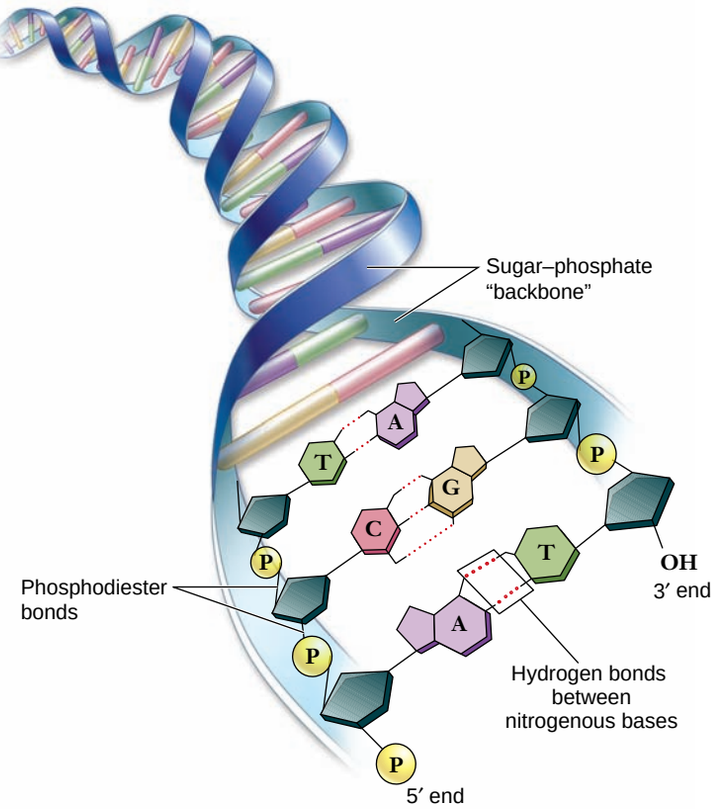


Figure 3.16 The structure of DNA. DNA consists of two polynucleotide chains running in opposite directions wrapped about a single helical axis. Hydrogen bond formation (dashed lines) between the nitrogenous bases, called base-pairing, causes the two chains of DNA to bind to each other and form a double helix.

A sentence written in English consists of a combination of the 26 different letters of the alphabet in a certain order; the code of a DNA molecule consists of different combinations of the four types of nucleotides in specific sequences, such as CGCTTACG. The information encoded in DNA is used in the everyday functioning of the organism and is passed on to the organism's descendants.

DNA molecules in organisms exist not as single chains folded into complex shapes, like proteins, but rather as two chains wrapped about each other in a long linear molecule in eukaryotes, and a circular molecule in most prokaryotes. The two strands of a DNA polymer wind around each other like the outside and inside rails of a spiral staircase. Such a spiral shape is called a helix, and a helix composed of two chains is called a **double helix.** Each step of DNA's helical staircase is composed of a base-pair. The pair consists of a base in one chain attracted by hydrogen bonds to a base opposite it on the other chain (figure 3.16).

The base-pairing rules are rigid: Adenine can pair only with thymine (in DNA) or with uracil (in RNA), and cytosine can pair only with guanine. The bases that participate in base-pairing are said to be **complementary** to each other. Additional details of the structure of DNA and how it interacts with RNA in the production of proteins are presented in chapters 14 and 15.

RNA is a transcript of a DNA strand

RNA is similar to DNA, but with two major chemical differences. First, RNA molecules contain ribose sugars, in which the C-2 is bonded to a hydroxyl group. (In DNA, this hydroxyl group is replaced by a hydrogen atom.) Second, RNA molecules use uracil in place of thymine. Uracil has the same structure as thymine, except that one of its carbons lacks a methyl ($—CH_3$) group.

Transcribing the DNA message into a chemically different molecule such as RNA allows the cell to distinguish between the original information-storage molecule and the transcript. DNA molecules are always double-stranded (except for a few single-stranded DNA viruses), whereas the RNA molecules transcribed from DNA are typically single-stranded (figure 3.17). These differences allow DNA to store hereditary information and RNA to use this information to specify the sequence of amino acids in proteins.

Other nucleotides are vital components of energy reactions

In addition to serving as subunits of DNA and RNA, nucleotide bases play other critical roles in the life of a cell. For example, adenine is a key component of the molecule **adenosine**

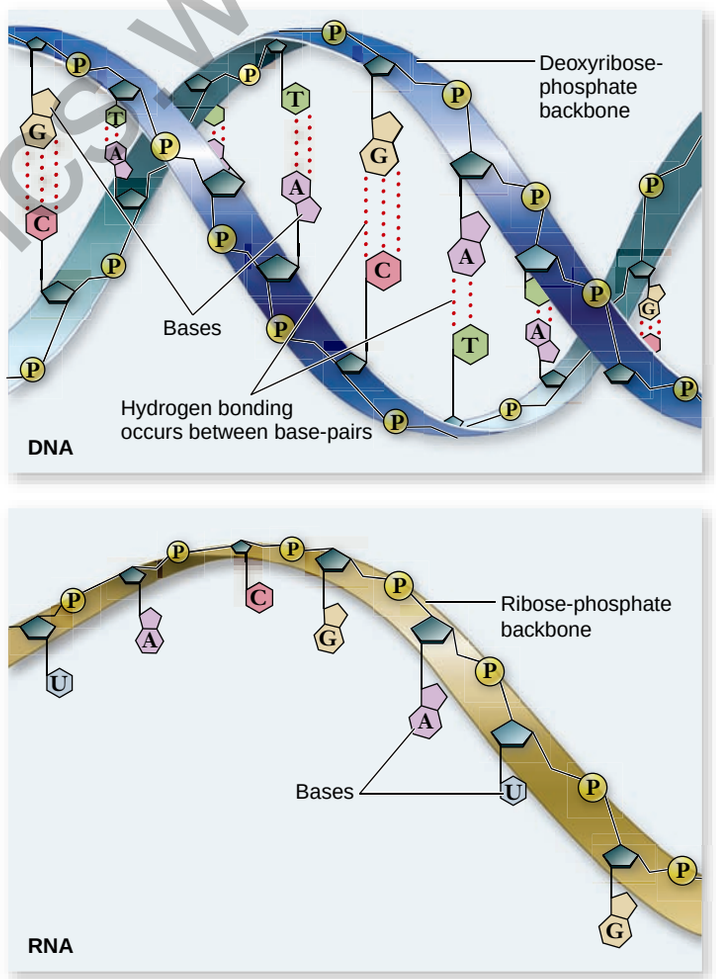


Figure 3.17 DNA versus RNA. DNA forms a double helix, uses deoxyribose as the sugar in its sugar–phosphate backbone, and uses thymine among its nitrogenous bases. RNA is usually single-stranded, uses ribose as the sugar in its sugar–phosphate backbone, and uses uracil in place of thymine.

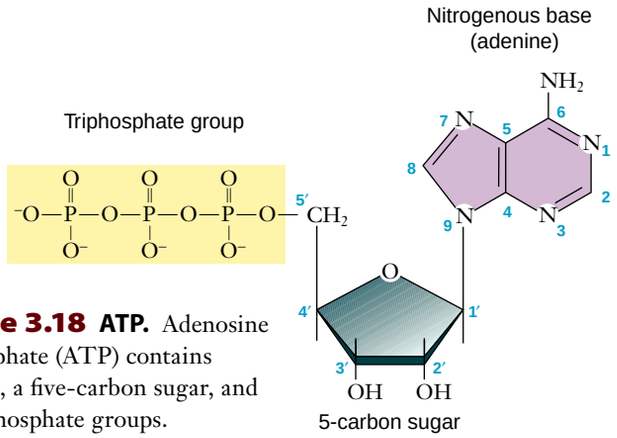


Figure 3.18 ATP. Adenosine triphosphate (ATP) contains adenine, a five-carbon sugar, and three phosphate groups.

triphosphate (**ATP;** figure 3.18)—the energy currency of the cell. Cells use ATP as energy in a variety of transactions, the way we use money in society. ATP is used to drive energetically unfavorable chemical reactions, to power transport across membranes, and to power the movement of cells.

Two other important nucleotide-containing molecules are **nicotinamide adenine dinucleotide (NAD$^+$)** and **flavin adenine dinucleotide (FAD).** These molecules function as electron carriers in a variety of cellular processes. You will see the action of these molecules in detail when we discuss photosynthesis and respiration (chapters 7–8).

Learning Outcomes Review 3.3

A nucleic acid is a polymer composed of alternating phosphate and five-carbon sugar groups with a nitrogenous base protruding from each sugar. In DNA, this sugar is deoxyribose. In RNA, the sugar is ribose. RNA also contains the base uracil instead of thymine. DNA is a double-stranded helix that stores hereditary information as a specific sequence of nucleotide bases. RNA is a single-stranded molecule consisting of a transcript of a DNA sequence that directs protein synthesis.

- ***If an RNA molecule is copied from a DNA strand, what is the relationship between the sequence of bases in RNA and each DNA strand?***

3.4 *Proteins: Molecules with Diverse Structures and Functions*

Learning Outcomes

1. ***Describe the possible levels of protein structure.***
2. ***Explain how motifs and domains contribute to protein structure.***
3. ***Understand the relationship between amino acid sequence and their three-dimensional structure.***

Proteins are the most diverse group of biological macromolecules, both chemically and functionally. Because proteins have so many different functions in cells we could not begin to list them all. We can, however, group these functions into the following seven categories. This list is a summary only, however; details are covered in later chapters.

1. **Enzyme catalysis.** Enzymes are biological catalysts that facilitate specific chemical reactions. Because of this property, the appearance of enzymes was one of the most important events in the evolution of life. Enzymes are three-dimensional globular proteins that fit snugly around the molecules they act on. This fit facilitates chemical reactions by stressing particular chemical bonds.
2. **Defense.** Other globular proteins use their shapes to "recognize" foreign microbes and cancer cells. These cell-surface receptors form the core of the body's endocrine and immune systems.
3. **Transport.** A variety of globular proteins transport small molecules and ions. The transport protein hemoglobin, for example, transports oxygen in the blood. Membrane transport proteins help move ions and molecules across the membrane.
4. **Support.** Protein fibers play structural roles. These fibers include keratin in hair, fibrin in blood clots, and collagen. The last one, collagen, forms the matrix of skin, ligaments, tendons, and bones and is the most abundant protein in a vertebrate body.
5. **Motion.** Muscles contract through the sliding motion of two kinds of protein filaments: actin and myosin. Contractile proteins also play key roles in the cell's cytoskeleton and in moving materials within cells.
6. **Regulation.** Small proteins called hormones serve as intercellular messengers in animals. Proteins also play many regulatory roles within the cell—turning on and shutting off genes during development, for example. In addition, proteins receive information, acting as cell-surface receptors.
7. **Storage.** Calcium and iron are stored in the body by binding as ions to storage proteins.

Table 3.2 summarizes these functions and includes examples of the proteins that carry them out in the human body.

Proteins are polymers of amino acids

Proteins are linear polymers made with 20 different amino acids. **Amino acids,** as their name suggests, contain an amino group (—NH_2) and an acidic carboxyl group (—COOH). The specific order of amino acids determines the protein's structure and function. Many scientists believe amino acids were among the first molecules formed on the early Earth. It seems highly likely that the oceans that existed early in the history of the Earth contained a wide variety of amino acids.

Amino acid structure

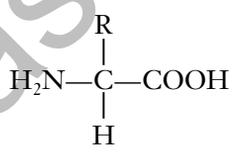
The generalized structure of an amino acid is shown here as amino and carboxyl groups bonded to a central carbon atom, with an additional hydrogen and a functional side group

TABLE 3.2 The Many Functions of Protein

Function	Class of Protein	Examples	Examples of Use
Enzyme catalysis	Enzymes	Glycosidases	Cleave polysaccharides
		Proteases	Break down proteins
		Polymerases	Synthesize nucleic acids
		Kinases	Phosphorylate sugars and proteins
Defense	Immunoglobulins	Antibodies	Mark foreign proteins for elimination
	Toxins	Snake venom	Blocks nerve function
	Cell-surface antigens	MHC* proteins	"Self" recognition
Transport	Circulating transporters	Hemoglobin	Carries O_2 and CO_2 in blood
		Myoglobin	Carries O_2 and CO_2 in muscle
		Cytochromes	Electron transport
	Membrane transporters	Sodium–potassium pump	Excitable membranes
		Proton pump	Chemiosmosis
		Glucose transporter	Transports glucose into cells
Support	Fibers	Collagen	Forms cartilage
		Keratin	Forms hair, nails
		Fibrin	Forms blood clots
Motion	Muscle	Actin	Contraction of muscle fibers
		Myosin	Contraction of muscle fibers
Regulation	Osmotic proteins	Serum albumin	Maintains osmotic concentration of blood
	Gene regulators	*lac* Repressor	Regulates transcription
	Hormones	Insulin	Controls blood glucose levels
		Vasopressin	Increases water retention by kidneys
		Oxytocin	Regulates uterine contractions and milk production
Storage	Ion-binding	Ferritin	Stores iron, especially in spleen
		Casein	Stores ions in milk
		Calmodulin	Binds calcium ions

*MHC, major histocompatibility complex.

indicated by R. These components completely fill the bonds of the central carbon:



The unique character of each amino acid is determined by the nature of the R group. Notice that unless the R group is an H atom, as in glycine, amino acids are chiral and can exist as two enantiomeric forms: D or L. In living systems, only the L-amino acids are found in proteins, and D-amino acids are rare.

