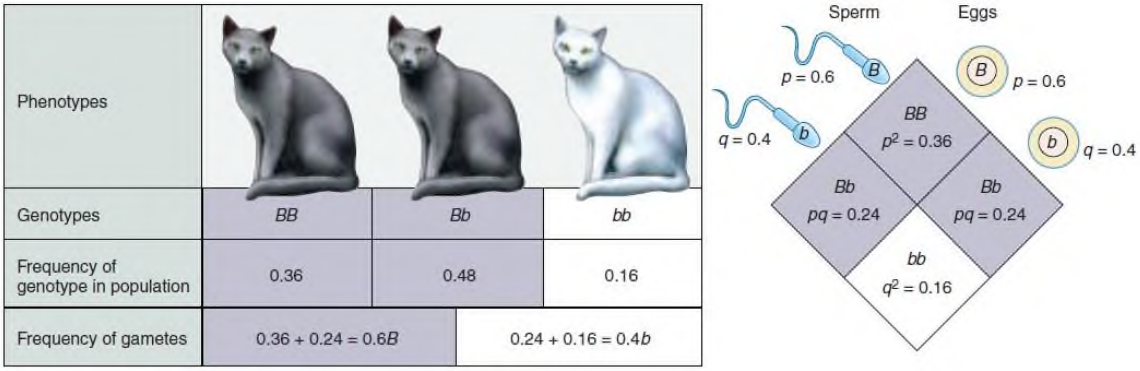


FIGURE 9.5. The Hardy–Weinberg equilibrium. In the absence of factors that alter them, the frequencies of gametes, genotypes, and phenotypes remain constant generation after generation.

In constructing this diagram, we have assumed that the union of sperm and egg in these cats is random, so that all combinations of *b* and *B* alleles occur. For this reason, the alleles are mixed randomly and represented in the next generation in proportion to their original representation. Each individual egg or sperm in each generation has a 0.6 chance of receiving a *B* allele ($p = 0.6$) and a 0.4 chance of receiving a *b* allele ($q = 0.4$).

In the next generation, therefore, the chance of combining two *B* alleles is $p2$, or 0.36 (that is, 0.6 _ 0.6), and approximately 36% of the individuals in the population will continue to have the *BB* genotype. The frequency of *bb* individuals is $q2$ (0.4 _ 0.4) and so will continue to be about 16%, and the frequency of *Bb* individuals will be 2*pq* (2 _0.6 _ 0.4), or approximately 48%. Phenotypically, if the population size remains at 100 cats, we will still see approximately 84 black individuals (with either *BB* or *Bb* genotypes) and 16 white individuals (with the *bb* genotype) in the population. Allele, genotype, and phenotype frequencies have remained unchanged from one generation to the next.

This simple relationship has proved extraordinarily useful in assessing actual situations. Consider the recessive allele responsible for the serious human disease cystic fibrosis. This allele is present in North Americans of Caucasian descent at a frequency q of about 22 per 1000 individuals, or 0.022. What proportion of North American Caucasians, therefore, is expected to express this trait? The frequency of double recessive individuals ($q2$) is expected to be 0.022 _ 0.022, or 1 in every 2000 individuals. What proportion is expected to be heterozygous carriers? If the frequency of the recessive allele q is 0.022, then the frequency of the dominant allele p must be 1 – 0.022, or 0.978. The frequency of heterozygous individuals ($2pq$) is thus expected to be 2 _ 0.978 _ 0.022, or 43 in every 1000 individuals.

The Hardy–Weinberg principle states that in a large population mating at random and in the absence of other forces that would change the proportions of the different alleles at a given locus, the process of sexual reproduction (meiosis and fertilization) alone will not change these proportions.

Why Do Allele Frequencies Change? According to the Hardy–Weinberg principle, both the allele and genotype frequencies in a large, random-mating population will remain constant from generation to generation if no mutation, no gene flow, and no selection occur. The stipulations tacked onto the end of the statement are important. In fact, they are the key to the importance of the Hardy–Weinberg principle, because individual allele frequencies often change in natural populations, with some alleles becoming more common and others decreasing in frequency. The Hardy–Weinberg principle establishes a convenient baseline against which to measure such changes. By looking at how various factors alter the proportions of homozygotes and heterozygotes, we can identify the forces affecting particular situations we observe.

Many factors can alter allele frequencies. Only five, however, alter the proportions of homozygotes and heterozygotes enough to produce significant deviations from the proportions predicted by the Hardy–Weinberg principle: mutation, gene flow (including both immigration into and emigration out of a given population), nonrandom mating, genetic drift (random change in allele frequencies, which is more likely in small populations), and selection (table

9.1). Of these, only selection produces adaptive evolutionary change because only in selection does the result depend on the nature of the environment. The other factors operate relatively independently of the environment, so the changes they produce are not shaped by environmental demands. Dominant alleles do not, in fact, replace recessive ones. Because their proportions do not change, the genotypes are said to be in **Hardy–Weinberg equilibrium.**

MOLECULAR-GENETIC METHOD

Direct analysis of DNA. Because the genetic material is composed of DNA, the ultimate characterization of a gene is the analysis of the DNA sequence itself. Many techniques, including gene cloning, are used to accomplish this. Cloning is a procedure by which an individual gene can be isolated and amplified (copied multiple times) to produce a pure sample for analysis.

One way of doing this is by inserting the gene of interest into a small bacterial chromosome and allowing bacteria to do the job of copying the inserted DNA. After the clone of a gene has been obtained, its nucleotide sequence can be determined, and hence important information about its structure and function can be obtained.

Table 9.1

Agents of Evolutionary Change

Factor	Description
Mutation	The ultimate source of variation. Individual mutations occur so rarely that mutation alone does not change allele frequency much.
Gene flow	A very potent agent of change. Populations exchange members.
Nonrandom mating	Inbreeding is the most common form. It does not alter allele frequency but decreases the proportion of heterozygotes.
Genetic drift	Statistical accidents. Usually occurs only in very small populations.
Selection	The only form that produces *adaptive* evolutionary changes.

Entire genomes of many organisms have been sequenced by extensions of the above techniques, thereby giving rise to a new discipline within genetics called **genomics,** the study of the structure, function, and evolution of whole

genomes. Part of genomics is **bioinformatics,** the mathematical analysis of the information content of genomes.

PCR (in vitro DNA amplification). Described as being to genes what Gutenberg's printing press was to the written word, PCR can amplify a desired DNA sequence of any origin (virus, bacteria, plant, or human) hundreds of millions of times in a matter of hours, a task that would have required several days with recombinant technology (Fig. 9.6).

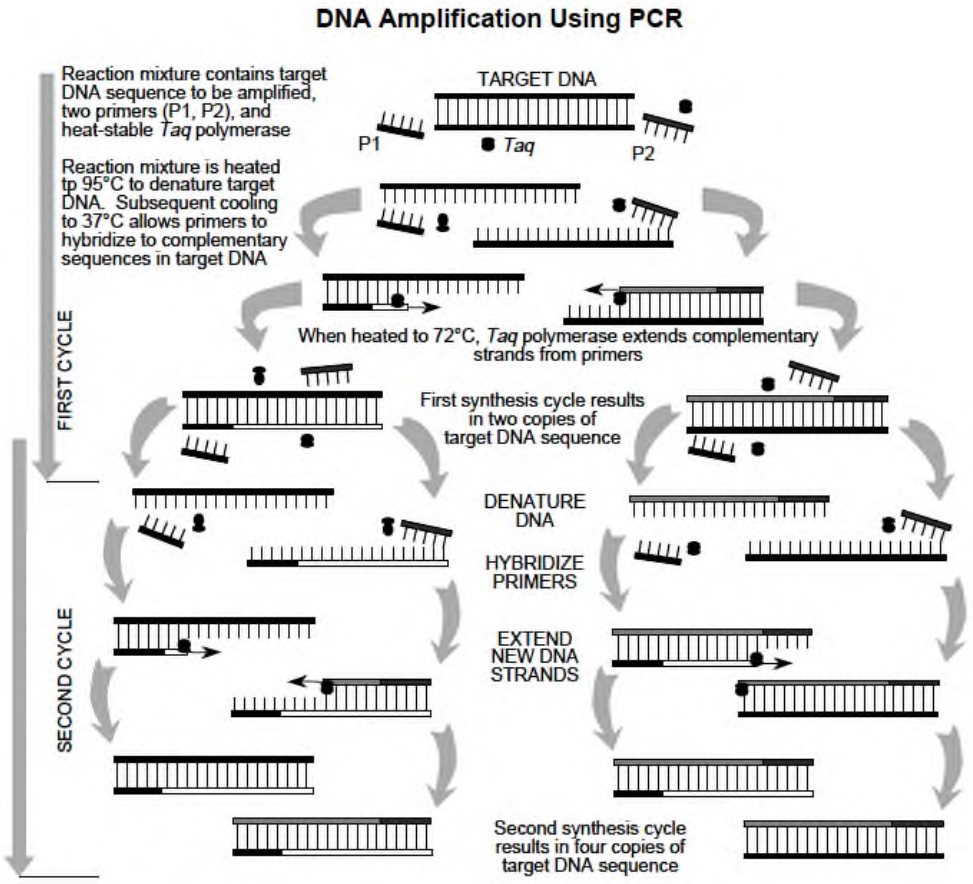


FIGURE 9.6. DNA Amplification using PCR.

PCR is especially valuable because the reaction is highly specific, easily automated, and capable of amplifying minute amounts of sample. For these reasons, PCR has also had a major impact on clinical medicine, genetic disease diagnostics, forensic science, and evolutionary biology. PCR is a process based on a specialized polymerase enzyme, which can synthesize a complementary strand to a given DNA strand in a mixture containing the 4 DNA bases and 2 DNA fragments (primers, each about 20 bases long) flanking the target se-

quence. The mixture is heated to separate the strands of doublestranded DNA containing the target sequence and then cooled to allow (1) the primers to find and bind to their complementary sequences on the separated strands and (2) the polymerase to extend the primers into new complementary strands. Repeated heating and cooling cycles multiply the target DNA exponentially, since each new double strand separates to become two templates for further synthesis. In about 1 hour, 20 PCR cycles can amplify the target by a millionfold.

Cutting genomic DNA. Most cutting is done using bacterial **restriction enzymes.** These enzymes cut at specific DNA target sequences, called *restriction sites,* and this property is one of the key features that make restriction enzymes suitable for DNA manipulation. Purely by chance, any DNA molecule, be it derived from virus, fly, or human, contains restriction enzyme target sites. Thus a restriction enzyme will cut the DNA into a set of **restriction fragments** determined by the locations of the restriction sites. Another key property of some restriction enzymes is that they make "sticky ends." Let's look at an example. The restriction enzyme *Eco*RI (from *E. coli*) recognizes the following sequence of six nucleotide pairs in the DNA of any organism:

5'-GAATTC-3'
3'-CTTAAG-5'.

Dozens of restriction enzymes with different sequence specificities are now known, some of which are listed in Figure 9.7.

One useful type of molecular chromosomal landmark, or marker, is a **restriction fragment length polymorphism (RFLP)**. Restriction enzymes are bacterial enzymes that cut DNA at specific base sequences in the genome. The target sequences have no biological significance in organisms other than bacteria—they occur purely by chance. Although the target sites generally occur quite consistently at specific locations, sometimes, on any one chromosome, a specific target site is missing or there is an extra site. If the presence or absence of such a restriction site flanks the sequence hybridized by a probe, then a Southern hybridization will reveal a length polymorphism, or RFLP.