The R group also determines the chemistry of amino acids. Serine, in which the R group is —CH_2OH, is a polar molecule. Alanine, which has —CH_3 as its R group, is nonpolar.

The 20 common amino acids are grouped into five chemical classes, based on their R group:

1. Nonpolar amino acids, such as leucine, often have R groups that contain —CH_2 or —CH_3.
2. Polar uncharged amino acids, such as threonine, have R groups that contain oxygen (or —OH).
3. Charged amino acids, such as glutamic acid, have R groups that contain acids or bases that can ionize.
4. Aromatic amino acids, such as phenylalanine, have R groups that contain an organic (carbon) ring with alternating single and double bonds. These are also nonpolar.
5. Amino acids that have special functions have unique properties. Some examples are methionine, which is often the first amino acid in a chain of amino acids; proline, which causes kinks in chains; and cysteine, which links chains together.

Each amino acid affects the shape of a protein differently, depending on the chemical nature of its side group. For example, portions of a protein chain with numerous nonpolar amino acids tend to fold into the interior of the protein by hydrophobic exclusion.

Peptide bonds

In addition to its R group, each amino acid, when ionized, has a positive amino (NH_3^+) group at one end and a negative carboxyl (COO^-) group at the other. The amino and carboxyl groups on a pair of amino acids can undergo a dehydration reaction to form a covalent bond. The covalent bond that links two amino acids is called a **peptide bond** (figure 3.19). The two amino acids linked by such a bond are not free to rotate around the N—C linkage because the peptide bond has a partial double-bond character. This is different from the N—C and C—C bonds to the central carbon of the amino acid. This lack of rotation about the peptide bond is one factor that determines the structural character of the coils and other regular shapes formed by chains of amino acids.

A protein is composed of one or more long unbranched chains. Each chain is called a **polypeptide** and is composed of amino acids linked by peptide bonds. The terms *protein* and *polypeptide* tend to be used loosely and may be confusing. For proteins that include only a single polypeptide chain, the two terms are synonymous.

The pioneering work of Frederick Sanger in the early 1950s provided the evidence that each kind of protein has a specific amino acid sequence. Using chemical methods to remove successive amino acids and then identify them, Sanger succeeded in determining the amino acid sequence of insulin. In so doing he demonstrated clearly that this protein had a defined sequence, which was the same for all insulin molecules in the solution. Although many different amino acids occur in nature, only 20 commonly occur in proteins. Figure 3.20 illustrates these 20 amino acids and their side groups.

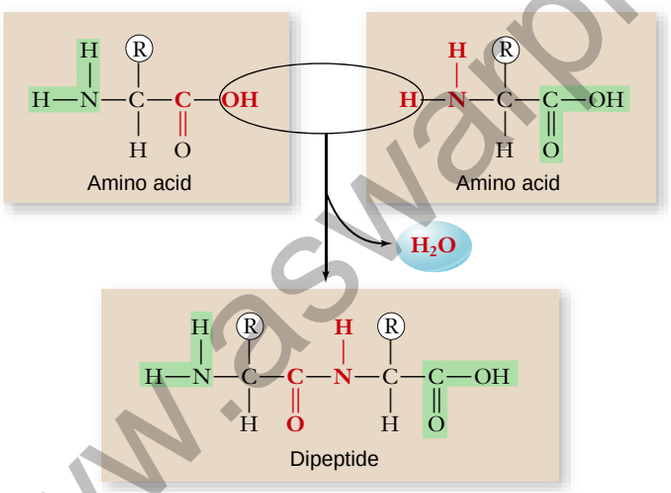


Figure 3.19 The peptide bond. A peptide bond forms when the amino end of one amino acid joins to the carboxyl end of another. Reacting amino and carboxyl groups are shown in red and nonreacting groups are highlighted in green. Notice that the resulting dipeptide still has an amino end and a carboxyl end. Because of the partial double-bond nature of peptide bonds, the resulting peptide chain cannot rotate freely around these bonds.

Proteins have levels of structure

The shape of a protein determines its function. One way to study the shape of something as small as a protein is to look at it with very short wavelength energy—in other words, with X-rays. X-rays can be passed through a crystal of protein to produce a diffraction pattern. This pattern can then be analyzed by a painstaking procedure that allows the investigator to build up a three-dimensional picture of the position of each atom. The first protein to be analyzed in this way was myoglobin, and the related protein hemoglobin was analyzed soon thereafter.

As more and more proteins were studied, a general principle became evident: In every protein studied, essentially all the internal amino acids are nonpolar ones—amino acids such as leucine, valine, and phenylalanine. Water's tendency to hydrophobically exclude nonpolar molecules literally shoves the nonpolar portions of the amino acid chain into the protein's interior (figure 3.21). This tendency forces the nonpolar amino acids into close contact with one another, leaving little empty space inside. Polar and charged amino acids are restricted to the surface of the protein, except for the few that play key functional roles.

The structure of proteins is usually discussed in terms of a hierarchy of four levels: *primary*, *secondary*, *tertiary*, and *quaternary* (figure 3.22). We will examine this view and then integrate it with a more modern approach arising from our increasing knowledge of protein structure.

Primary structure: amino acid sequence

The **primary structure** of a protein is its amino acid sequence. Because the R groups that distinguish the amino acids play no role in the peptide backbone of proteins, a protein can consist of any sequence of amino acids. Thus, because any of 20 different amino acids might appear at any position, a protein containing 100 amino acids could form any of 20^{100} different amino acid sequences (that's the same as 10^{130}, or 1 followed by 130 zeros—more than the number of atoms known in the universe). This important property of proteins permits great diversity.

Consider the protein hemoglobin, the protein your blood uses to transport oxygen. Hemoglobin is composed of two α-globin peptide chains and two β-globin peptide chains. The α-globin chains differ from the β-globin ones in the sequence of amino acids. Furthermore, any alteration in the normal sequence of either of the types of globin proteins, even by a single amino acid, can have drastic effects on how the protein functions.

Secondary structure: Hydrogen bonding patterns

The amino acid side groups are not the only portions of proteins that form hydrogen bonds. The peptide groups of the main chain can also do so. These hydrogen bonds can be with water or with other peptide groups. If the peptide groups formed too many hydrogen bonds with water, the proteins would tend to behave like a random coil and wouldn't produce

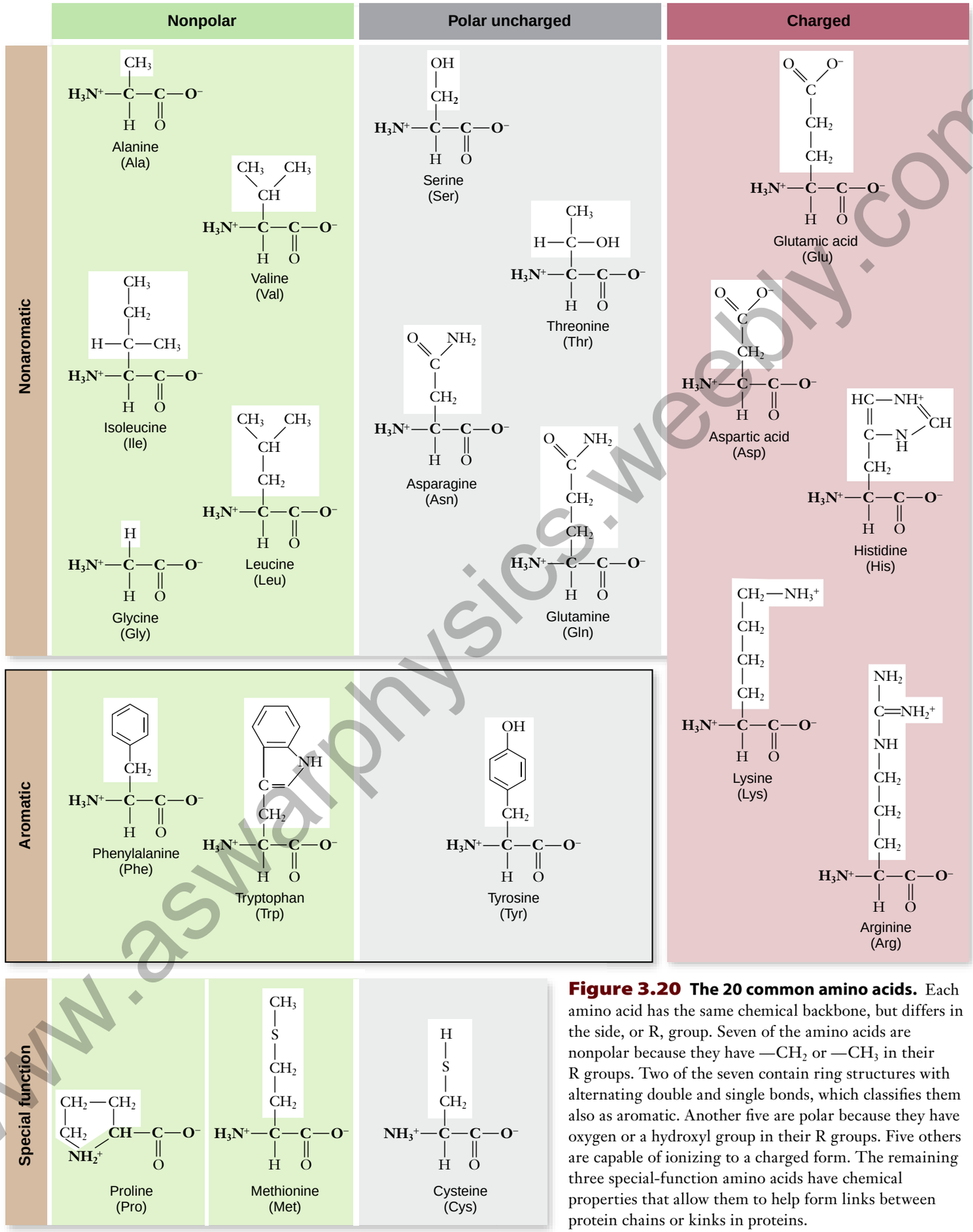
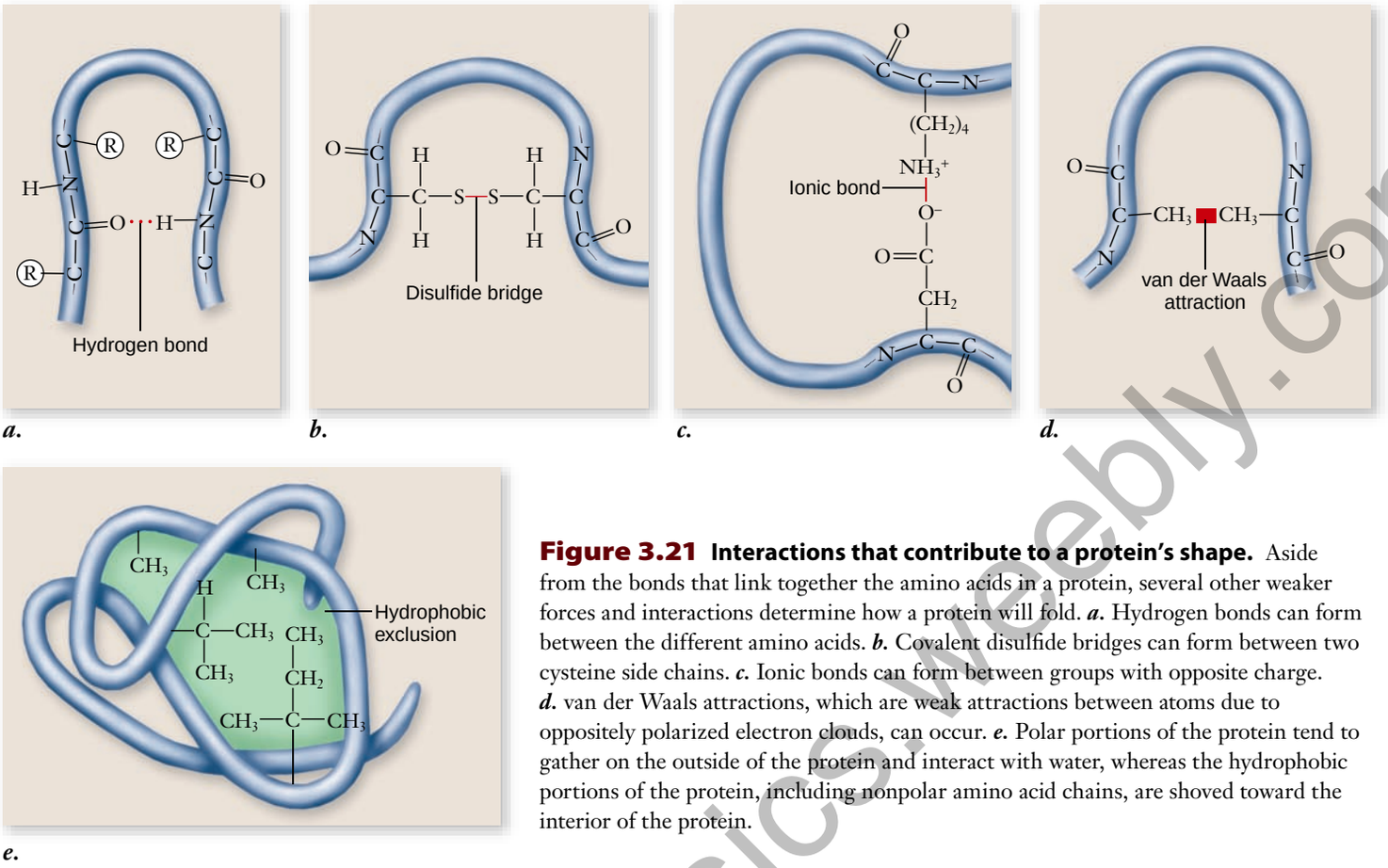


Figure 3.20 The 20 common amino acids. Each amino acid has the same chemical backbone, but differs in the side, or R, group. Seven of the amino acids are nonpolar because they have —CH_2 or —CH_3 in their R groups. Two of the seven contain ring structures with alternating double and single bonds, which classifies them also as aromatic. Another five are polar because they have oxygen or a hydroxyl group in their R groups. Five others are capable of ionizing to a charged form. The remaining three special-function amino acids have chemical properties that allow them to help form links between protein chains or kinks in proteins.