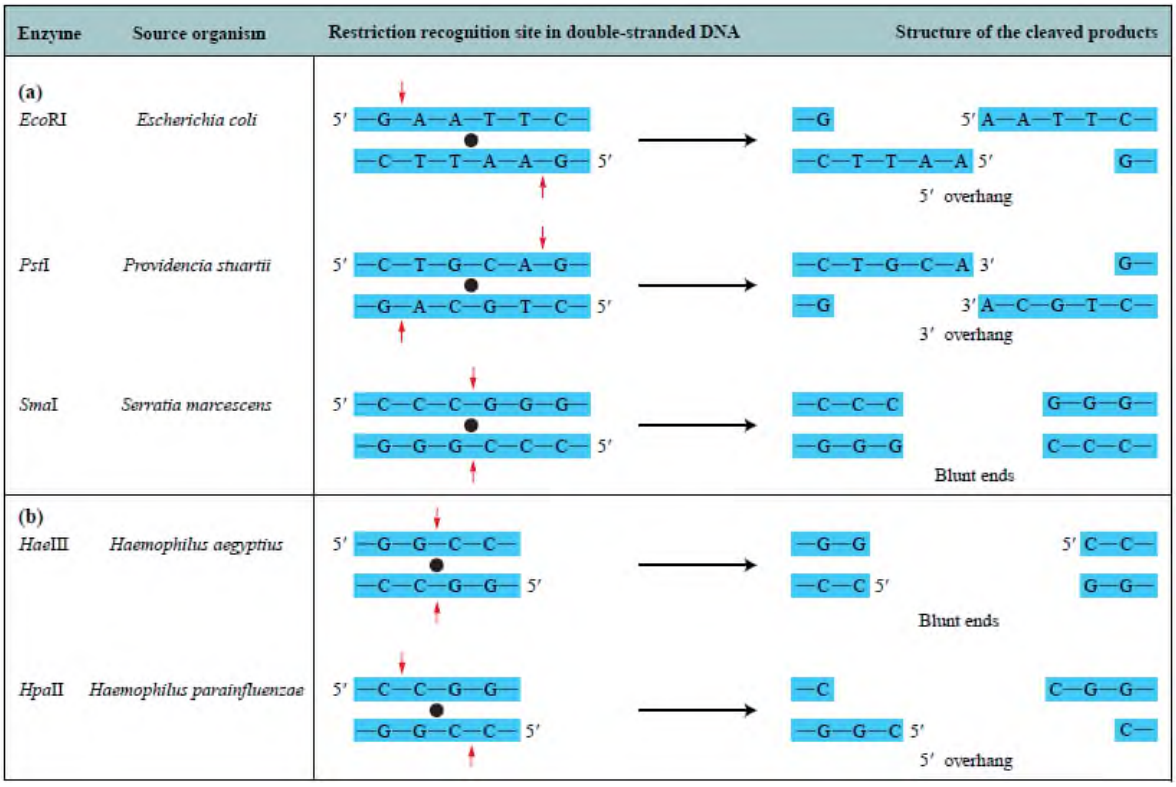


FIGURE 9.7. The specificity and results of restriction enzyme cleavage. The 5’ end of each DNA strand and the site of cleavage (small red arrows) are indicated. The large dot indicates the site of rotational symmetry of each recognition site. Note that the recognition sites differ for different enzymes. In addition, the positions of the cut sites may differ for different enzymes, producing single-stranded overhangs (sticky ends) at the 5’ or 3’ end of each double-stranded DNA molecule or producing blunt ends if the cut sites are not offset. (a) Three hexanucleotide (six-cutter) recognition sites and the restriction enzymes that cleave them. Note that one site produces a 5’ overhang, another a 3’ overhang, and the third a blunt end. (b) Examples of enzymes that have tetranucleotide (four-cutter) recognition sites.

One useful type of molecular chromosomal landmark, or marker, is a **restriction fragment length polymorphism (RFLP)**. Restriction enzymes are bacterial enzymes that cut DNA at specific base sequences in the genome. The target sequences have no biological significance in organisms other than bacteria—they occur purely by chance. Although the target sites generally occur quite consistently at specific locations, sometimes, on any one chromosome, a specific target site is missing or there is an extra site. If the presence or absence of such a restriction site flanks the sequence hybridized by a probe, then a Southern hybridization will reveal a length polymorphism, or RFLP.

DNA sequencing exploits base-pair complementarity together with an understanding of the basic biochemistry of DNA replication. Several techniques have been developed, but one of them is by far most used. It is called **dideoxy sequencing** or, sometimes, **Sanger sequencing** after its inventor. The term *dideoxy* comes from a special modified nucleotide, called a dideoxynucleotide triphosphate (generically, a ddNTP). This modified nucleotide is key to the Sanger technique because of its ability to block continued DNA synthesis. The products of such dideoxy sequencing reactions are shown in Figure 9.8.

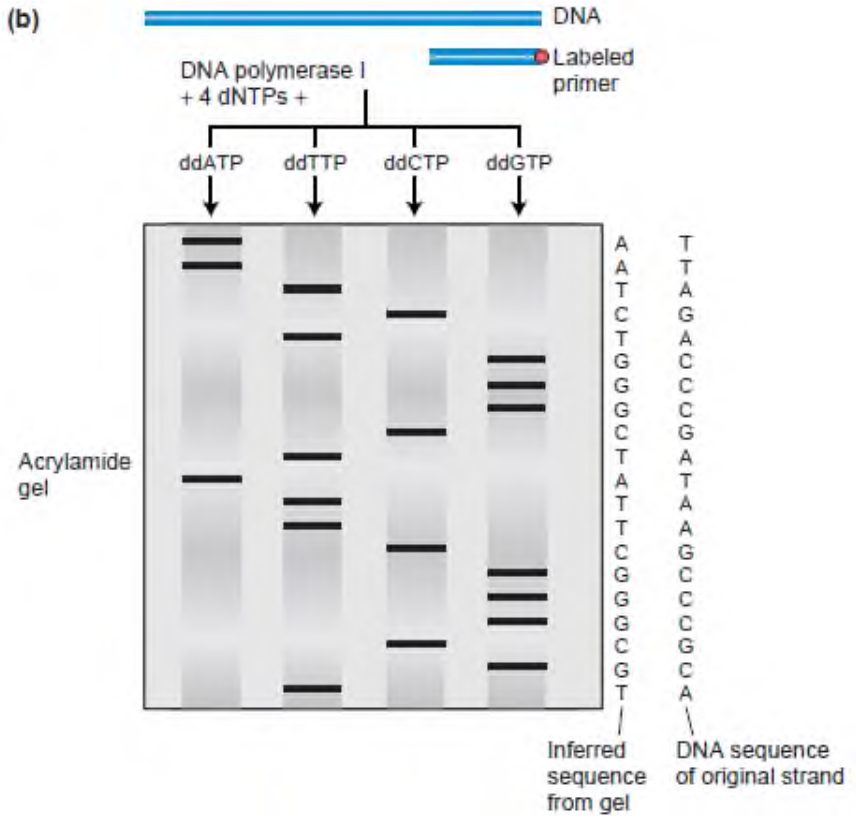


FIGURE 9.8. The dideoxy sequencing method (Sanger sequencing).

A labeled primer (designed from the flanking vector sequence) is used to initiate DNA synthesis. The addition of four different dideoxy nucleotides (ddATP is shown here) randomly arrests synthesis. (b) The resulting fragments are separated electrophoretically and subjected to autoradiography. The inferred sequence is shown at the right. [Parts a and b from J. D. Watson, M. Gilman, J. Witkowski, and M. Zoller, *Recombinant DNA*, 2d ed. Copyright 1992 by Scientific American Books; part c is from Loida Escote-Carlson.]

Figure 9.9 illustrates a readout of automated sequencing.

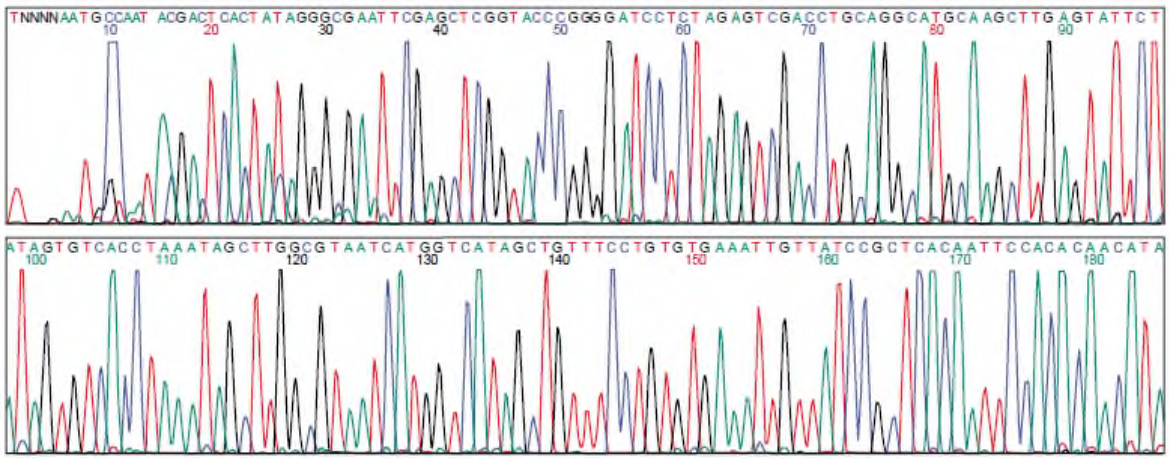


FIGURE 9.9. Printout from an automatic sequencer that uses fluorescent dyes. Each of the four colors represents a different base. N represents a base that cannot be assigned, because peaks are too low. Note that, if this were a gel as in Figure 13.5, each of these peaks would correspond to one of the dark bands on the gel; in other words, these colored peaks represent a different readout of the same sort of data as are produced on a sequencing gel. Each colored peak represents a different-size fragment of DNA, ending with a fluorescent base that was detected by the fluorescent scanner of the automated DNA sequencer; the four different colors represent the four bases of DNA.

Polymorphism is an interesting genetic phenomenon. Population geneticists have been surprised at how much polymorphism there is in natural populations of plants and animals generally. Furthermore, even though the genetics of polymorphisms is straightforward, there are very few polymorphisms for which there is satisfac tory explanation for the coexistence of the morphs. But polymorphism is rampant at every level of genetic analysis, even at the DNA level; indeed, polymorphisms observed at the DNA level have been invaluable as land marks to help geneticists find their way around the chromosomes of complex organisms.

PRENATAL DIAGNOSTICS AND PREVENTING BIRTH DEFECTS

It is believed that at least 1 in 16 newborns has a birth defect, either minor or serious, and the actual percentage may be even higher. It is estimated that only 20% of all birth defects are due to heredity. Those that are hereditary can sometimes be detected before birth. Amniocentesis allows the fetus to be tested for abnormalities of development; chorionic villi sampling allows the

embryo to be tested; and a new method has been developed for screening eggs to be used for in vitro fertilization (Fig. 9.10).