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

Figure 3.21 Interactions that contribute to a protein's shape. Aside from the bonds that link together the amino acids in a protein, several other weaker forces and interactions determine how a protein will fold. ***a.*** Hydrogen bonds can form between the different amino acids. ***b.*** Covalent disulfide bridges can form between two cysteine side chains. ***c.*** Ionic bonds can form between groups with opposite charge. ***d.*** van der Waals attractions, which are weak attractions between atoms due to oppositely polarized electron clouds, can occur. ***e.*** Polar portions of the protein tend to gather on the outside of the protein and interact with water, whereas the hydrophobic portions of the protein, including nonpolar amino acid chains, are shoved toward the interior of the protein.

the kinds of globular structures that are common in proteins. Linus Pauling suggested that the peptide groups could interact with one another if the peptide was coiled into a spiral that he called the **α helix.** We now call this sort of regular interaction of groups in the peptide backbone **secondary structure.** Another form of secondary structure can occur between regions of peptide aligned next to each other to form a planar structure called a **β sheet.** These can be either parallel or antiparallel depending on whether the adjacent sections of peptide are oriented in the same direction, or opposite direction.

These two kinds of secondary structure create regions of the protein that are cylindrical (α helices) and planar (β sheets). A protein's final structure can include regions of each type of secondary structure. For example, DNA-binding proteins usually have regions of α helix that can lay across DNA and interact directly with the bases of DNA. Porin proteins that form holes in membranes are composed of β sheets arranged to form a pore in the membrane. Finally in hemoglobin, the α- and β-globin peptide chains that make up the final molecule each have characteristic regions of secondary structure.

Tertiary structure: Folds and links

The final folded shape of a globular protein is called its **tertiary structure.** This tertiary structure contains regions that have secondary structure and determines how these are further arranged in space to produce the overall structure. A protein is initially driven into its tertiary structure by hydrophobic exclusion from water. Ionic bonds between oppositely charged R groups bring regions into close proximity, and disulfide bonds (covalent links between two cysteine R groups) lock particular regions together. The final folding of a protein is determined by its primary structure—the chemical nature of its side groups (see figure 3.21 and 3.22). Many small proteins can be fully unfolded ("denatured") and will spontaneously refold into their characteristic shape. Other larger proteins tend to associate together and form insoluble clumps when denatured, such as the film that can form when you heat milk for hot chocolate.

The tertiary structure is stabilized by a number of forces including hydrogen bonding between R groups of different amino acids, electrostatic attraction between R groups with opposite charge (also called salt bridges), hydrophobic exclusion of nonpolar R groups, and covalent bonds in the form of disulfides. The stability of a protein, once it has folded into its tertiary shape, is strongly influenced by how well its interior fits together. When two nonpolar chains in the interior are very close together, they experience a form of molecular attraction called van der Waals forces. Individually quite weak, these forces can add up to a strong attraction when many of them come into play, like the combined strength of hundreds of hooks and loops on a strip of Velcro. These forces are effective only over short distances, however. No "holes" or cavities exist in the interior of proteins. The variety of different nonpolar amino acids, with a different-sized R group with its own distinctive shape, allows nonpolar chains to fit very precisely within the protein interior.

It is therefore not surprising that changing a single amino acid can drastically alter the structure, and thus the function of a

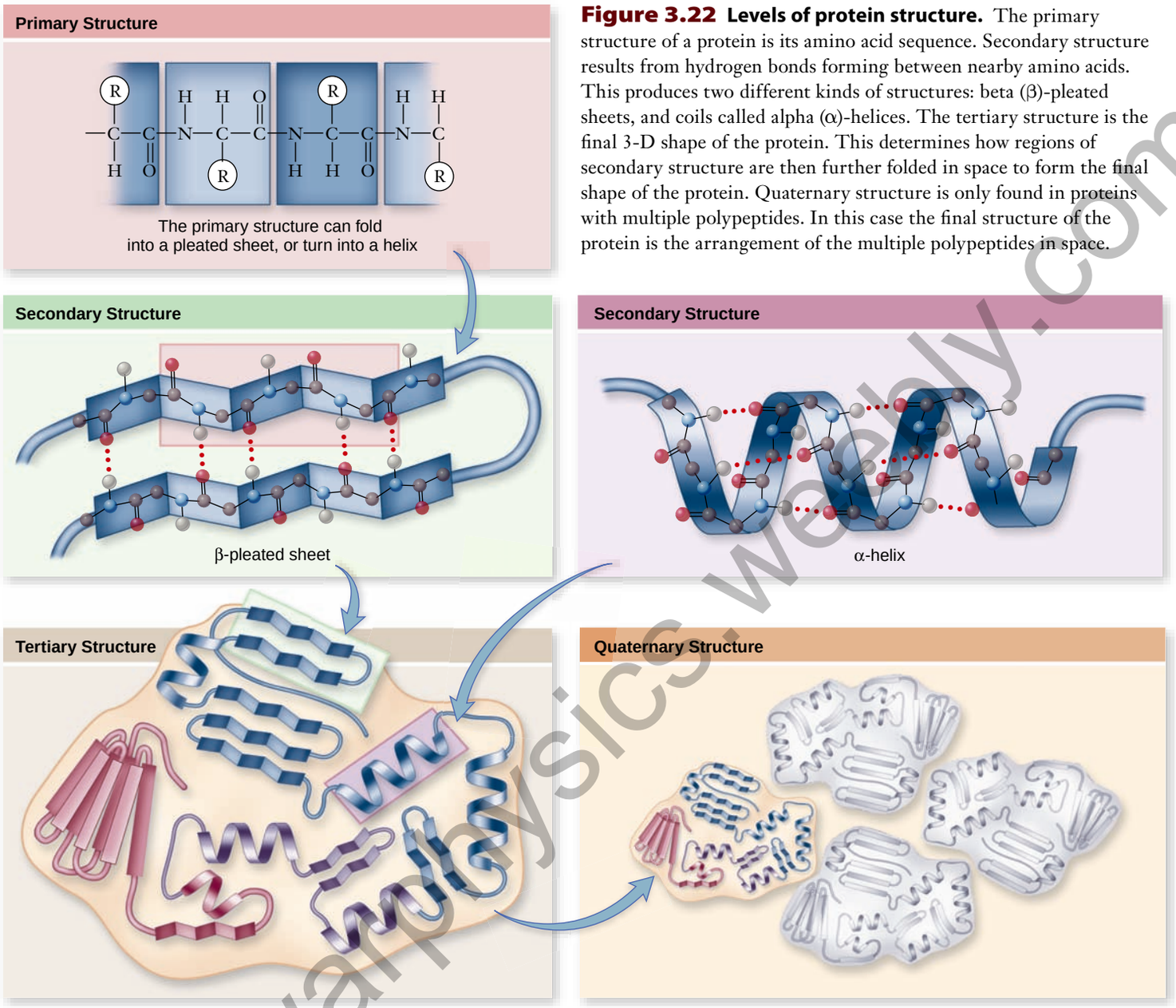


Figure 3.22 Levels of protein structure. The primary structure of a protein is its amino acid sequence. Secondary structure results from hydrogen bonds forming between nearby amino acids. This produces two different kinds of structures: beta (β)-pleated sheets, and coils called alpha (α)-helices. The tertiary structure is the final 3-D shape of the protein. This determines how regions of secondary structure are then further folded in space to form the final shape of the protein. Quaternary structure is only found in proteins with multiple polypeptides. In this case the final structure of the protein is the arrangement of the multiple polypeptides in space.

protein. The sickle cell version of hemoglobin (HbS), for example, is a change of a single glutamic acid for a valine in the β-globin chain. This change substitutes a charged amino acid for a nonpolar one on the surface of the protein, leading the protein to become sticky and form clumps. Another variant of hemoglobin called HbE, actually the most common in human populations, causes a change from glutamic acid to lysine at a different site in the β-globin chain. In this case the structural change is not as dramatic, but it still impairs function, resulting in blood disorders called anemia and thalassemia. More than 700 structural variants of hemoglobin are known, with up to 7% of the world's population being carriers of forms that are medically important.

Quaternary structure: Subunit arrangements

When two or more polypeptide chains associate to form a functional protein, the individual chains are referred to as subunits of the protein. The arrangement of these subunits is termed its **quaternary structure**. In proteins composed of subunits, the interfaces where the subunits touch one another are often nonpolar, and they play a key role in transmitting information between the subunits about individual subunit activities.

Remember that the protein hemoglobin is composed of two α-chain subunits and two β-chain subunits. Each α- and β-globin chain has a primary structure consisting of a specific sequence of amino acids. This then assumes a characteristic secondary structure consisting of α helices and β sheets that are then arranged into a specific tertiary structure for each α- and β-globin subunit. Lastly, these subunits are then arranged into their final quaternary structure. This is the final structure of the protein. For proteins that consist of only a single peptide chain, the enzyme lysozyme for example, the tertiary structure is the final structure of the protein.

Motifs and domains are additional structural characteristics

To directly determine the sequence of amino acids in a protein is a laborious task. Although the process has been automated, it remains slow and difficult.

The ability to sequence DNA changed this situation rather suddenly. Sequencing DNA was a much simpler process, and even before it was automated, the number of known sequences rose quickly. With the advent of automation, the known sequences increased even more dramatically. Today the entire sequence of hundreds of bacterial genomes and more than a dozen animal genomes, including that of humans, has been determined. Because the DNA sequence is directly related to amino acid sequence in proteins, biologists now have a large database of protein sequences to compare and analyze. This new information has also stimulated thought about the logic of the genetic code and whether underlying patterns exist in protein structure. Our view of protein structure has evolved with this new information. Researchers still view the four-part hierarchical structure as important, but two new terms have entered the biologist's vocabulary: motif and domain.

Motifs

As biologists discovered the 3-D structure of proteins (an even more laborious task than determining the sequence), they noticed similarities between otherwise dissimilar proteins. These similar structures are called **motifs,** or sometimes "supersecondary structure." The term *motif* is borrowed from the arts and refers to a recurring thematic element in music or design.

One very common protein motif is the β-α-β motif, which creates a fold or crease; the so-called "Rossmann fold" at the core of nucleotide-binding sites in a wide variety of proteins. A second motif that occurs in many proteins is the β barrel, which is a β sheet folded around to form a tube. A third type of motif, the helix-turn-helix, consists of two α helices separated by a bend. This motif is important because many proteins use it to bind to the DNA double helix (figure 3.23; see also chapter 16).

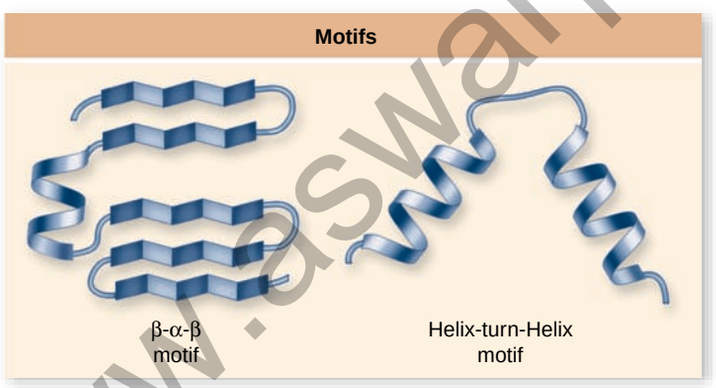
Motifs indicate a logic to structure that investigators still do not understand. Do they simply represent a reuse by evolution of something that already works, or are they an optimal solution to a problem, such as how to bind a nucleotide? One way to think about it is that if amino acids are letters in the language of proteins, then motifs represent repeated words or phrases. Motifs have been useful in determining the function of unknown proteins. Databases of protein motifs are used to search new unknown proteins. Finding motifs with known functions may allow an investigator to infer the function of a new protein.

Domains

Domains of proteins are functional units within a larger structure. They can be thought of as substructure within the tertiary structure of a protein (see figure 3.23). To continue the metaphor: Amino acids are letters in the protein language, motifs are words or phrases, and domains are paragraphs.

Most proteins are made up of multiple domains that perform different parts of the protein's function. In many cases, these domains can be physically separated. For example, transcription factors (discussed in chapter 16) are proteins that bind to DNA and initiate its transcription. If the DNA-binding region is exchanged with a different transcription factor, then the specificity of the factor for DNA can be changed without changing its ability to stimulate transcription. Such "domain-swapping" experiments have been performed with many transcription factors, and they indicate, among other things, that the DNA-binding and activation domains are functionally separate.

These functional domains of proteins may also help the protein to fold into its proper shape. As a polypeptide chain



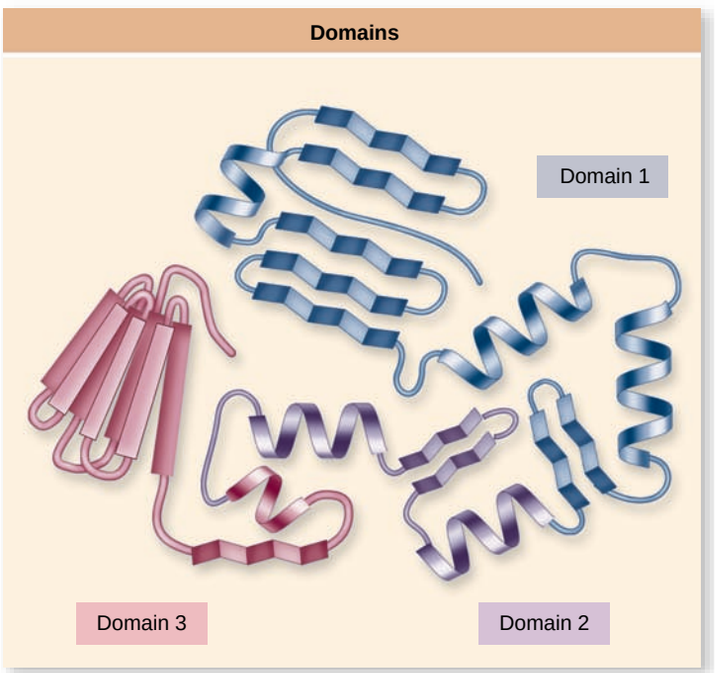


Figure 3.23 Motifs and domains. The elements of secondary structure can combine, fold, or crease to form motifs. These motifs are found in different proteins and can be used to predict function. Proteins also are made of larger domains, which are functionally distinct parts of a protein. The arrangement of these domains in space is the tertiary structure of a protein.

folds, the domains take their proper shape, each more or less independently of the others. This action can be demonstrated experimentally by artificially producing the fragment of a polypeptide that forms the domain in the intact protein, and showing that the fragment folds to form the same structure as it exhibits in the intact protein. A single polypeptide chain connects the domains of a protein, like a rope tied into several adjacent knots.

Domains can also correspond to the structure of the genes that encode them. Later, in chapter 15, you will see that genes in eukaryotes are often in pieces within the genome, and these pieces, called *exons*, sometimes encode the functional domains of a protein. This finding led to the idea of evolution acting by shuffling protein-encoding domains.

The process of folding relies on chaperone proteins

Until recently, scientific investigators thought that newly made proteins fold spontaneously, randomly trying out different configurations as hydrophobic interactions with water shoved nonpolar amino acids into the protein's interior until the final structure was arrived at. We now know this view is too simple. Protein chains can fold in so many different ways that trial and error would simply take too long. In addition, as the open chain folds its way toward its final form, nonpolar "sticky" interior portions are exposed during intermediate stages. If these intermediate forms are placed in a test tube in an environment identical to that inside a cell, they stick to other, unwanted protein partners, forming a gluey mess.

How do cells avoid having their proteins clump into a mass? A vital clue came in studies of unusual mutations that prevent viruses from replicating in bacterial cells. It turns out that the virus proteins produced inside the cells could not fold properly. Further study revealed that normal cells contain **chaperone proteins,** which help other proteins to fold correctly.

Molecular biologists have now identified many proteins that act as molecular chaperones. This class of proteins has multiple subclasses, and representatives have been found in essentially every organism that has been examined. Furthermore, these proteins seem to be essential for viability as well, illustrating their fundamental importance. Many are heat shock proteins, produced in greatly increased amounts when cells are exposed to elevated temperature. High temperatures cause proteins to unfold, and heat shock chaperone proteins help the cell's proteins to refold properly.

One class of these proteins, called chaperonins, has been extensively studied. In the bacterium *Escherichia coli* (*E. coli*), one example is the essential protein GroE chaperonin. In mutants in which the GroE chaperonin is inactivated, fully 30% of the bacterial proteins fail to fold properly. Chaperonins associate to form a large macromolecular complex that resembles a cylindrical container. Proteins can move into the container, and the container itself can change its shape considerably (figure 3.24). Experiments have shown that an improperly folded protein can enter the chaperonin and be refolded. Although we don't know exactly how this happens, it seems to involve changes in the hydrophobicity of the interior of the chamber.

The flexibility of the structure of chaperonins is amazing. We tend to think of proteins as being fixed structures, but this is clearly not the case for chaperonins and this flexibility is necessary for their function. It also illustrates that even domains that may be very widely separated in a very large protein are still functionally connected. The folding process within a chaperonin harnesses the hydrolysis of ATP to power these changes in structure necessary for function. This entire process can occur in a cyclic manner until the appropriate structure is achieved. Cells use these chaperonins both to accomplish the original folding of some proteins and to restore the structure of incorrectly folded ones.

Some diseases may result from improper folding

Chaperone protein deficiencies may be implicated in certain diseases in which key proteins are improperly folded. Cystic fibrosis

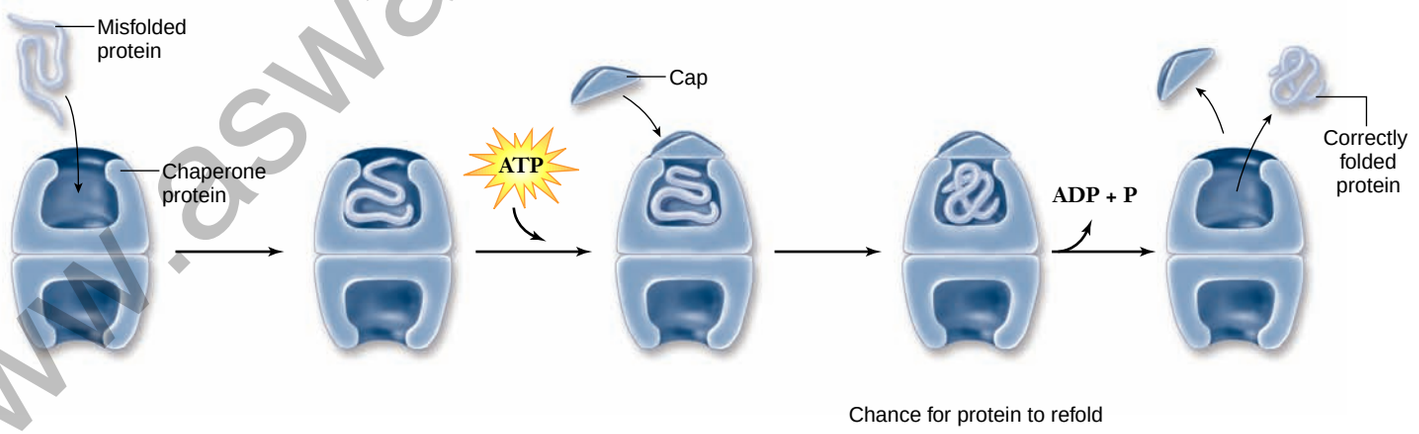


Figure 3.24 How one type of chaperone protein works. This barrel-shaped chaperonin is from the GroE family of chaperone proteins. It is composed of two identical rings each with seven identical subunits, each of which has three distinct domains. An incorrectly folded protein enters one chamber of the barrel, and a cap seals the chamber. Energy from the hydrolysis of ATP fuels structural alterations to the chamber, changing it from hydrophobic to hydrophilic. This change allows the protein to refold. After a short time, the protein is ejected, either folded or unfolded, and the cycle can repeat itself.

is a hereditary disorder in which a mutation disables a vital protein that moves ions across cell membranes. As a result, people with cystic fibrosis have thicker than normal mucus. This results in breathing problems, lung disease, and digestive difficulties, among other things. One interesting feature of the molecular analysis of this disease has been the number of different mutations found in human populations. One diverse class of mutations all result in problems with protein folding. The number of different mutations that can result in improperly folded proteins may be related to the fact that the native protein often fails to fold properly.

Denaturation inactivates proteins

If a protein's environment is altered, the protein may change its shape or even unfold completely. This process is called **denaturation** (figure 3.25). Proteins can be denatured when the pH, temperature, or ionic concentration of the surrounding solution changes.

Denatured proteins are usually biologically inactive. This action is particularly significant in the case of enzymes. Because practically every chemical reaction in a living organism is catalyzed by a specific enzyme, it is vital that a cell's enzymes work properly.

The traditional methods of food preservation, salt curing and pickling, involve denaturation of proteins. Prior to the general availability of refrigerators and freezers, the only practical

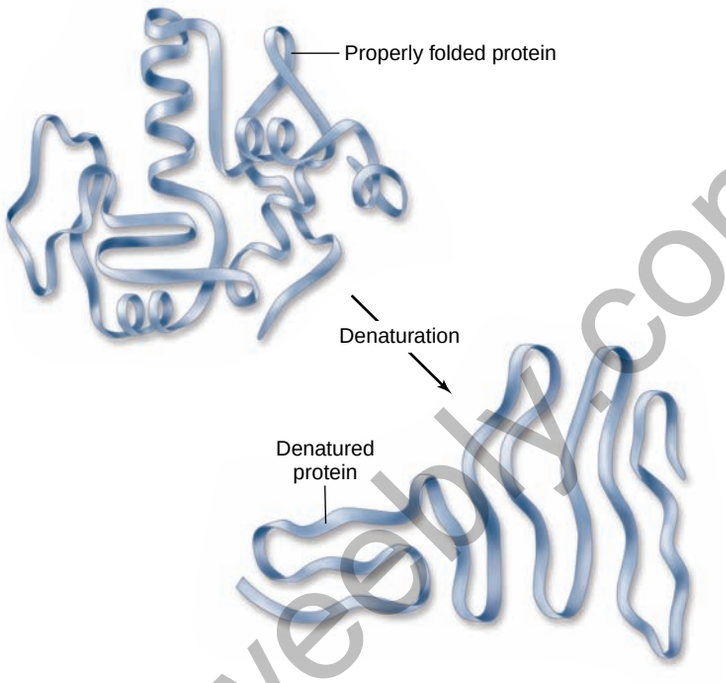


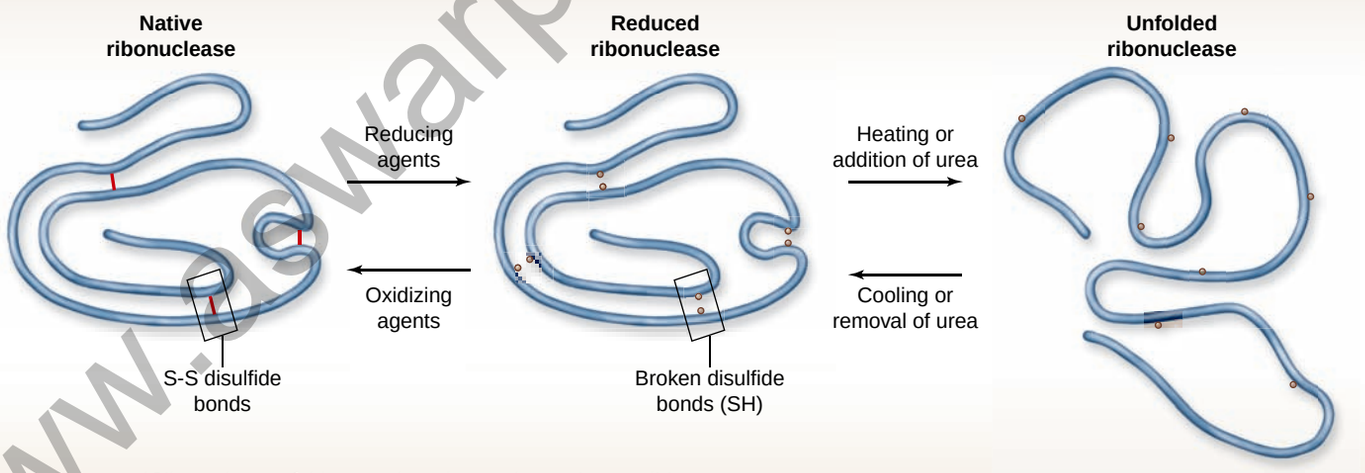
Figure 3.25 Protein denaturation. Changes in a protein's environment, such as variations in temperature or pH, can cause a protein to unfold and lose its shape in a process called denaturation. In this denatured state, proteins are biologically inactive.