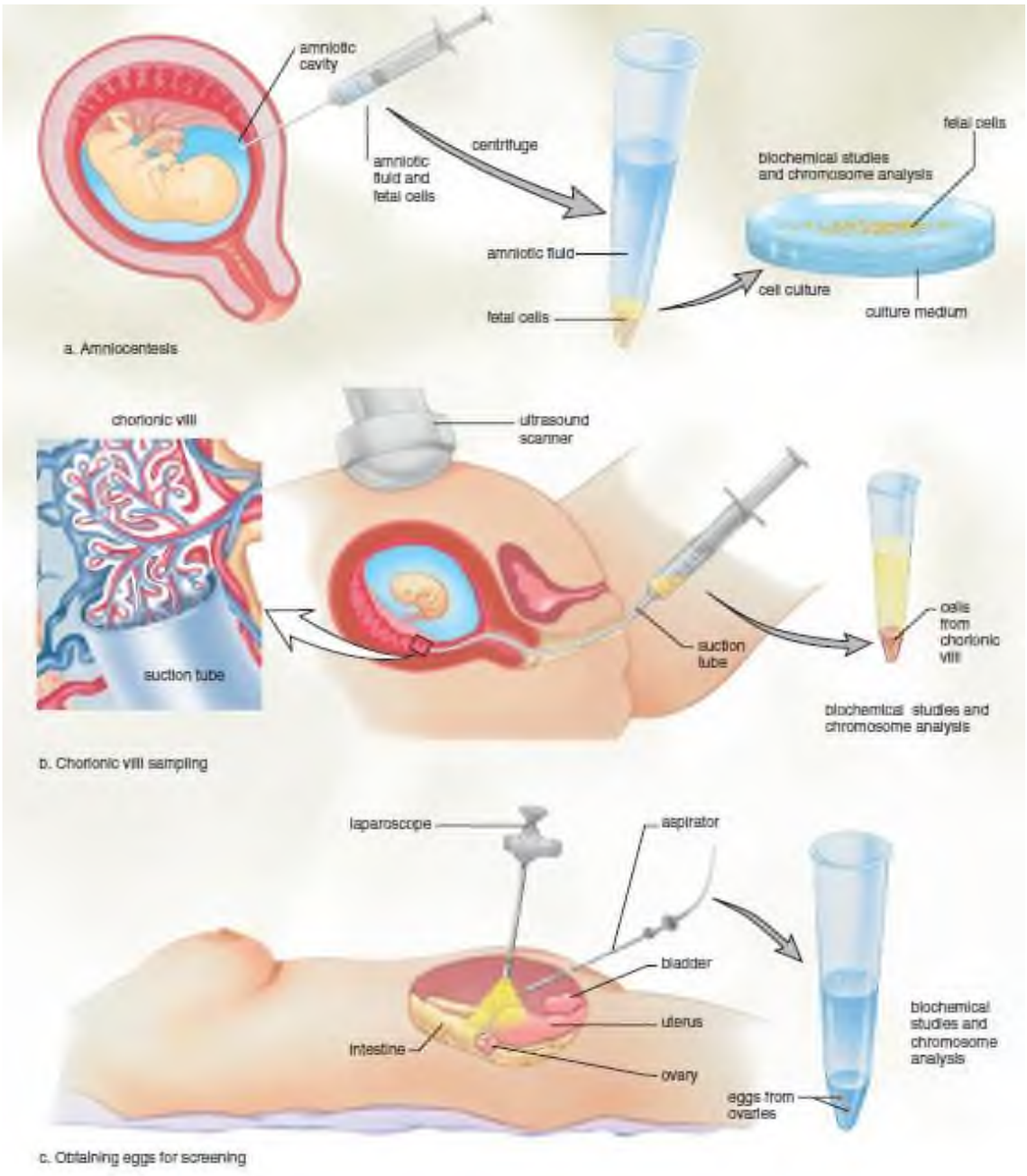


FIGURE 9.10. Three methods for genetic-defect testing before birth. (a) Amniocentesis, (b) Chorionic villi sampling, (c) Screening eggs.

Amniocentesis is usually performed from the 15th to the 17th week of pregnancy. A long needle is passed through the abdominal wall to withdraw a small amount of amniotic fluid, along with fetal cells. Since there are only a few cells in the amniotic fluid, testing may be delayed as long as four weeks

until cell culture produces enough cells for testing purposes. About 40 tests are available for different defects.

Chorionic villi sampling is usually performed from the 5th to the 12th week of pregnancy. The doctor inserts a long, thin tube through the vagina into the uterus. With the help of ultrasound, which gives a picture of the uterine contents, the tube is placed between the lining of the uterus and the chorion. Then a sampling of the chorionic villi cells is obtained by suction. Chromosome analysis and biochemical tests for genetic defects can be done immediately on these cells. However, chorionic villi sampling poses a greater threat to the unborn child than does amniocentesis.

Screening eggs for genetic defects is a new technique. Preovulatory eggs are removed by aspiration after a laparoscope (optical telescope) is inserted into the abdominal cavity through a small incision in the region of the navel. The first polar body is tested. If the woman is heterozygous *(Aa)* and the defective gene *(a)* is found in the polar body, the egg must have received the normal gene *(A)*. Normal eggs then undergo in vitro fertilization and are placed in the prepared uterus. At present, only one in ten attempts results in a birth, but it is known ahead of time that the child will be normal for the genetic traits tested.

Key Questions:

1. Main methods of human genetics.
2. Pedigree analysis.
3. Types of inheritance of human traits: autosomal-dominant, autosomal-recessive, X-linked, Y-linked. Features of family trees at different types of inheritance.
4. Studying of a sexual chromatin (Barr bodies analysis).
5. Cytogenetic method. Routine-staining of chromosomes, differential staining and FISH – method).
6. Population-genetical analysis.
7. The Hardy-Weinberg equilibrium.

8. Driving forces of evolution
9. Molecular-genetic method.
10. DNA Sequence Polymorphism.
11. Medical-genetic consultation.
12. Prenatal diagnostics.

Examples of Review questions:

NN	Questions	Right answers
1	WHICH METHOD IS USED TO DETECT TYPE OF INHERITANCE? 1) pedigree analisis 2) cytogenetical method 3) twin-method 4) none of the above	**1**
2	HOLTZINGER'S EQUATION IS USED TO ESTIMATE ___ . 1) the proportion of environment 2) the type of inheritance 3) the proportion of heredity	**3**
3	WHICH METHOD IS USED TO DETECT CHROMOSOMAL ABNORMALITIES? 1) pedigree analisis 2) cytogenetical method 3) twin-method 4) none of the above	**2**
4	WHICH METHOD IS USED TO DETECT THE FREQUENCY OF HETEROZYGOTE CARRIERS OF SOME HEREDITARY DISEASES WITHING POPULATION? 1) pedigree analysis 2) cytogenetical 3) biochemical 4) moleqular-genetics 5) population-genetic	**5**
5	NEWBORN GENETIC SCREENING FOR PKU ___ . 1) is very expensive 2) detects phenylketones in urine 3) has not led to the prevention of mental retardation 4) resulting from this disorder 5) uses bacterial growth to detect excess phenylalanine in blood	**2**

CHAPTER 10. DEVELOPMENT BIOLOGY. EMBRYONIC DEVELOPMENT. DEVELOPMENT AFTER BIRTH

FERTILIZATION

During fertilization, a single sperm enters the egg. The head of a sperm has a membrane-bounded acrosome filled with enzymes. When released, these enzymes digest a pathway for the sperm through the zona pellucida. After it binds to the plasma membrane of the egg, a sperm enters the egg. When the sperm nucleus fuses with the egg nucleus, fertilization is complete.

During fertilization, several sperm penetrate the corona radiata, several sperm attempt to penetrate the zona pellucida, and one sperm enters the egg and their nuclei fuse. The acrosome plays a role in allowing sperm to penetrate the zona pellucida. After a sperm head binds tightly to the zona pellucida, the acrosome releases digestive enzymes that forge a pathway for the sperm through the zona pellucida. When a sperm binds to the egg, their plasma membranes fuse, and this sperm (the head, the middle piece, and usually the tail) enters into the egg. Fusion of the sperm nucleus and the egg nucleus follows. When fertilization is complete, the egg is termed a zygote, and when the zygote begins dividing, it is called an **embryo.** The developing embryo travels very slowly down the oviduct to the uterus, where it implants itself in the endometrium (Fig. 10.1).

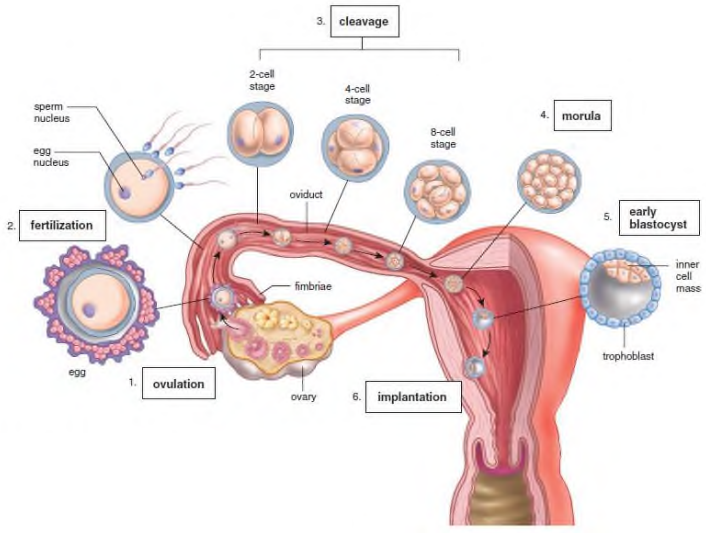


FIGURE 10.1. Human development before implantation. At ovulation, the egg leaves the ovary. Fertilization occurs in the upper one-third of the oviduct. The zygote is termed an embryo when cell division (cleavage) begins. The embryo implants itself in the endometrium.

EMBRYONIC DEVELOPMENT

Development Before Birth. This section considers the major processes and events that take place from the time of fertilization to the time of birth. Embryonic development includes these processes:

1. Cleavage Immediately after fertilization, the zygote begins to divide so that at first there are 2, then 4, 8, 16, and 32 cells, and so forth. Increase in size does not accompany these divisions.

2. Morphogenesis Morphogenesis refers to the shaping of the embryo and is first evident when certain cells are seen to move, or migrate, in relation to other cells. By these movements, the embryo begins to assume various shapes.

3. Differentiation Differentiation occurs as cells take on a specific structure and function. For example, nerve cells have long processes that conduct nerve impulses, and muscle cells contain contractile elements.

4. Growth During most of embryonic development, cell division is accompanied by an increase in the size of the daughter cells, and growth (in the true sense of the term) takes place.

The following processes can be observed in the early developmental stages, which humans share with all animals (Fig. 10.2).

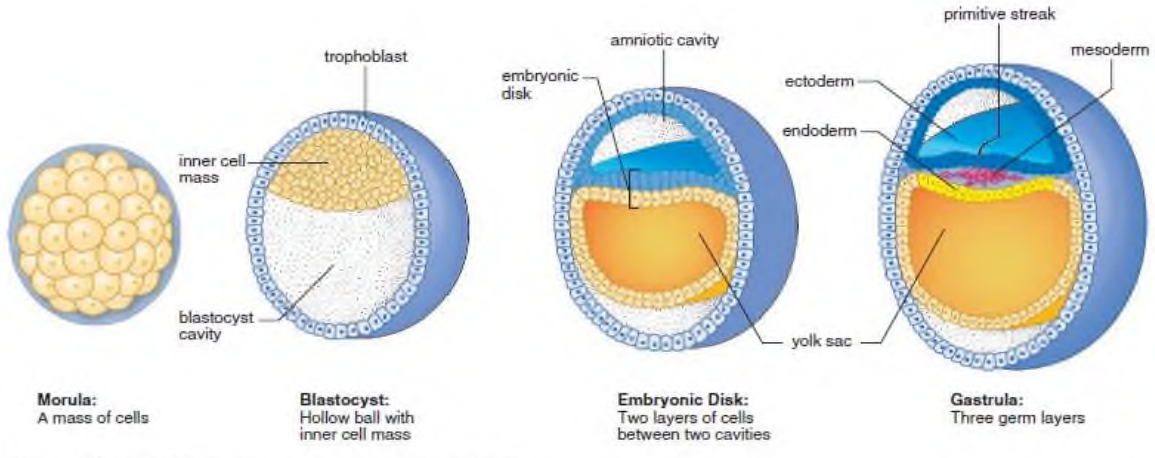


FIGURE 10.2. Early developmental stages in cross section. Cleavage results in the inner cell mass. Morphogenesis occurs as cells rearrange themselves, and differentiation is first exemplified by the formation of three different germ layers.

Morula. Cleavage is a process that occurs during the first stage of development. During cleavage, cell division without growth results in a mass of tiny cells. The cells are uniform in size because the cytoplasm has been equally divided among them. This solid mass of cells is called a morula, which means a bunch of berries.

Blastula. Morphogenesis begins as the cells of the morula form an empty ball of cells called the blastula. All animal blastulas have an empty cavity, but since the human blastula is called a **blastocyst,** the cavity is called the blastocyst cavity. In humans, an inner cell mass becomes an embryonic disk composed of two layers of cells. The lower layer of cells becomes the yolk sac. Acavity called the amniotic cavity occurs above the embryonic disk.

Gastrula. Gastrulation is a movement of cells that results in a gastrula, an embryo composed of three differentiated tissue layers. These tissue layers, called **ectoderm, mesoderm,** and **endoderm,** are known as the embryonic germ layers because they give rise to all the other tissues and organs of the body (Table 10.1).

Table 10.1

Embryonic Germ Layers and Organ Development

Ectoderm	Mesoderm	Endoderm
Skin epidermis, including hair, nails, and sweat glands	All muscles	Lining of digestive tract, trachea, bronchi, lungs, gallbladder, and urethra
Nervous system, including brain, spinal cord, ganglia, and nerves	Dermis of skin	Liver
Retina, lens, and cornea of eye	All connective tissue, including bone, cartilage, and blood	Pancreas
Inner ear	Blood vessels	Thyroid, parathyroid, and thymus glands
Lining of nose, mouth, and anus	Kidneys	Urinary bladder
Tooth enamel	Reproductive organs	

Neurula. Mesodermal cells that turn in at the primitive node become the notochord, a dorsal supporting rod. (In humans, the notochord is later replaced

by the vertebral column.) The nervous system develops from ectoderm located just above the notochord. A neural plate thickens into neural folds that fuse, forming a neural tube. The neural tube develops into the spinal cord and the brain. Neurulation involves **induction,** a process by which one tissue influences the development of another tissue. Experiments have shown that the nervous system does not form unless there is a notochord present. Today, investigators believe that induction explains the process of differentiation.

Induction requires direct contact or the production of a chemical by one tissue that most likely activates certain genes in the cells of the other tissue. These genes then direct how differentiation is to occur. Midline mesoderm not contributing to the formation of the notochord becomes two longitudinal masses of tissue. From these blocklike portions of mesoderm, called somites, the muscles of the body and the vertebrae of the spine develop. The coelom, an embryonic body cavity that forms at this time, is completely lined by mesoderm. In humans, the coelom becomes the thoracic and abdominal cavities. Figure 10.3 gives a generalized cross section of the embryo indicating the location of the three germ layers.

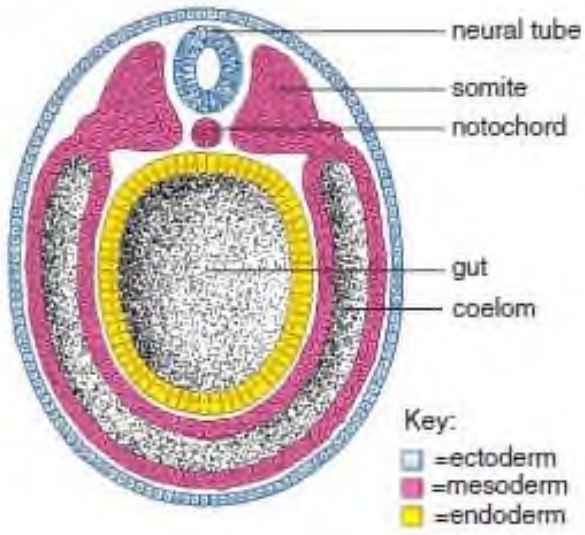


FIGURE 10.3. A generalized cross section of the embryo.

Embryonic development is the second week through the eighth week (Fig. 10.4), and **fetal development** is the third month through the ninth month of human development.

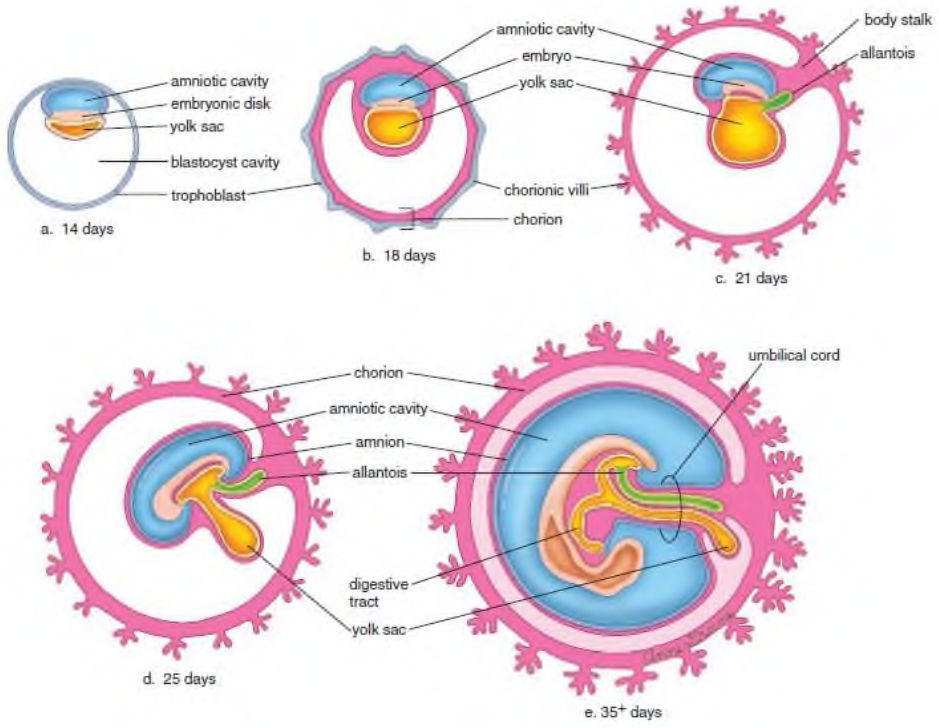


FIGURE 10.4. Embryonic development. (a) At first, no organs are present in the embryo, only tissues. The amniotic cavity is above the embryo, and the yolk sac is below. (b) The chorion is developing villi, so important to exchange between mother and child. (c) The allantois and yolk sac are two more extraembryonic membranes. (d) These extraembryonic membranes are positioned inside the body stalk as it becomes the umbilical cord. (e) At 35+ days, the embryo has a head region and a tail region. The umbilical cord takes blood vessels between the embryo and the chorion (placenta).

EXTRAEMBRYONIC MEMBRANES

One of the major events in early development is the establishment of the extraembryonic membranes (Fig. 10.5).

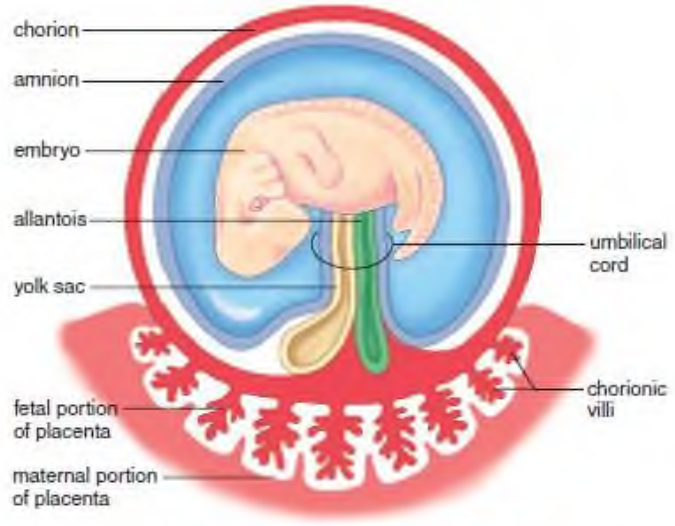


FIGURE 10.5. The extraembryonic membranes. The chorion and amnion surround the embryo. The two other extraembryonic membranes, the yolk sac and allantois, contribute to the umbilical cord.

The term **extraembryonic membranes** is apt because these membranes extend out beyond the embryo.

The **chorion,** the outer extraembryonic membrane.

One of the membranes, the **amnion,** provides a fluid environment for the developing embryo and fetus. It is a remarkable fact that all animals, even land-dwelling humans, develop in a watery medium. One authority describes the functions of amniotic fluid in this way: It prevents the walls of the uterus from cramping the fetus and allows it unhampered growth and movement. It encompasses the fetus with a fluid of constant temperature which is a marvelous insulator against cold and heat.

The **yolk sac** is another extraembryonic membrane. Yolk is a nutrient material utilized by other animal embryos—the yellow of a chick's egg is yolk. However, in humans, the yolk sac contains no yolk and is the first site of red blood cell formation. Part of this membrane becomes incorporated into the umbilical cord.

Another extraembryonic membrane, the **allantois,** contributes to the circulatory system: its blood vessels become umbilical blood vessels that transport fetal blood to and from the placenta. The chorion becomes part of the **placenta** (Fig. 10.6), where the fetal blood exchanges gases, nutrients, and wastes with the maternal blood.

Fetal Circulation. Fetal circulation involves the placenta, which begins forming once the embryo is implanted fully. The placenta has a fetal side contributed by the chorion and a maternal side consisting of uterine tissues. Notice in Figure 10.6 how projections called **chorionic villi** are immersed in maternal blood. The blood of the mother and the fetus never mix since exchange always takes place across the placenta.

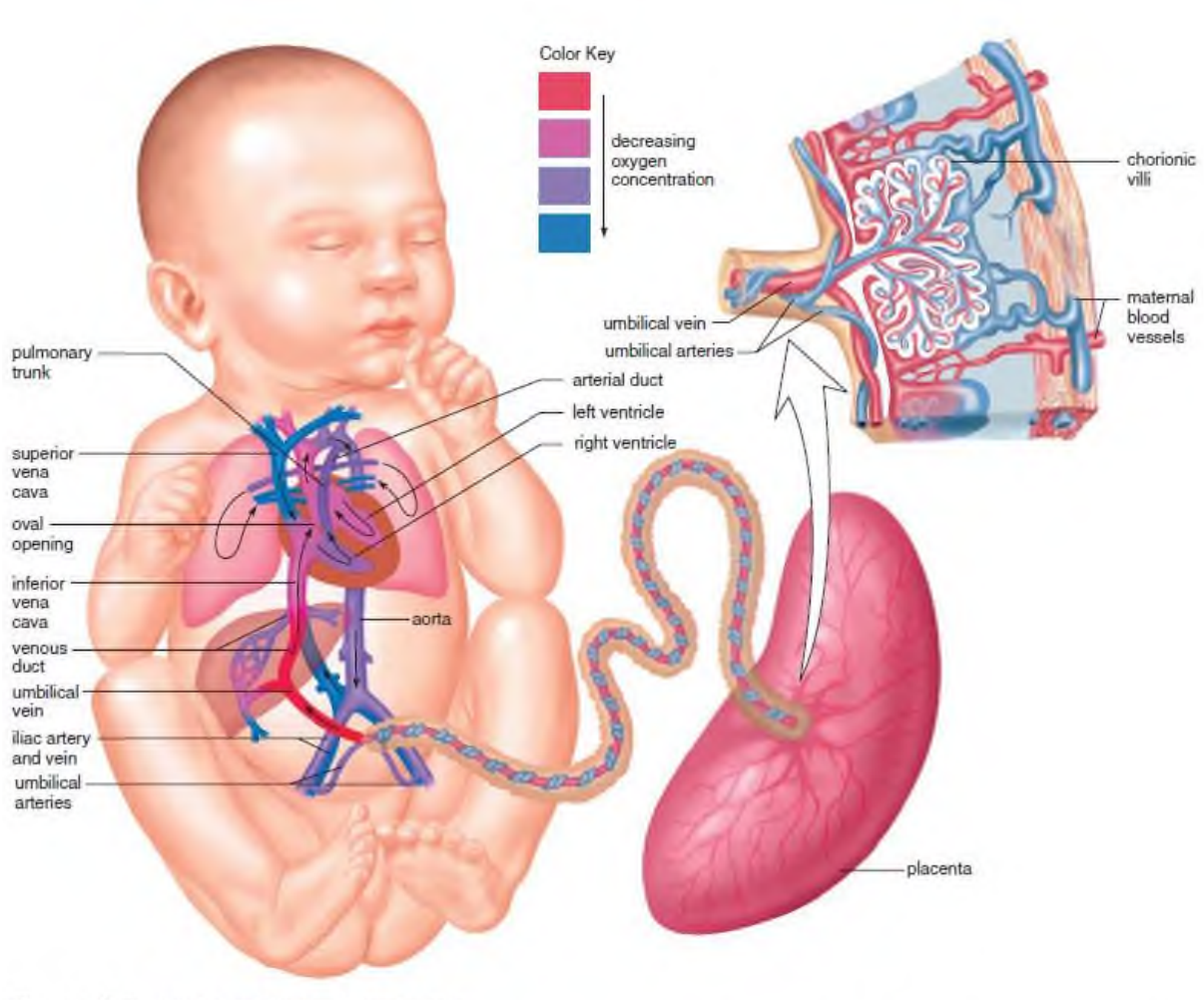


FIGURE 10.6. Fetal circulation and the placenta. The lungs are not functional in the fetus, and the blood passes directly from the right atrium to the left atrium or from the right ventricle to the aorta. The umbilical arteries take fetal blood to the placenta where exchange of molecules between fetal and maternal blood takes place across the walls of the chorionic villi. Oxygen and nutrient molecules diffuse into the fetal blood, and carbon dioxide and urea diffuse from the fetal blood. The umbilical vein returns blood from the placenta to the fetus.