SCIENTIFIC THINKING

Hypothesis: *The 3-D structure of a protein is the thermodynamically stable structure. It depends only on the primary structure of the protein and the solution conditions.*

Prediction: *If a protein is denatured and allowed to renature under native conditions, it will refold into the native structure.*

Test: *Ribonuclease is treated with a reducing agent to break disulfide bonds and is then treated with urea to completely unfold the protein. The disulfide bonds are reformed under nondenaturing conditions to see if the protein refolds properly.*



Result: *Denatured Ribonuclease refolds properly under nondenaturing conditions.*

Conclusion: *The hypothesis is supported. The information in the primary structure (amino acid sequence) is sufficient for refolding to occur. This implies that protein folding results in the thermodynamically stable structure.*

Further Experiments: *If the disulfide bonds were allowed to reform under denaturing conditions, would we get the same result? How can we rule out that the protein had not been completely denatured and therefore retained some structure?*

Figure 3.26 Primary structure determines tertiary structure.

way to keep microorganisms from growing in food was to keep the food in a solution containing a high concentration of salt or vinegar, which denatured the enzymes of most microorganisms and prevented them from growing on the food.

Most enzymes function within a very narrow range of environmental conditions. Blood-borne enzymes that course through a human body at a pH of about 7.4 would rapidly become denatured in the highly acidic environment of the stomach. Conversely, the protein-degrading enzymes that function at a pH of 2 or less in the stomach would be denatured in the relatively basic pH of the blood. Similarly, organisms that live near oceanic hydrothermal vents have enzymes that work well at these extremes of temperature (over 100°C). They cannot survive in cooler waters, because their enzymes do not function properly at lower temperatures. Any given organism usually has a tolerance range of pH, temperature, and salt concentration. Within that range, its enzymes maintain the proper shape to carry out their biological functions.

When a protein's normal environment is reestablished after denaturation, a small protein may spontaneously refold into its natural shape, driven by the interactions between its nonpolar amino acids and water (figure 3.26). This process is termed *renaturation*, and it was first established for the enzyme ribonuclease (RNase). The renaturation of RNase led to the doctrine that primary structure determines tertiary structure. Larger proteins can rarely refold spontaneously, however, because of the complex nature of their final shape, so this simple idea needs to be qualified.

The fact that some proteins can spontaneously renature implies that tertiary structure is strongly influenced by primary structure. In an extreme example, the *E. coli* ribosome can be taken apart and put back together experimentally. Although this process requires temperature and ion concentration shifts, it indicates an amazing degree of self-assembly. That complex structures can arise by self-assembly is a key idea in the study of modern biology.

It is important to distinguish denaturation from **dissociation.** For proteins with quaternary structure, the subunits may be dissociated without losing their individual tertiary structure. For example, the four subunits of hemoglobin may dissociate into four individual molecules (two α-globins and two β-globins) without denaturation of the folded globin proteins. They readily reassume their four-subunit quaternary structure.

Learning Outcomes Review 3.4

Proteins are molecules with diverse functions. They are constructed from 20 different kinds of amino acids. Protein structure can be viewed at four levels: (1) the amino acid sequence, or primary structure; (2) coils and sheets, called secondary structure; (3) the three-dimensional shape, called tertiary structure; and (4) individual polypeptide subunits associated in a quaternary structure. Different proteins often have similar substructures called motifs and can be broken down into functional domains. Proteins have a narrow range of conditions in which they fold properly; outside that range, proteins tend to unfold (denaturation). Under some conditions, denatured proteins can refold and become functional again (renaturation).

- ***How does our knowledge of protein structure help us to predict the function of unknown proteins?***

3.5 *Lipids: Hydrophobic Molecules*

Learning Outcomes

1. ***Understand the structure of triglycerides.***
2. ***Explain how fats function as energy-storage molecules.***
3. ***Apply knowledge of the structure of phospholipids to the formation of membranes.***

Lipids are a somewhat loosely defined group of molecules with one main chemical characteristic: They are insoluble in water. Storage fats such as animal fat are one kind of lipid. Oils such as those from olives, corn, and coconut are also lipids, as are waxes such as beeswax and earwax. Even some vitamins are lipids!

Lipids have a very high proportion of nonpolar carbon–hydrogen (C—H) bonds, and so long-chain lipids cannot fold up like a protein to confine their nonpolar portions away from the surrounding aqueous environment. Instead, when they are placed in water, many lipid molecules spontaneously cluster together and expose what polar (hydrophilic) groups they have to the surrounding water, while confining the nonpolar (hydrophobic) parts of the molecules together within the cluster. You may have noticed this effect when you add oil to a pan containing water, and the oil beads up into cohesive drops on the water's surface. This spontaneous assembly of lipids is of paramount importance to cells, as it underlies the structure of cellular membranes.

Fats consist of complex polymers of fatty acids attached to glycerol

Many lipids are built from a simple skeleton made up of two main kinds of molecules: fatty acids and glycerol. Fatty acids are long-chain hydrocarbons with a carboxylic acid (COOH) at one end. Glycerol is a three-carbon polyalcohol (three —OH groups). Many lipid molecules consist of a glycerol molecule with three fatty acids attached, one to each carbon of the glycerol backbone. Because it contains three fatty acids, a fat molecule is commonly called a **triglyceride** (the more accurate chemical name is *triacylglycerol*). This basic structure is depicted in figure 3.27. The three fatty acids of a triglyceride need not be identical, and often they are very different from one another. The hydrocarbon chains of fatty acids vary in length. The most common are even-numbered chains of 14 to 20 carbons. The many C—H bonds of fats serve as a form of long-term energy storage.

If all of the internal carbon atoms in the fatty acid chains are bonded to at least two hydrogen atoms, the fatty acid is said to be **saturated,** which refers to its having all the hydrogen atoms possible (see figure 3.27). A fatty acid that has double bonds between one or more pairs of successive carbon atoms is said to be **unsaturated.** Fatty acids with one double bond are called monounsaturated, and those with more than one double bond are termed **polyunsaturated.** Most naturally occurring unsaturated fatty acids have double bonds with a cis configuration where the carbon chain is on the same side before and after the double bond (double bonds in fatty acids in 3.27*b* are all cis).

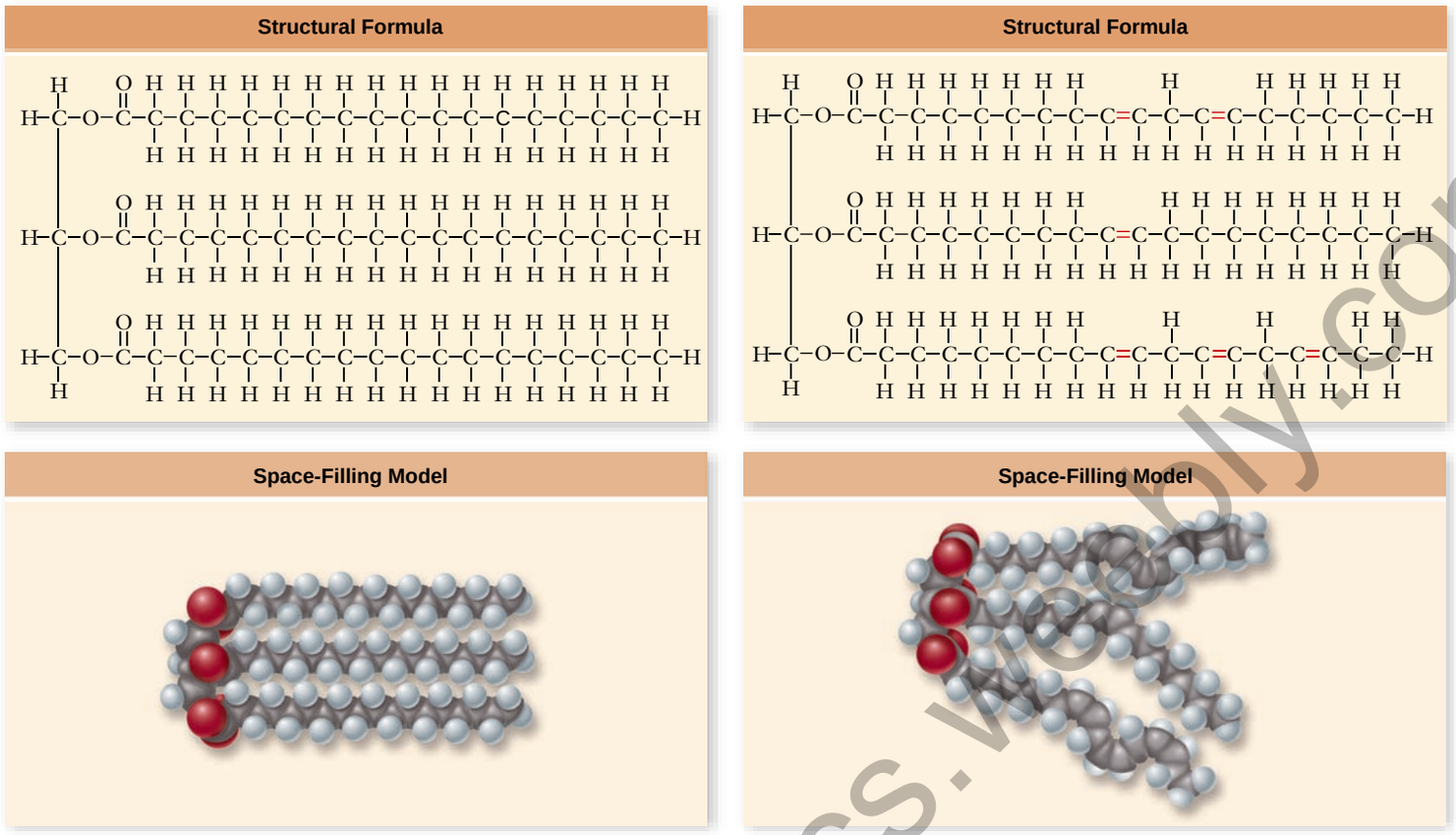


Figure 3.27 Saturated and unsaturated fats. *a.* A saturated fat is composed of triglycerides that contain three saturated fatty acids (the kind that have no double bonds). A saturated fat therefore has the maximum number of hydrogen atoms bonded to its carbon chain. Most animal fats are saturated. *b.* Unsaturated fat is composed of triglycerides that contain three unsaturated fatty acids (the kind that have one or more double bonds). These have fewer than the maximum number of hydrogen atoms bonded to the carbon chain. This example includes both a monounsaturated and two polyunsaturated fatty acids. Plant fats are typically unsaturated. The many kinks of the double bonds prevent the triglyceride from closely aligning, which makes them liquid oils at room temperature.

When fats are partially hydrogenated industrially, this can produce double bonds with a trans configuration where the carbon chain is on opposite sides before and after the double bond. These are the so called trans fats. These have been linked to elevated levels of low-density lipoprotein (LDL) "bad cholesterol" and lowered levels of high-density lipoprotein (HDL) "good cholesterol." This condition is thought to be associated with an increased risk for coronary heart disease.

Having double bonds changes the behavior of the molecule because free rotation cannot occur about a C═C double bond as it can with a C—C single bond. This characteristic mainly affects melting point: that is, whether the fatty acid is a solid fat or a liquid oil at room temperature. Fats containing polyunsaturated fatty acids have low melting points because their fatty acid chains bend at the double bonds, preventing the fat molecules from aligning closely with one another. Most saturated fats, such as animal fat or those in butter, are solid at room temperature.

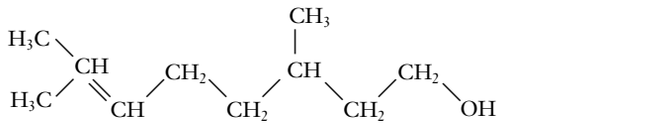
Placed in water, triglycerides spontaneously associate together, forming fat globules that can be very large relative to the size of the individual molecules. Because fats are insoluble in water, they can be deposited at specific locations within an organism, such as in vesicles of adipose tissue.

Organisms contain many other kinds of lipids besides fats (figure 3.28). *Terpenes* are long-chain lipids that are components of many biologically important pigments, such as chlorophyll and the visual pigment retinal. Rubber is also a terpene. *Steroids*, another class of lipid, are composed of four carbon rings. Most animal cell membranes contain the steroid cholesterol. Other steroids, such as testosterone and estrogen, function as hormones in multicellular animals. *Prostaglandins* are a group of about 20 lipids that are modified fatty acids, with two nonpolar "tails" attached to a five-carbon ring. Prostaglandins act as local chemical messengers in many vertebrate tissues. Later chapters explore the effects of some of these complex fatty acids.

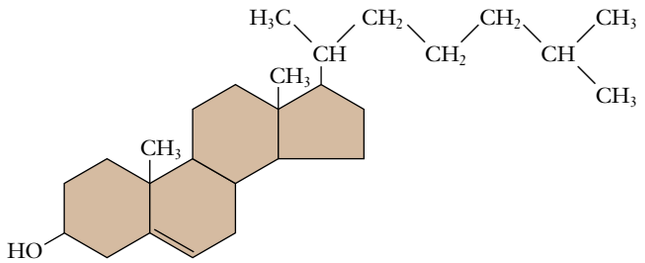
Fats are excellent energy-storage molecules

Most fats contain over 40 carbon atoms. The ratio of energy-storing C—H bonds in fats is more than twice that of carbohydrates (see section 3.2), making fats much more efficient molecules for storing chemical energy. On average, fats yield about 9 kilocalories (kcal) of chemical energy per gram, as compared with about 4 kcal/g for carbohydrates.

Most fats produced by animals are saturated (except some fish oils), whereas most plant fats are unsaturated (see



a. Terpene (citronellol)



b. Steroid (cholesterol)

Figure 3.28 Other kinds of lipids. *a.* Terpenes are found in biological pigments, such as chlorophyll and retinal, and ***(b)*** steroids play important roles in membranes and as the basis for a class of hormones involved in chemical signaling.

figure 3.27). The exceptions are the tropical plant oils (palm oil and coconut oil), which are saturated even though they are liquid at room temperature. An oil may be converted into a solid fat by chemically adding hydrogen. Most peanut butter is usually artificially hydrogenated to make the peanut fats solidify, preventing them from separating out as oils while the jar sits on the store shelf. However, artificially hydrogenating unsaturated fats produces the *trans*-fatty acids described above.

When an organism consumes excess carbohydrate, it is converted into starch, glycogen, or fats reserved for future use. The reason that many humans in developed countries gain weight as they grow older is that the amount of energy they need decreases with age, but their intake of food does not. Thus, an increasing proportion of the carbohydrates they ingest is converted into fat.

A diet heavy in fats is one of several factors thought to contribute to heart disease, particularly atherosclerosis. In atherosclerosis, sometimes referred to as "hardening of the arteries," fatty substances called plaque adhere to the lining of blood vessels, blocking the flow of blood. Fragments of a plaque can break off from a deposit and clog arteries to the brain, causing a stroke.

Phospholipids form membranes

Complex lipid molecules called **phospholipids** are among the most important molecules of the cell because they form the core of all biological membranes. An individual phospholipid can be thought of as a substituted triglyceride, that is, a triglyceride with a phosphate replacing one of the fatty acids. The basic structure of a phospholipid includes three kinds of subunits:

1. *Glycerol*, a three-carbon alcohol, in which each carbon bears a hydroxyl group. Glycerol forms the backbone of the phospholipid molecule.
2. *Fatty acids*, long chains of —CH_2 groups (hydrocarbon chains) ending in a carboxyl (—COOH) group. Two fatty acids are attached to the glycerol backbone in a phospholipid molecule.
3. *A phosphate group* (—PO_4^{2-}) attached to one end of the glycerol. The charged phosphate group usually has a charged organic molecule linked to it, such as choline, ethanolamine, or the amino acid serine.

The phospholipid molecule can be thought of as having a polar "head" at one end (the phosphate group) and two long, very nonpolar "tails" at the other (figure 3.29). This structure is essential for how these molecules function, although it first

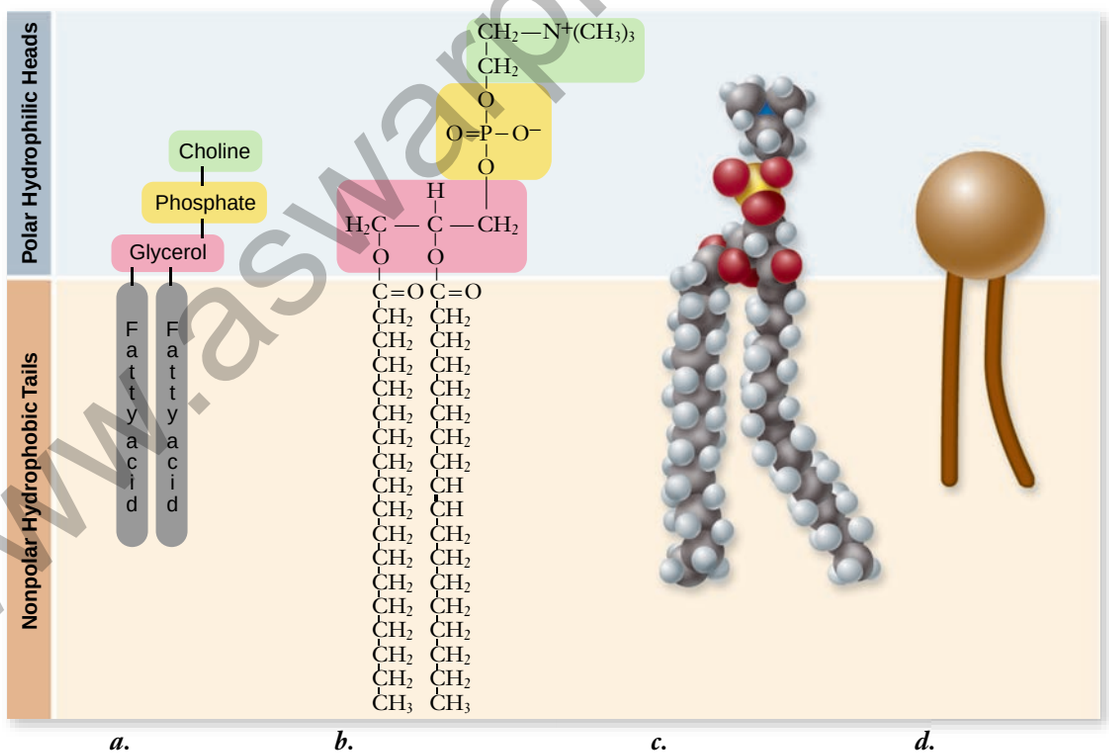


Figure 3.29 Phospholipids. The phospholipid phosphatidylcholine is shown as ***(a)*** a schematic, ***(b)*** a formula, ***(c)*** a space-filling model, and ***(d)*** an icon used in depictions of biological membranes.

appears paradoxical. Why would a molecule need to be soluble in water, but also not soluble in water? The formation of a membrane shows the unique properties of such a structure.

In water, the nonpolar tails of nearby lipid molecules aggregate away from the water, forming spherical *micelles*, with the tails facing inward (figure 3.30*a*). This is actually how detergent molecules work to make grease soluble in water. The grease is soluble within the nonpolar interior of the micelle and the polar surface of the micelle is soluble in water. With phospholipids, a more complex structure forms in which two layers of molecules line up, with the hydrophobic tails of each layer pointing toward one another, or inward, leaving the hydrophilic heads oriented outward, forming a bilayer (figure 3.30*b*). Lipid bilayers are the basic framework of biological membranes, discussed in detail in chapter 5.

Learning Outcomes Review 3.5

Triglycerides are made of fatty acids linked to glycerol. Fats can contain twice as many C—H bonds as carbohydrates and thus they store energy efficiently. Because the C—H bonds in lipids are nonpolar, they are not water-soluble and aggregate together in water. Phospholipids replace one fatty acid with a hydrophilic phosphate group. This allows them to spontaneously form bilayers, which are the basis of biological membranes.

- ***Why do phospholipids form membranes while triglycerides form insoluble droplets?***

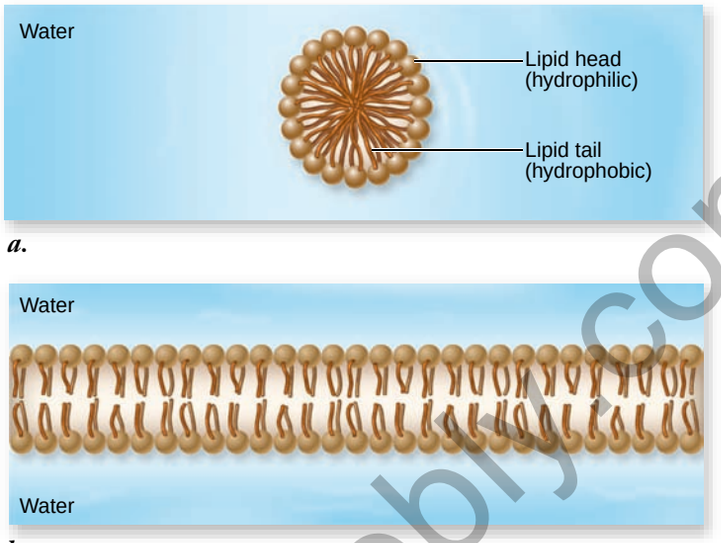
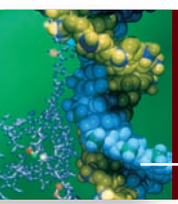


Figure 3.30 Lipids spontaneously form micelles or lipid bilayers in water. In an aqueous environment, lipid molecules orient so that their polar (hydrophilic) heads are in the polar medium, water, and their nonpolar (hydrophobic) tails are held away from the water. ***a.*** Droplets called micelles can form, or ***(b)*** phospholipid molecules can arrange themselves into two layers; in both structures, the hydrophilic heads extend outward and the hydrophobic tails inward. This second example is called a phospholipid bilayer.



Chapter Review

3.1 Carbon: The Framework of Biological Molecules

Carbon, the backbone of all biological molecules, can form four covalent bonds and make long chains. Hydrocarbons consist of carbon and hydrogen, and their bonds store considerable energy.

Functional groups account for differences in molecular properties.

Functional groups are small molecular entities that confer specific chemical characteristics when attached to a hydrocarbon.

Carbon and hydrogen have similar electronegativity so C—H bonds are not polar. Oxygen and nitrogen have greater electronegativity, leading to polar bonds.

Isomers have the same molecular formulas but different structures.

Structural isomers are molecules with the same formula but different structures; stereoisomers differ in how groups are attached. Enantiomers are mirror-image stereoisomers.

Biological macromolecules include carbohydrates, nucleic acids, proteins, and lipids.

Most important biological macromolecules are polymers—long chains of monomer units. Biological polymers are formed by elimination of water (H and OH) from two monomers (dehydration reaction). They are broken down by adding water (hydrolysis).

3.2 Carbohydrates: Energy Storage and Structural Molecules

The empirical formula of a carbohydrate is $(CH_2O)_n$. Carbohydrates are used for energy storage and as structural molecules.

Monosaccharides are simple sugars.

Simple sugars contain three to six or more carbon atoms. Examples are glyceraldehyde (3 carbons), deoxyribose (5 carbons), and glucose (6 carbons).

Sugar isomers have structural differences.

The general formula for six-carbon sugars is $C_6H_{12}O_6$, and many isomeric forms are possible. Living systems often have enzymes for converting isomers from one to the other.

Disaccharides serve as transport molecules in plants and provide nutrition in animals.

Plants convert glucose into the disaccharide sucrose for transport within their bodies. Female mammals produce the disaccharide lactose to nourish their young.

Polysaccharides provide energy storage and structural components.

Glucose is used to make three important polymers: glycogen (in animals), and starch and cellulose (in plants). Chitin is a related structural material found in arthropods and many fungi.

3.3 Nucleic Acids: Information Molecules

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are polymers composed of nucleotide monomers. Cells use nucleic acids for information storage and transfer.

Nucleic acids are nucleotide polymers.

Nucleic acids contain four different nucleotide bases. In DNA these are adenine, guanine, cytosine, and thymine. In RNA, thymine is replaced by uracil.

DNA carries the genetic code.

DNA exists as a double helix held together by specific base pairs: adenine with thymine and guanine with cytosine. The nucleic acid sequence constitutes the genetic code.

RNA is a transcript of a DNA strand.

RNA is made by copying DNA. This transcript is then used as a template to make proteins.

Other nucleotides are vital components of energy reactions.

Adenosine triphosphate (ATP) provides energy in cells; NAD^+ and FAD transport electrons in cellular processes.

3.4 Proteins: Molecules with Diverse Structures and Functions

Most enzymes are proteins. Proteins also provide defense, transport, motion, and regulation, among many other roles.

Proteins are polymers of amino acids.

Amino acids are joined by peptide bonds to make polypeptides. The 20 common amino acids are characterized by R groups that determine their properties.

Proteins have levels of structure.

Protein structure is defined by the following hierarchy: primary (amino acid sequence), secondary (hydrogen bonding patterns), tertiary (three-dimensional folding), and quaternary (associations between two or more polypeptides).

Motifs and domains are additional structural characteristics.

Motifs are similar structural elements found in dissimilar proteins. They can create folds, creases, or barrel shapes. Domains are functional subunits or sites within a tertiary structure.

The process of folding relies on chaperone proteins.

Chaperone proteins assist in the folding of proteins. Heat shock proteins are an example of chaperone proteins.

Some diseases may result from improper folding.

Some forms of cystic fibrosis and Alzheimer disease are associated with misfolded proteins.

Denaturation inactivates proteins.

Denaturation refers to an unfolding of tertiary structure, which usually destroys function. Some denatured proteins may recover function when conditions are returned to normal. This implies that primary structure strongly influences tertiary structure.

Disassociation refers to separation of quaternary subunits with no changes to their tertiary structure.

3.5 Lipids: Hydrophobic Molecules

Lipids are insoluble in water because they have a high proportion of nonpolar C—H bonds.

Fats consist of complex polymers of fatty acids attached to glycerol.