Carbon dioxide and other wastes move from the fetal side to the maternal side, and nutrients and oxygen move from the maternal side to the fetal side of the placenta by diffusion. The **umbilical cord,** which stretches between the placenta and the fetus, is the lifeline of the fetus because it contains the umbilical arteries and veins. These vessels transport waste molecules (carbon dioxide and urea) to the placenta for disposal and take oxygen and nutrient molecules from the placenta to the rest of the fetal circulatory system. By the tenth week, the placenta is formed fully and begins to produce progesterone and estrogen.

These hormones have two effects due to their negative feedback effect on the mother's hypothalamus and anterior pituitary. They prevent any new follicles from maturing, and they maintain the endometrium. There is usually no menstruation during pregnancy. Harmful chemicals in the mother's blood can cross the placenta, and this is of particular concern during the embryonic period, when various structures are first forming. Each organ or part seems to have a sensitive period during which a substance can alter its normal function. The Health Focus on pages 370–71 concerns the origination of birth defects and explains ways to detect genetic defects before birth.

Fetal circulation shunts blood away from the lungs, toward and away from the placenta within the umbilical blood vessels located within the umbilical cord. Exchange of substances between fetal blood and maternal blood takes place at the placenta, which forms from the chorion and uterine tissue.

Birth. The uterus has contractions throughout pregnancy. At first, these are light, lasting about 20–30 seconds and occurring every 15–20 minutes. Near the end of pregnancy, the contractions may become stronger and more frequent so that a woman may think that she is in labor. However, the onset of true labor is marked by uterine contractions that occur regularly every 15–20 minutes and last for 40 seconds or more. A positive feedback mechanism can explain the onset and continuation of labor. Uterine contractions are induced by a stretching of the cervix, which also brings about the release of oxytocin from the posterior pituitary. Oxytocin stimulates the uterine muscles, both directly and through the action of prostaglandins. Uterine contractions push the fetus downward, and the cervix stretches even more. This cycle keeps repeating itself until birth occurs. Stages of birth shows Figure 10.7.

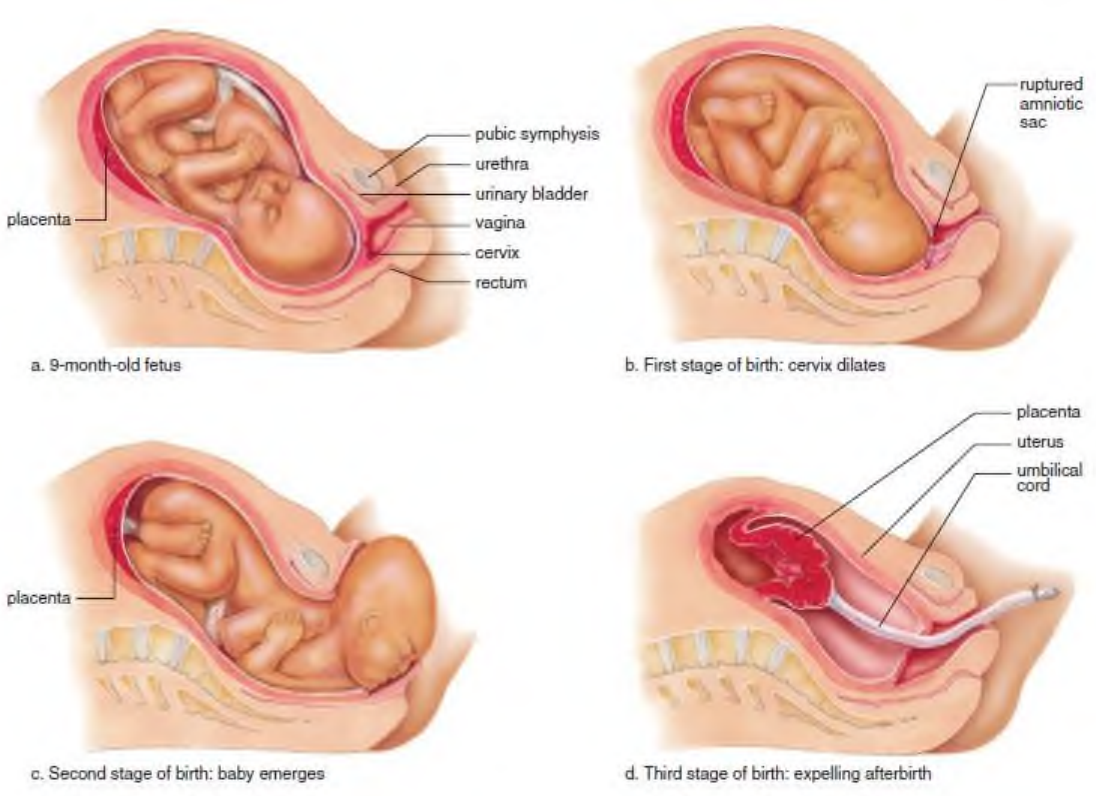


Figure 10.7. Three stages of parturition (birth). (a) Position of fetus just before birth begins. (b) Dilation of cervix. (c) Birth of baby. (d) Expulsion of afterbirth.

DEVELOPMENT AFTER BIRTH

Development does not cease once birth has occurred but continues throughout the stages of life: infancy, childhood, adolescence, and adulthood. **Aging** encompasses these progressive changes that contribute to an increased risk of infirmity, disease, and death. Today, there is great interest in **gerontology,** the study of aging, because there are now more older individuals in our society than ever before, and the number is expected to rise dramatically. In the next half-century, the number of people over age 75 will rise from the present 8 million to 14.5 million, and the number over age 80 will rise from 5 million to 12 million. The human life span is judged to be a maximum of 120–125 years. The present goal of gerontology is not necessarily to increase the life span, but to increase the health span, the number of years that an individual enjoys the full functions of all body parts and processes.

Theories of Aging. There are many theories about what causes aging. Three of these are considered here.

Genetic in Origin. Several lines of evidence indicate that aging has a genetic basis: (1) The number of times a cell divides is species-specific. The maximum number of times human cells divide is around 50. Perhaps as we grow older, more and more cells are unable to divide, and instead, they undergo degenerative changes and die. (2) Some cell lines may become nonfunctional long before the maximum number of divisions has occurred. Whenever DNA replicates, mutations can occur, and this can lead to the production of nonfunctional proteins. Eventually, the number of inadequately functioning cells can build up, which contributes to the aging process. (3) The children of long-lived parents tend to live longer than those of short-lived parents. Recent work suggests that when an animal produces fewer free radicals, it lives longer. Free radicals are unstable molecules that carry an extra electron. In order to stabilize themselves, free radicals donate an electron to another molecule like DNA or proteins (e.g., enzymes) or lipids found in plasma membranes. Eventually these molecules are unable to function, and the cell is destroyed. There are genes that code for antioxidant enzymes that detoxify free radicals. This research suggests that animals with particular forms of these genes—and therefore more efficient antioxidant enzymes—live longer.

Key Questions:

1. Main stages of ontogenesis.
2. Fertilization – the initial stage of development of a new organism.
3. Cleavage as process of formation of a multicellular germ.
4. Cleavage types.
5. Communication of a structure of egg with crushing type.
6. Gastrulation as process of formation of a multilayered germ.
7. Ways of a gastrulation.
8. Primary organogenesis.
9. Differentiation of germinal leaves.
10. Features of an early embryo development of the person.
11. Provisional bodies of chordates.
12. Post-embryonic ontogenesis at the person, his periodization.
13. Regularities of formation definitive of structures.

14. Puberty and reproduction.
15. Aging as natural stage of ontogenesis.
16. Regularities of aging.
17. Main hypotheses of aging.
18. Death as biological phenomenon, natural stage of ontogenesis.

Examples of Review questions:

NN	Questions	Right answers
1	THE SHAPING OF THE EMBRYO AND IS FIRST EVIDENT WHEN CERTAIN CELLS ARE SEEN TO MOVE, OR MIGRATE, IN RELATION TO OTHER CELLS. BY THESE MOVEMENTS, THE EMBRYO BEGINS TO ASSUME VARIOUS SHAPES. THIS PROCESS IS CALLED… . 1) cleavage 2) morphogenesis 3) differentiation 4) growth	**2**
2	THE STAGE OF EARLY DEVELOPMENT WHEN EMBRYO COMPOSED OF THREE DIFFERENTIATED TISSUE LAYERS IS CALLED … . 1) morula 2) blastula 3) gastrula 4) neurula	**3**
3	NERVOUS SYSTEM, INCLUDING BRAIN, IS DEVELOPED FROM … . 1) ectoderm 2) mesoderm 3) entoderm	**1**
4	A PROCESS BY WHICH ONE TISSUE INFLUENCES THE DEVELOPMENT OF ANOTHER TISSUE IS CALLED … . 1) morphogenesis 2) differentiation 3) growth 4) induction	**4**
5	ONE OF THE EXTRAEMBRYONIC MEMBRANES PROVIDES A FLUID ENVIRONMENT FOR THE DEVELOPING EMBRYO AND FETUS. IT IS … . 1) chorion 2) amnion 3) yolk sac 4) allantois 5) placenta	**2**

CHAPTER 11. ECOLOGY AND BIOSPHERE

THE NATURE OF ECOSYSTEMS

When the earth was formed, the outer crust was covered by ocean and barren land. Over time, aquatic organisms filled the seas, and terrestrial organisms colonized the land so that eventually there were many complex communities of living things. A **community** is made up of all the **populations** in a particular area, such as a forest or pond. When we study a community, we are considering only the populations of organisms that make up that community, but when we study an ecosystem, we are concerned with the community and its physical environment. Table 11.1 defines these important terms in the study of ecology and shows how they relate to the biosphere as a whole.

Table 11.1

Ecological Terms

Term	Definition
Ecology	Study of the interactions of organisms with each other and with the physical environment
Population	All the members of the same species that inhabit a particular area
Community	All the populations that are found in a particular area
Ecosystem	A community and its physical environment, including both nonliving (abiotic) and living (biotic) components
Biosphere	All the communities on earth whose members exist in air and water and on land

The process of succession from either bare rock or disturbed land results in a climax community.

The dynamic nature of communities is shown by their changing nature when succession occurs. Climax communities are threatened by disturbances.

Biotic Components of an Ecosystem. An ecosystem is a community of organisms plus the physical environment. Each population in an ecosystem has a habitat and a niche. Some populations are producers and some are consumers. Producers are autotrophs that produce their own organic food. Consumers are

heterotrophs that take in organic food. Consumers may be herbivores, carnivores, omnivores, or decomposers (Fig. 11.1).

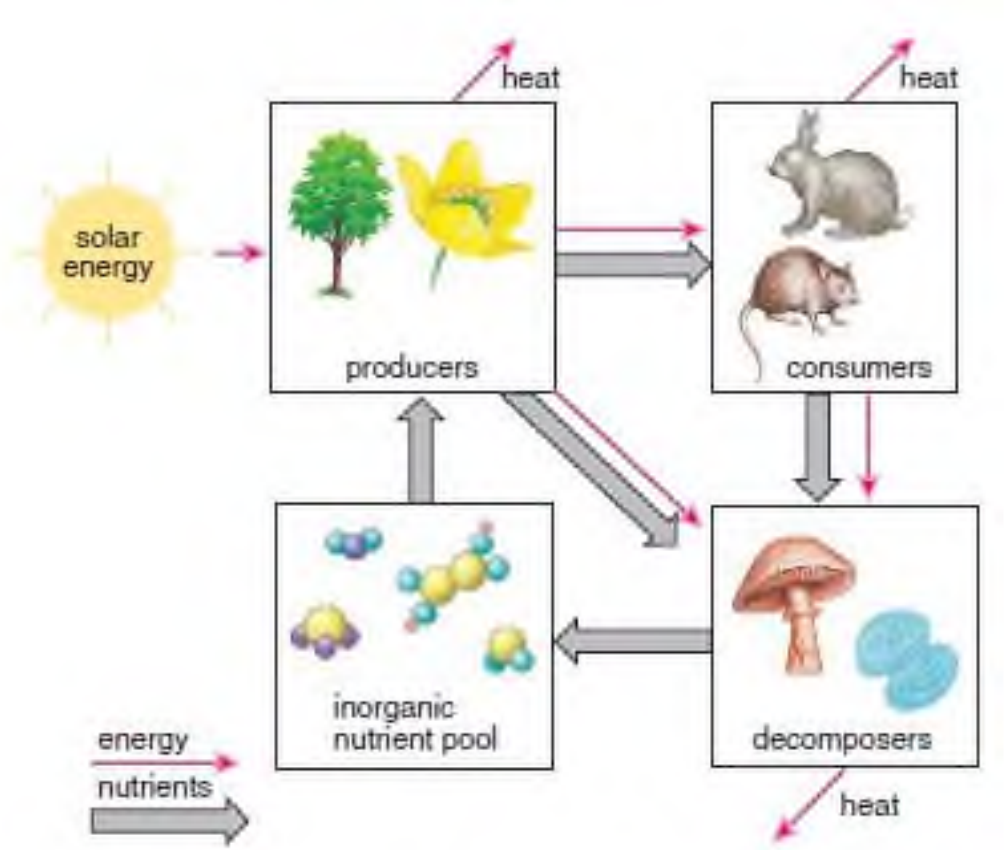


FIGURE 11.1 Nature of an ecosystem. Chemicals cycle, but energy flows through an ecosystem. As energy transformations repeatedly occur, all the energy derived from the sun eventually dissipates as heat.

An ecosystem possesses both nonliving (abiotic) and living (biotic) components. The abiotic components include resources, such as sunlight and inorganic nutrients, and conditions, such as type of soil, water availability, prevailing temperature, and amount of wind. The biotic components of an ecosystem are the various populations of organisms. Each population in an ecosystem has a habitat and a niche. The **habitat** of an organism is its place of residence—that is, where it can be found, such as under a log or at the bottom of a pond. The **niche** of an organism is its profession or total role in the community. Adescription of an organism's niche includes its interactions with the physical environment and with the other organisms in the community. Woodpeckers feed on parasitic grubs from a tree that also provides a habitat for the woodpecker's young.

The populations in an ecosystem are often categorized as producers or consumers. **Producers** produce organic nutrients and are autotrophic organisms. **Autotrophic organisms** are able to carry on photosynthesis and make organic nutrients for themselves (and indirectly for the other populations as well). In terrestrial ecosystems, the producers are predominantly green plants, while in freshwater and marine ecosystems, the dominant producers are various species of algae. **Consumers** consume organic nutrients and are heterotrophic organisms. **Heterotrophic organisms** feed on producers directly or on organisms that have fed on producers. It is possible to distinguish four types of consumers, depending on their food source:

- **herbivores** feed directly on green plants; they are termed primary consumers;
- **carnivores** feed only on other animals and are thus secondary or tertiary consumers;
- **omnivores** feed on both plants and animals;
- **decomposers** (bacteria and fungi) feed on and thereby break down **detritus**, the remains of plants and animals following their death;
- therefore, a caterpillar feeding on a leaf is a herbivore; a green heron feeding on a fish is a carnivore; and a human being eating both leafy green vegetables and beef is an omnivore. The bacteria and fungi of decay are important detritus feeders, but so are other soil organisms, such as earthworms and various small arthropods. The importance of the latter can be demonstrated by placing leaf litter in bags with mesh too fine to allow soil animals to enter; the leaf litter does not decompose well, even though bacteria and fungi are present. Small soil organisms precondition the detritus so that bacteria and fungi can break it down to inorganic matter that producers can use again.

Food Webs and Trophic Levels. The principles we have been discussing can now be applied to an actual ecosystem—a forest. The feeding relationships in an ecosystem are interconnected as shown in Figure 11.2.

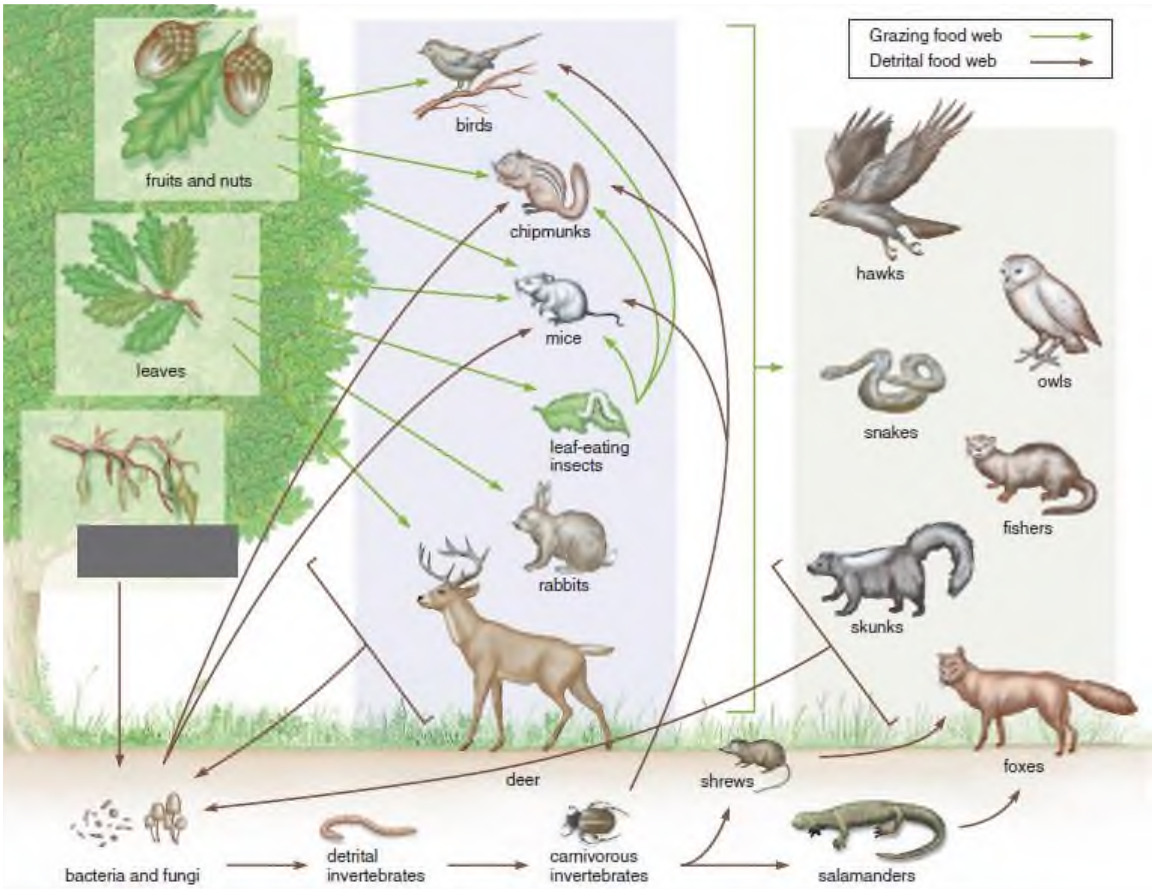


FIGURE 11.2. Forest food webs. Two linked food webs are shown for a forest ecosystem: a grazing food web and a detrital food web.

Therefore, they create a **food web;** the upper part of Figure 11.2 is a **grazing food web** because it begins with trees, such as the oak trees depicted. A **detrital food web** begins with detritus, partially decayed matter in the soil. Insects in the form of caterpillars feed on leaves, while mice, rabbits, and deer feed on leaf tissue at or near the ground. Birds, chipmunks, and mice feed on fruits and nuts, but they are in fact omnivores because they also feed on caterpillars. These herbivores and omnivores all provide food for a number of different carnivores.

In the detrital food web, detritus, along with the bacteria and fungi of decay, is food for larger decomposers. Because some of these, like shrews and salamanders, become food for aboveground carnivores, the detrital and the grazing food webs are joined. We naturally tend to think that aboveground plants like trees are the largest storage form of organic matter and energy, but this is not necessarily the case. In this particular forest, the organic matter lying

on the forest floor and mixed into the soil contains much more energy than does the leaf matter of living trees. The soil contains over twice as much energy as the leaves of the trees. Therefore, more energy in a forest may be funneling through the detrital food web than through the grazing food web.

Trophic Levels. You can see that Figure 11.2 would allow us to link organisms one to another in a straight line manner, according to who eats whom. Such diagrams are called **food chains** (Fig. 11.3).

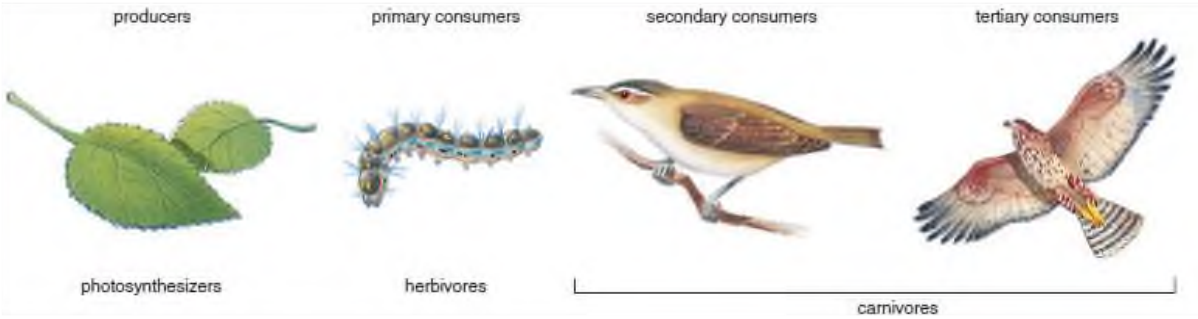


FIGURE 13.3. Food chain.

For example, in the grazing food web we can find this **grazing food chain:**

leaves — caterpillars — tree birds — hawks.

And in the detrital food web we could find this **detrital food chain:**

dead organic matter — soil microbes — earthworms, etc.

A trophic level is composed of all the organisms that feed at a particular link in a food chain. In the grazing food web the trees are primary producers (first trophic level), the first series of animals are primary consumers (second trophic level), and the next group of animals are secondary consumers (third trophic level).

Ecological Pyramids. Ecologists portray the energy relationships between trophic levels in the form of ***ecological pyramids,*** diagrams whose building blocks designate the various trophic levels (Fig. 11.4). (We need to keep in mind that sometimes organisms don’t fit into only one trophic level. For exam-

ple, chipmunks feed on fruits and nuts, but they also feed on leaf-eating insects.)

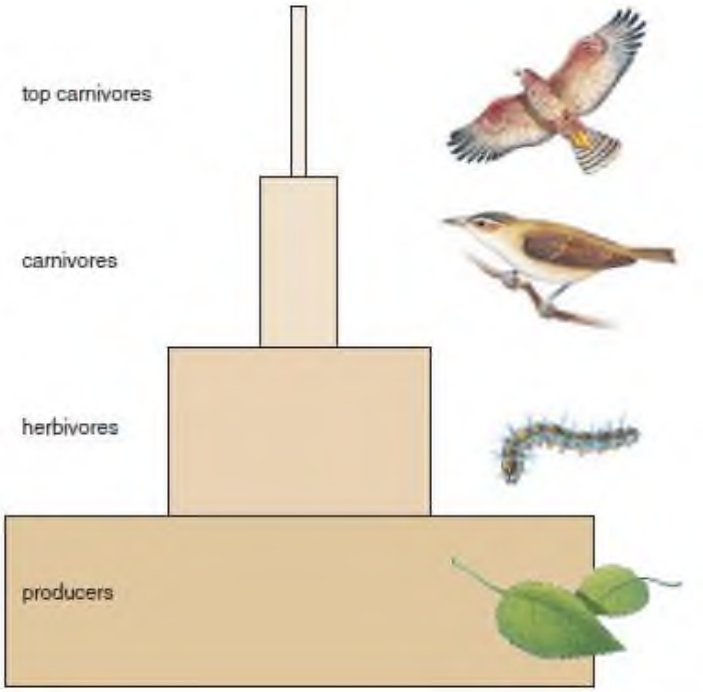


FIGURE 11.4. Ecological pyramid. An ecological pyramid shows the relationship between either the number of organisms, the biomass, or the amount of energy theoretically available at each trophic level.