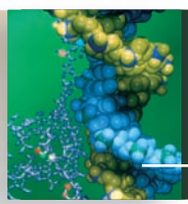
Many lipids exist as triglycerides, three fatty acids connected to a glycerol molecule. Saturated fatty acids contain the maximum number of hydrogen atoms. Unsaturated fatty acids contain one or more double bonds between carbon atoms.

Fats are excellent energy-storage molecules.

The energy stored in the C—H bonds of fats is more than twice that of carbohydrates: 9 kcal/g compared with 4 kcal/g. For this reason, excess carbohydrate is converted to fat for storage.

Phospholipids form membranes

Phospholipids contain two fatty acids and one phosphate attached to glycerol. In phospholipid-bilayer membranes, the phosphate heads are hydrophilic and cluster on the two faces of the membrane, and the hydrophobic tails are in the center.



Review Questions

UNDERSTAND

1. How is a polymer formed from multiple monomers?
   a. From the growth of the chain of carbon atoms
   b. By the removal of an —OH group and a hydrogen atom
   c. By the addition of an —OH group and a hydrogen atom
   d. Through hydrogen bonding
2. Why are carbohydrates important molecules for energy storage?
   a. The C—H bonds found in carbohydrates store energy.
   b. The double bonds between carbon and oxygen are very strong.
   c. The electronegativity of the oxygen atoms means that a carbohydrate is made up of many polar bonds.
   d. They can form ring structures in the aqueous environment of a cell.
3. Plant cells store energy in the form of _____, and animal cells store energy in the form of _____.
   a. fructose; glucose
   b. disaccharides; monosaccharides
   c. cellulose; chitin
   d. starch; glycogen
4. Which carbohydrate would you find as part of a molecule of RNA?
   a. Galactose
   b. Deoxyribose
   c. Ribose
   d. Glucose
5. A molecule of DNA or RNA is a polymer of
   a. monosaccharides.
   b. nucleotides.
   c. amino acids.
   d. fatty acids.

6. What makes cellulose different from starch?
   a. Starch is produced by plant cells, and cellulose is produced by animal cells.
   b. Cellulose forms long filaments, and starch is highly branched.
   c. Starch is insoluble, and cellulose is soluble.
   d. All of the above.
7. What monomers make up a protein?
   a. Monosaccharides
   b. Nucleotides
   c. Amino acids
   d. Fatty acids
8. A triglyceride is a form of _____ composed of _____.
   a. lipid; fatty acids and glucose
   b. lipid; fatty acids and glycerol
   c. carbohydrate; fatty acids
   d. lipid; cholesterol

APPLY

1. Amino acids are linked together to form a protein by
   a. phosphodiester bonds.
   b. β-(1⟶4) linkages.
   c. peptide bonds.
   d. hydrogen bonds.
2. Which of the following is NOT a difference between DNA and RNA?
   a. Deoxyribose sugar versus ribose sugar
   b. Thymine versus uracil
   c. Double-stranded versus single-stranded
   d. Phosphodiester versus hydrogen bonds
3. Which part of an amino acid has the greatest influence on the overall structure of a protein?
   a. The (—NH_2) amino group
   b. The R group
   c. The (—COOH) carboxyl group
   d. Both a and c
4. A mutation that alters a single amino acid within a protein can alter
   a. the primary level of protein structure.
   b. the secondary level of protein structure.
   c. the tertiary level of protein structure.
   d. all of the above.
5. Two different proteins have the same domain in their structure. From this we can infer that they have
   a. the same primary structure.
   b. similar function.
   c. very different functions.
   d. the same primary structure but different function.
6. What chemical property of lipids accounts for their insolubility in water?
   a. The COOH group of fatty acids
   b. The large number of nonpolar C—H bonds
   c. The branching of saturated fatty acids
   d. The C═C bonds found in unsaturated fatty acids
7. The spontaneous formation of a lipid bilayer in an aqueous environment occurs because
   a. the polar head groups of the phospholipids can interact with water.
   b. the long fatty acid tails of the phospholipids can interact with water.
   c. the fatty acid tails of the phospholipids are hydrophobic.
   d. both a and c.

SYNTHESIZE

1. How do the four biological macromolecules differ from one another? How does the structure of each relate to its function?
2. Hydrogen bonds and hydrophobic interactions each play an important role in stabilizing and organizing biological macromolecules. Consider the four macromolecules discussed in this chapter. Describe how these affect the form and function of each type of macromolecule. Would a disruption in the hydrogen bonds affect form and function? Hydrophobic interactions?
3. Plants make both starch and cellulose. Would you predict that the enzymes involved in starch synthesis could also be used by the plant for cellulose synthesis? Construct an argument to explain this based on the structure and function of the enzymes and the polymers synthesized.

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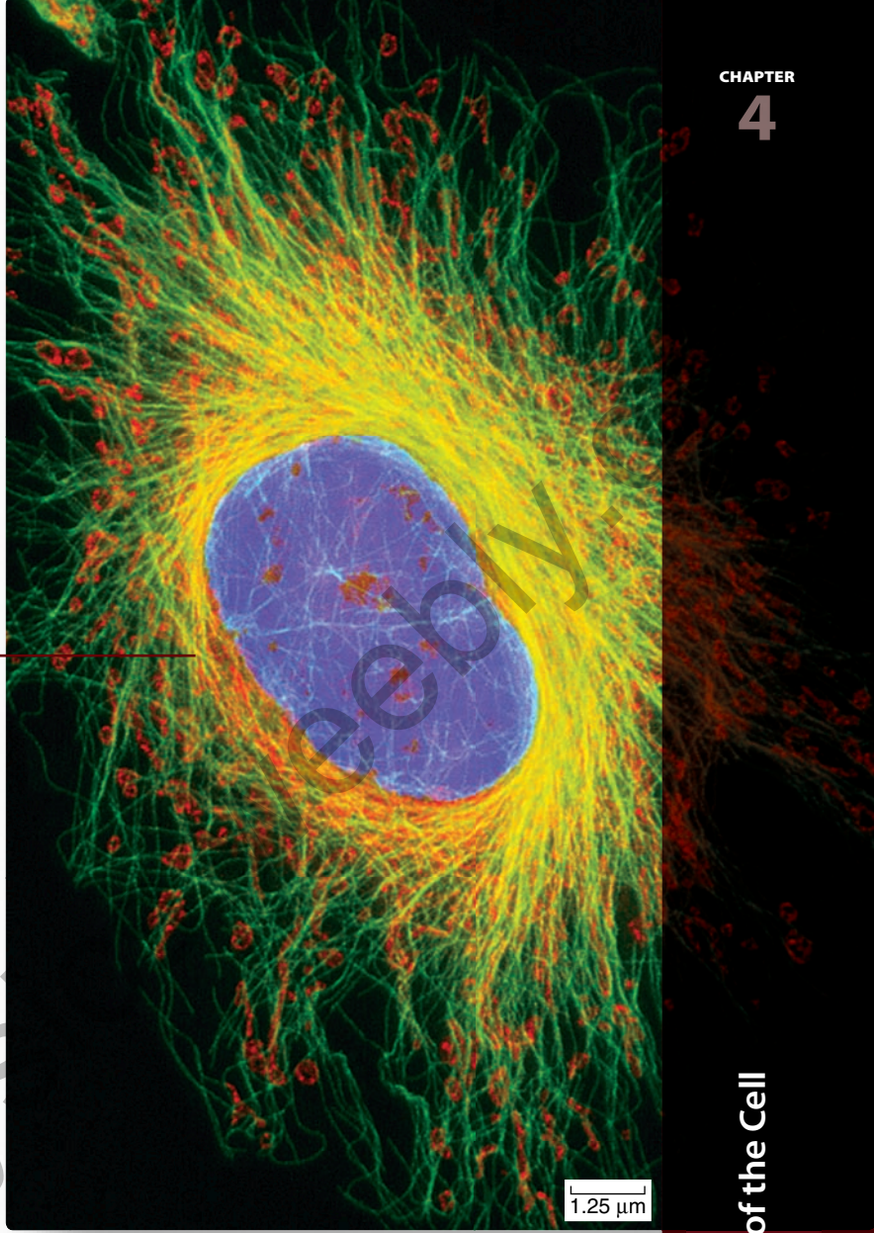
Chapter 4

Cell Structure



Chapter Outline

Introduction

All organisms are composed of cells. The gossamer wing of a butterfly is a thin sheet of cells and so is the glistening outer layer of your eyes. The hamburger or tomato you eat is composed of cells, and its contents soon become part of your cells. Some organisms consist of a single cell too small to see with the unaided eye. Others, such as humans, are composed of many specialized cells, such as the fibroblast cell shown in the striking fluorescence micrograph on this page. Cells are so much a part of life that we cannot imagine an organism that is not cellular in nature. In this chapter, we take a close look at the internal structure of cells. In chapters 4 to 10, we will focus on cells in action—how they communicate with their environment, grow, and reproduce.

4.1 Cell Theory

Learning Outcomes

1. ***Explain the cell theory.***
2. ***Describe the factors that limit cell size.***
3. ***Categorize structural and functional similarities in cells.***

Cells are characteristically microscopic in size. Although there are exceptions, a typical eukaryotic cell is 10 to 100 micrometers (µm) (10 to 100 millionths of a meter) in diameter, while most prokaryotic cells are only 1 to 10 µm in diameter.

Because cells are so small, they were not discovered until the invention of the microscope in the 17th century. Robert Hooke was the first to observe cells in 1665, naming the shapes he saw in cork *cellulae* (Latin, "small rooms"). This is known to us as *cells*. Another early microscopist, Anton van Leeuwenhoek first observed living cells, which he termed "animalcules," or little animals. After these early efforts, a century and a half

passed before biologists fully recognized the importance of cells. In 1838, botanist Matthias Schleiden stated that all plants "are aggregates of fully individualized, independent, separate beings, namely the cells themselves." In 1839, Theodor Schwann reported that all animal tissues also consist of individual cells. Thus, the cell theory was born.

Cell theory is the unifying foundation of cell biology

The cell theory was proposed to explain the observation that all organisms are composed of cells. It sounds simple, but it is a far-reaching statement about the organization of life.

In its modern form, the *cell theory* includes the following three principles:

1. All organisms are composed of one or more cells, and the life processes of metabolism and heredity occur within these cells.
2. Cells are the smallest living things, the basic units of organization of all organisms.
3. Cells arise only by division of a previously existing cell.

Although life likely evolved spontaneously in the environment of early Earth, biologists have concluded that no additional cells are originating spontaneously at present. Rather, life on Earth represents a continuous line of descent from those early cells.

Cell size is limited

Most cells are relatively small for reasons related to the diffusion of substances into and out of cells. The rate of diffusion is affected by a number of variables, including (1) surface area available for diffusion, (2) temperature, (3) concentration gradient of diffusing substance, and (4) the distance over which diffusion must occur. As the size of a cell increases, the length of time for diffusion from the outside membrane to the interior of the cell increases as well. Larger cells need to synthesize more macromolecules, have correspondingly higher energy requirements, and produce a greater quantity of waste. Molecules used for energy and biosynthesis must be transported through the membrane. Any metabolic waste produced must be removed, also passing through the membrane. The rate at which this transport occurs depends on both the distance to the membrane and the area of membrane available. For this reason, an organism made up of many relatively small cells has an advantage over one composed of fewer, larger cells.

The advantage of small cell size is readily apparent in terms of the **surface area-to-volume ratio.** As a cell's size increases, its volume increases much more rapidly than its surface area. For a spherical cell, the surface area is proportional to the square of the radius, whereas the volume is proportional to the cube of the radius. Thus, if the radii of two cells differ by a factor of 10, the larger cell will have 10^2, or 100 times, the surface area, but 10^3, or 1000 times, the volume of the smaller cell (figure 4.1).

The cell surface provides the only opportunity for interaction with the environment, because all substances enter and exit a cell via this surface. The membrane surrounding the cell plays a key role in controlling cell function. Because small cells have more surface area per unit of volume than large ones, control over cell contents is more effective when cells are relatively small.

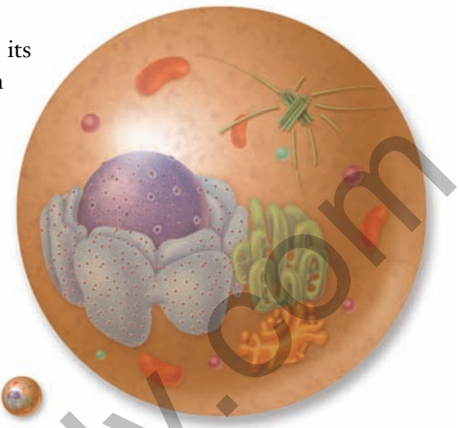


Figure 4.1 Surface area-to-volume ratio. As a cell gets larger, its volume increases at a faster rate than its surface area. If the cell radius increases by 10 times, the surface area increases by 100 times, but the volume increases by 1000 times. A cell's surface area must be large enough to meet the metabolic needs of its volume.

Cell radius (r)	1 unit	10 unit
Surface area ($4\pi r^2$)	12.57 unit^2	1257 unit^2
Volume ($\frac{4}{3}\pi r^3$)	4.189 unit^3	4189 unit^3
Surface Area / Volume	3	0.3

Although most cells are small, some quite large cells do exist. These cells have apparently overcome the surface area-to-volume problem by one or more adaptive mechanisms. For example, some cells, such as skeletal muscle cells, have more than one nucleus, allowing genetic information to be spread around a large cell. Some other large cells, such as neurons, are long and skinny, so that any given point within the cell is close to the plasma membrane. This permits diffusion between the inside and outside of the cell to still be rapid.