A pyramid of numbers simply tells how many organisms there are at each trophic level. It's easy to see that a pyramid of numbers could be completely misleading. For example, in Figure 13.2 you would expect each tree to contain numerous caterpillars; therefore, there would be more herbivores than autotrophs! The problem, of course, has to do with size. Autotrophs can be tiny, like microscopic algae, or they can be big, like beech trees; similarly, herbivores can be as small as caterpillars or as large as elephants.

Pyramids of biomass eliminate size as a factor since **biomass** is the number of organisms multiplied by their weight. You would certainly expect the biomass of the producers to be greater than the biomass of the herbivores, and that of the herbivores to be greater than that of the carnivores. In aquatic ecosystems such as lakes and open

seas, where algae are the only producers, the herbivores may have a greater biomass than the producers when you take their measurements. Why? The reason is that over time, the algae reproduce rapidly, but they are also consumed at a

high rate. Pyramids like this one, that have more herbivores than producers, are called inverted pyramids:

Energy Flow and Chemical Cycling. Energy flows through an ecosystem. Producers transform solar energy into food for themselves and all consumers. As herbivores feed on plants (or algae), and carnivores feed on herbivores, some energy is converted to heat. Feces, urine, and dead bodies become food for decomposers. Eventually, all the solar energy that enters an ecosystem is converted to heat, and thus ecosystems require a continual supply of solar energy. Inorganic nutrients are not lost from the biosphere as is energy. They recycle within and between ecosystems. Decomposers return some proportion of inorganic nutrients to autotrophs, and other portions are imported or exported between ecosystems in global cycles. Ecosystems contain food webs, and a diagram of a food web shows how the various organisms are connected by eating relationships. In a grazing food web, food chains begin with a producer. In a detrital food web, food chains begin with detritus. The two food webs are joined when the same consumer is a link in both a grazing and detrital food chain. A trophic level is all the organisms that feed at a particular link in a food chain. Ecological pyramids show trophic levels stacked one on top of the other like building blocks. Generally they show that biomass and energy content decrease from one trophic level to the next. Most pyramids pertain to grazing food webs and largely ignore the detrital food web portion of an ecosystem.

Global Biogeochemical Cycles. Biogeochemical cycles contain reservoirs, which are components of ecosystems, such as fossil fuels, sediments, and rocks, that contain elements available on a limited basis to living things. Pools are components of ecosystems, such as the atmosphere, soil, and water, which are ready sources of nutrients for living things. In the water cycle, evaporation over the ocean is not compensated for by rainfall. Evaporation from terrestrial ecosystems includes transpiration from plants. Rainfall over land results in bodies of fresh water plus groundwater, including aquifers. Eventually, all water returns to the oceans (Fig. 11.5).

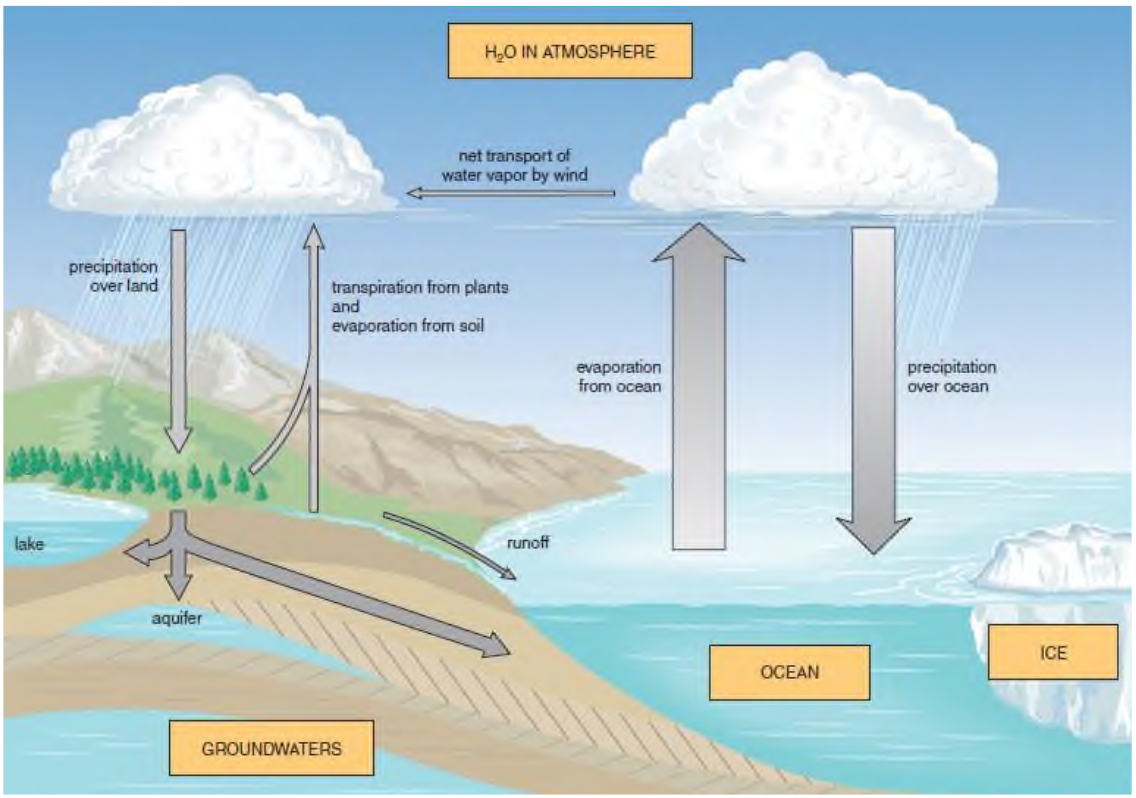


FIGURE 11.5. The water (hydrologic) cycle. In the water cycle, fresh water evaporates from the bodies of water. Precipitation on land enters the ground, surface waters, or aquifers. Water ultimately returns to the ocean—even the quantity that remains in aquifers for some time.

In the carbon cycle, organisms add as much carbon dioxide to the atmosphere as they remove (Fig. 11.6).

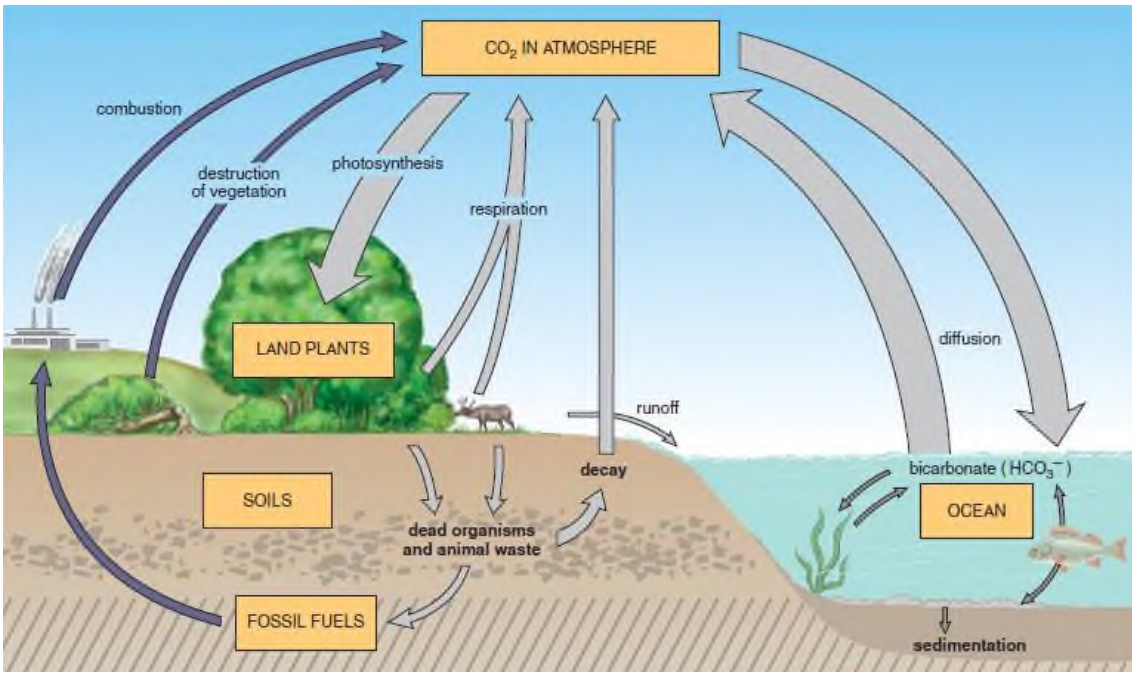


FIGURE 11.6. The carbon cycle. Purple arrows represent human activities; gray arrows represent natural events.

Shells in ocean sediments, organic compounds in living and dead organisms, and fossil fuels are reservoirs for carbon. Human activities such as burning fossil fuels and trees are adding carbon dioxide to the atmosphere. Like the panes of a greenhouse, carbon dioxide and other gases allow the sun's rays to pass through but impede the release of infrared wavelengths. It is predicted that a buildup of these "greenhouse gases" will lead to a global warming. The effects of global warming could be a rise in sea level and a change in climate patterns, with disastrous effects.

In the nitrogen cycle, the biotic community, which includes several types of bacteria, keeps recycling nitrogen back to the producers (Fig. 11.7).

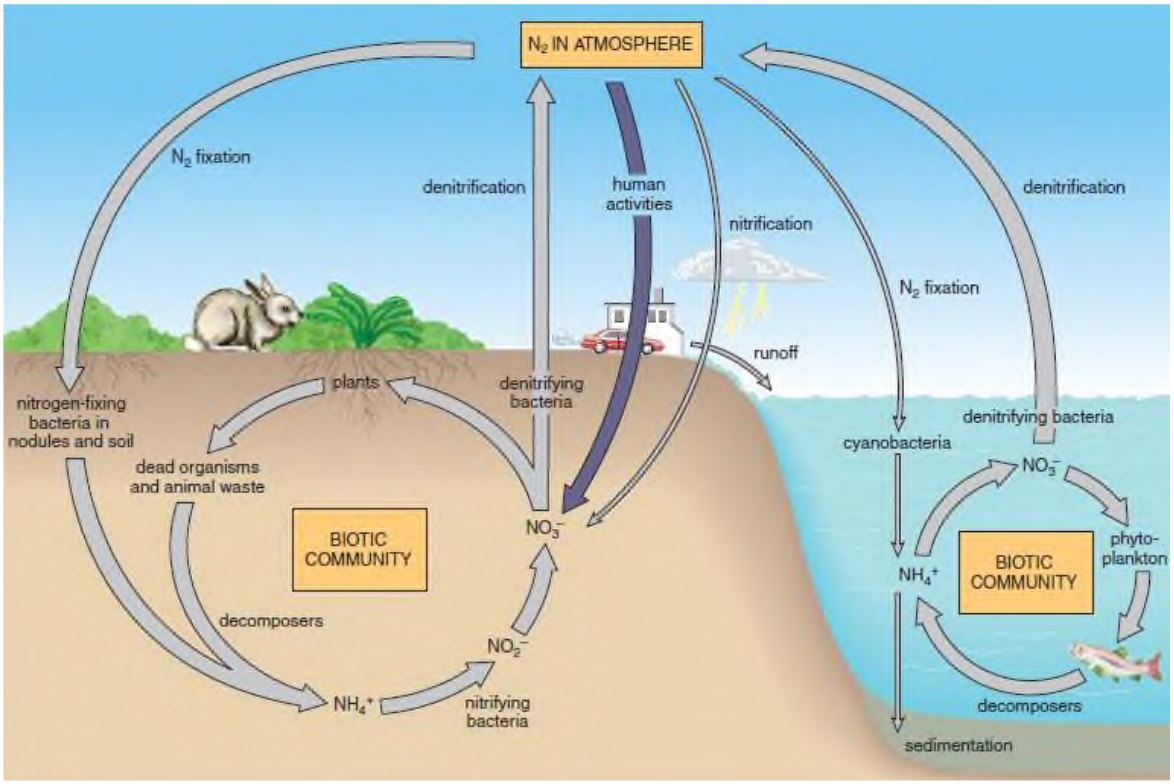


FIGURE 11.7. The nitrogen cycle.

Certain bacteria in water, soil, and root nodules, can fix atmospheric nitrogen. Other bacteria return nitrogen to the atmosphere. Human activities convert atmospheric nitrogen to fertilizer, which is broken down by soil bacteria; humans also burn fossil fuels. In this way, a large quantity of nitrogen oxide (NOx) and sulfur dioxide (SO2) is added to atmosphere where it reacts with water vapor to form acids that contribute to acid deposition. Acid deposition

can kill lakes and forests and corrode marble, metal, and stonework. Nitrogen oxides and hydrocarbons (HC) react to form smog, which contains ozone and PAN (peroxyacetylnitrate). These oxidants are harmful to animal and plant life.

In the phosphorus cycle, the biotic community recycles phosphorus back to the producers, and only limited quantities are made available by the weathering of rocks (Fig. 11.8).

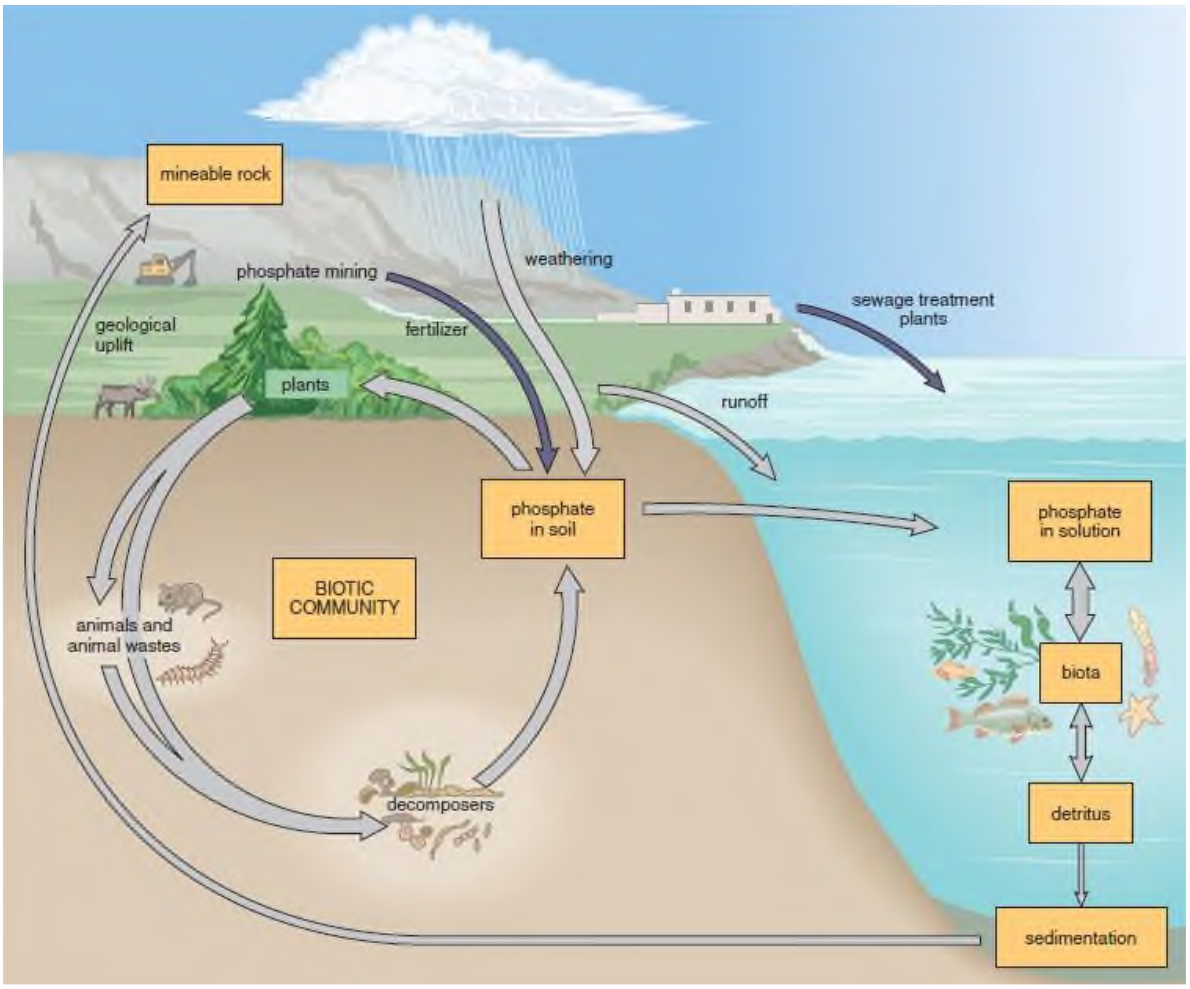


FIGURE 11.8. The phosphorus cycle.

Phosphates are mined for fertilizer production; when phosphates and nitrates enter lakes and ponds, overenrichment occurs. Many kinds of wastes enter the rivers and then flow to the oceans, which have now become degraded from added pollutants.

Ozone Shield Depletion. The earth's atmosphere is divided into layers. The troposphere envelops us as we go about our day-to-day lives. When ozone (O3) is present in the troposphere (called ground-level ozone), it is considered a pollutant because it adversely affects a plant's ability to grow and our ability to

breathe oxygen (O_2). In the stratosphere, some 50 kilometers above the earth, ozone forms the **ozone shield,** a layer of ozone that absorbs much of the ultraviolet (UV) rays of the sun so that fewer rays strike the earth.

UV radiation causes mutations that can lead to skin cancer and can make the lens of the eye develop cataracts. It also is believed to adversely affect the immune system and our ability to resist infectious diseases. Crop and tree growth is impaired, and UV radiation also kills off small plants (phytoplankton) and tiny shrimplike animals (krill) that sustain oceanic life. Without an adequate ozone shield, our health and food sources are threatened.

Ozone shield depletion in recent years is, therefore, of serious concern. It became apparent in the 1980s that depletion of ozone had occurred worldwide and that there was a severe depletion (40–50% of the ozone) above the Antarctic every spring. A vortex of cold wind (a whirlpool in the atmosphere) circles the pole during the winter months, creating ice crystals in which chemical reactions occur that break down ozone.

Severe depletions of the ozone layer are commonly called "ozone holes." Detection devices now tell us that the ozone hole above the Antarctic is about the size of the United States and growing (Fig. 11.9).

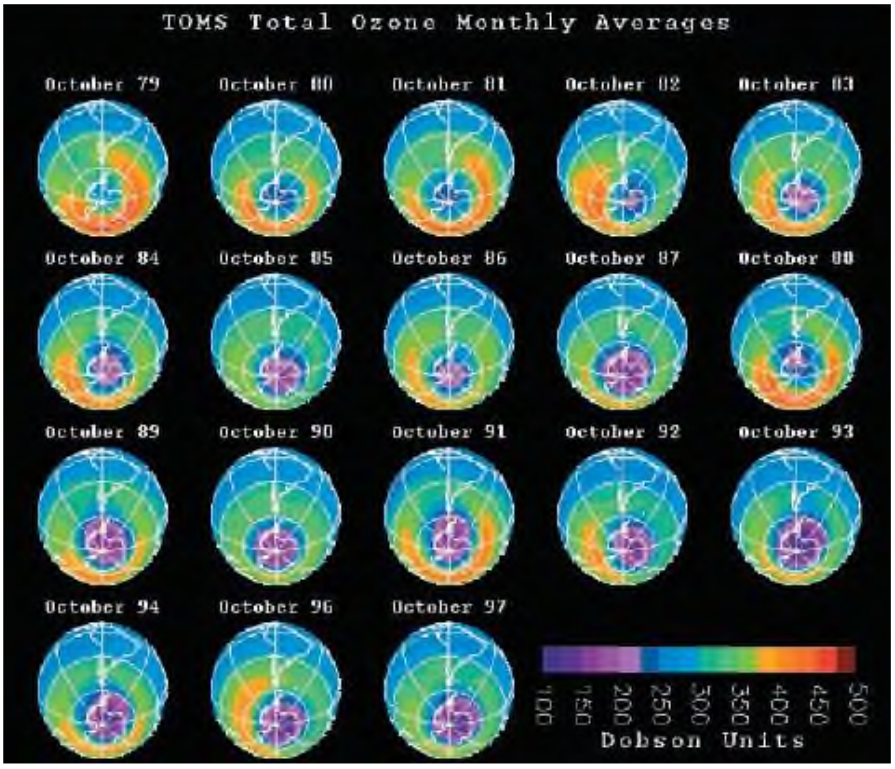


FIGURE 11.9. Ozone shield depletion.

These satellite observations show that the amount of ozone over the South Pole between October 1979 and October 1997 fell by more than 50%. Green represents an average amount of ozone, blue less, and purple still less. Yellow, orange, and red represent above-average amounts of ozone. Of even greater concern, an ozone hole has now appeared above the Arctic as well, and ozone holes could also occur within northern and southern latitudes, where many people live. Whether or not these holes develop depends on prevailing winds, weather conditions, and the type of particles in the atmosphere. A United Nations Environment Program report predicts a 26% rise in cataracts and nonmelanoma skin cancers for every 10% drop in the ozone level. A 26% increase translates into 1.75 million additional cases of cataracts and 300,000 more skin cancers every year, worldwide.

The cause of ozone depletion can be traced to the release of chlorine atoms (Cl) into the stratosphere. Chlorine atoms combine with ozone and strip away the oxygen atoms, one by one. One atom of chlorine can destroy up to 100,000 molecules of ozone before settling to the earth's surface as chloride years later. These chlorine atoms come from the breakdown of **chlorofluorocarbons** (CFCs), chemicals much in use by humans. The best known CFC is Freon, a coolant found in refrigerators and air conditioners. CFCs are also used as cleaning agents and foaming agents during the production of styrofoam used in coffee cups, egg artons, insulation, and paddings. Formerly, CFCs were used as propellants in spray cans, but this application is now banned in the United States and several European countries. Most of the countries of the world have stopped using CFCs. The United States halted production in 1995. Computer projections suggest that an 85% reduction in CFC emissions is needed to stabilize CFC levels in the atmosphere. Otherwise, they keep on increasing. There are many available CFC substitutes that will not release chlorine atoms (nor bromine atoms) to harm the ozone shield.

Key Questions:

1. What is succession, and how does it result in a climax community?
2. Distinguish between the abiotic and biotic components of an ecosystem. What are the aspects of niche for a plant? An animal?
3. Distinguish between autotrophs and heterotrophs, and describe four different types of heterotrophs found in natural ecosystems. Explain the terms producer and consumer.
4. Tell why energy must flow but chemicals can cycle in an ecosystem.
5. Describe two types of food webs and two types of food chains typically found in terrestrial ecosystems. Which of these typically moves more energy through an ecosystem?
6. What is a trophic level? An ecological pyramid?
7. Give examples of reservoirs and exchange pools in biogeochemical cycles.
8. Draw one diagram to illustrate the water cycle and another to represent the carbon cycle.
9. How and why is the global climate expected to change, and what are the predicted consequences of this change?
10. Draw a diagram of the nitrogen cycle. What types of bacteria are involved in this cycle?
11. What causes acid deposition, and what are its effects? How does photochemical smog develop, and what is a thermal inversion?
12. Draw a diagram of the phosphorus cycle.
13. What are several ways in which fresh water and marine water can be polluted? What is biological magnification?

Examples of Review questions:

NN	Questions	Right answers
1	HETEROTROPH THAT FEEDS ON PLANT MATERIAL IS CALLED AS … . 1) producer 2) consumer 3) decomposer 4) 4. herbivore	**4**
2	AUTOTROPH THAT MANUFACTURES ORGANIC NUTRIENTS IS CALLED AS … . 1) producer 2) consumer 3) decomposer 4) 4. herbivore	**1**
3	HETEROTROPH THAT BREAKS DOWN DETRITUS AS A SOURCE OF NUTRIENTS IS CALLED AS … . 1) producer 2) consumer 3) decomposer 4) 4. herbivore	**3**
4	ANY TYPE OF HETEROTROPH THAT FEEDS ON PLANT MATERIAL OR ON OTHER ANIMALS IS CALLED AS … . 1) producer 2) consumer 3) decomposer 4) 4 herbivore	**2**
5	DURING THE PROCESS OF DENITRIFICATION, NITRATE IS CONVERTED TO … . 1) ammonium 2) nitrogen gas 3) nitrates 4) nitrite	**2**

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