Microscopes allow visualization of cells and components

Other than egg cells, not many cells are visible to the naked eye (figure 4.2). Most are less than 50 μm in diameter, far smaller than the period at the end of this sentence. So, to visualize cells we need the aid of technology. The development of microscopes and their refinement over the centuries has allowed us to continually explore cells in greater detail.

The resolution problem

How do we study cells if they are too small to see? The key is to understand why we can't see them. The reason we can't see such small objects is the limited resolution of the human eye. *Resolution* is the minimum distance two points can be apart and still be distinguished as two separate points. When two objects are closer together than about 100 μm, the light reflected from each strikes the same photoreceptor cell at the rear of the eye. Only when the objects are farther than 100 μm apart can the light from each strike different cells, allowing your eye to resolve them as two distinct objects rather than one.

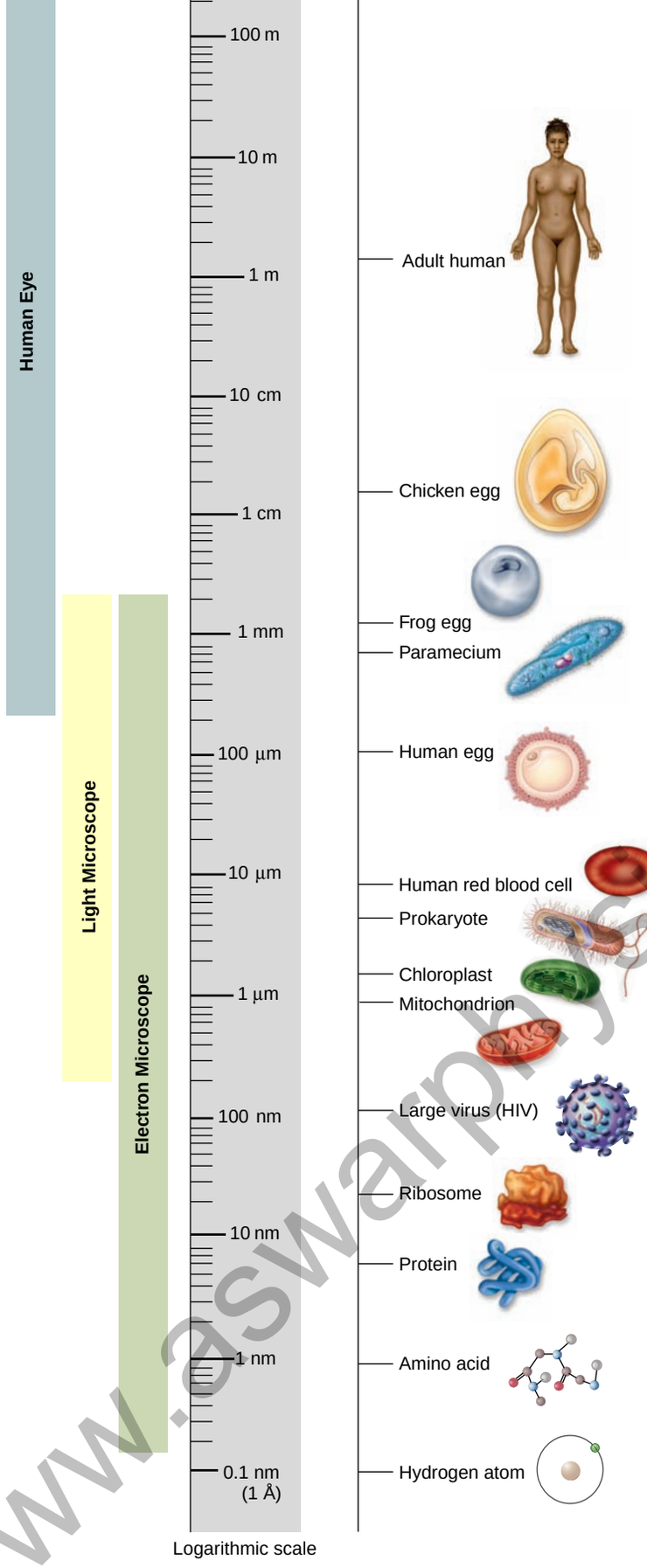


Figure 4.2 The size of cells and their contents. Except for vertebrate eggs, which can typically be seen with the unaided eye, most cells are microscopic in size. Prokaryotic cells are generally 1 to 10 µm across.
1 m = 10^2 cm = 10^3 mm = 10^6 µm = 10^9 nm

Types of microscopes

One way to overcome the limitations of our eyes is to increase magnification so that small objects appear larger. The first microscopists used glass lenses to magnify small cells and cause them to appear larger than the 100-µm limit imposed by the human eye. The glass lens increases focusing power. Because the glass lens makes the object appear closer, the image on the back of the eye is bigger than it would be without the lens.

Modern *light microscopes*, which operate with visible light, use two magnifying lenses (and a variety of correcting lenses) to achieve very high magnification and clarity (table 4.1). The first lens focuses the image of the object on the second lens, which magnifies it again and focuses it on the back of the eye. Microscopes that magnify in stages using several lenses are called *compound microscopes*. They can resolve structures that are separated by at least 200 nanometers (nm).

Light microscopes, even compound ones, are not powerful enough to resolve many of the structures within cells. For example, a cell membrane is only 5 nm thick. Why not just add another magnifying stage to the microscope to increase its resolving power? This doesn't work because when two objects are closer than a few hundred nanometers, the light beams reflecting from the two images start to overlap each other. The only way two light beams can get closer together and still be resolved is if their wavelengths are shorter. One way to avoid overlap is by using a beam of electrons rather than a beam of light. Electrons have a much shorter wavelength, and an *electron microscope*, employing electron beams, has 1000 times the resolving power of a light microscope. *Transmission electron microscopes*, so called because the electrons used to visualize the specimens are transmitted through the material, are capable of resolving objects only 0.2 nm apart—which is only twice the diameter of a hydrogen atom!

A second kind of electron microscope, the *scanning electron microscope*, beams electrons onto the surface of the specimen. The electrons reflected back from the surface, together with other electrons that the specimen itself emits as a result of the bombardment, are amplified and transmitted to a screen, where the image can be viewed and photographed. Scanning electron microscopy yields striking three-dimensional images. This technique has improved our understanding of many biological and physical phenomena (see table 4.1).

Using stains to view cell structure

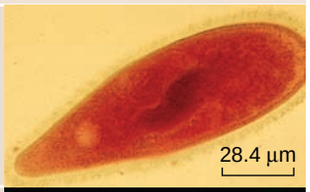
Although resolution remains a physical limit, we can improve the images we see by altering the sample. Certain chemical stains increase the contrast between different cellular components. Structures within the cell absorb or exclude the stain differentially, producing contrast that aids resolution.

Stains that bind to specific types of molecules have made these techniques even more powerful. This method uses antibodies that bind, for example, to a particular protein. This process, called *immunohistochemistry*, uses antibodies generated in animals such as rabbits or mice. When these animals are injected with specific proteins, they produce antibodies that bind to the injected protein. The antibodies are then purified and chemically bonded to enzymes, to stains, or to fluorescent molecules. When cells are incubated in a solution containing the antibodies, the antibodies

TABLE 4.1 Microscopes

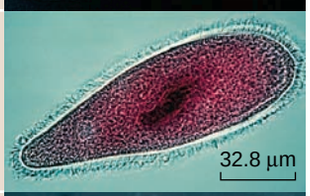
LIGHT MICROSCOPES

Bright-field microscope:
Light is transmitted through a specimen, giving little contrast. Staining specimens improves contrast but requires that cells be fixed (not alive), which can distort or alter components.

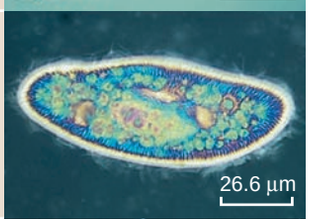


Dark-field microscope:
Light is directed at an angle toward the specimen. A condenser lens transmits only light reflected off the specimen. The field is dark, and the specimen is light against this dark background.

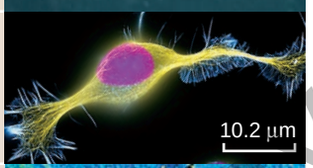
Phase-contrast microscope:
Components of the microscope bring light waves out of phase, which produces differences in contrast and brightness when the light waves recombine.



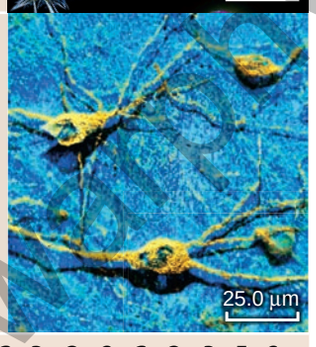
Differential-interference–contrast microscope:
Polarized light is split into two beams that have slightly different paths through the sample. Combining these two beams produces greater contrast, especially at the edges of structures.



Fluorescence microscope:
Fluorescent stains absorb light at one wavelength, then emit it at another. Filters transmit only the emitted light.

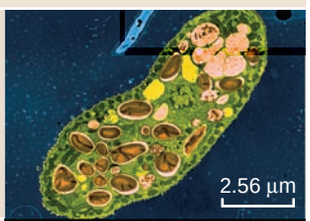


Confocal microscope:
Light from a laser is focused to a point and scanned across the fluorescently stained specimen in two directions. This produces clear images of one plane of the specimen. Other planes of the specimen are excluded to prevent the blurring of the image. Multiple planes can be used to reconstruct a 3-D image.

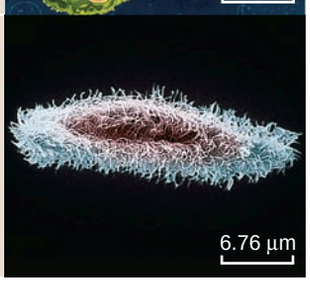


ELECTRON MICROSCOPES

Transmission electron microscope:
A beam of electrons is passed through the specimen. Electrons that pass through are used to expose film. Areas of the specimen that scatter electrons appear dark. False coloring enhances the image.



Scanning electron microscope:
An electron beam is scanned across the surface of the specimen, and electrons are knocked off the surface. Thus, the topography of the specimen determines the contrast and the content of the image. False coloring enhances the image.



bind to cellular structures that contain the target molecule and can be seen with light microscopy. This approach has been used extensively in the analysis of cell structure and function.

All cells exhibit basic structural similarities

The general plan of cellular organization varies between different organisms, but despite these modifications, all cells resemble one another in certain fundamental ways. Before we begin a detailed examination of cell structure, let's first summarize four major features all cells have in common: (1) a nucleoid or nucleus where genetic material is located, (2) cytoplasm, (3) *ribosomes* to synthesize proteins, and (4) a plasma membrane.

Centrally located genetic material

Every cell contains DNA, the hereditary molecule. In **prokaryotes,** the simplest organisms, most of the genetic material lies in a single circular molecule of DNA. It typically resides near the center of the cell in an area called the **nucleoid.** This area is not segregated, however, from the rest of the cell's interior by membranes.

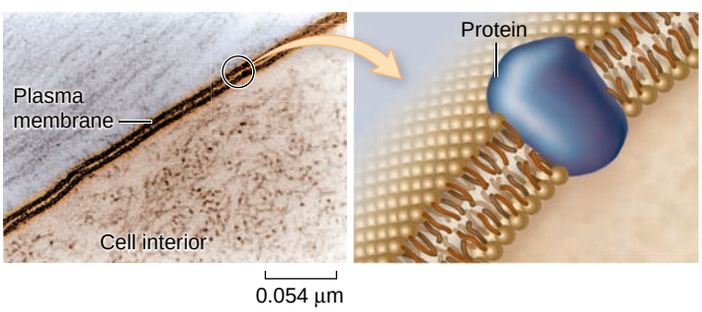
By contrast, the DNA of eukaryotes, which are more complex organisms, is contained in the nucleus, which is surrounded by a double-membrane structure called the nuclear envelope. In both types of organisms, the DNA contains the genes that code for the proteins synthesized by the cell. (Details of nucleus structure are described later in the chapter.)

The cytoplasm

A semifluid matrix called the **cytoplasm** fills the interior of the cell. The cytoplasm contains all of the sugars, amino acids, and proteins the cell uses to carry out its everyday activities. Although it is an aqueous medium, cytoplasm is more like jello than water due to the high concentration of proteins and other macromolecules. We call any discrete macromolecular structure in the cytoplasm specialized for a particular function an **organelle.** The part of the cytoplasm that contains organic molecules and ions in solution is called the **cytosol** to distinguish it from the larger organelles suspended in this fluid.

The plasma membrane

The **plasma membrane** encloses a cell and separates its contents from its surroundings. The plasma membrane is a phospholipid bilayer about 5 to 10 nm (5 to 10 billionths of a meter) thick, with proteins embedded in it. Viewed in cross section with the electron microscope, such membranes appear as two dark lines separated by a lighter area. This distinctive appearance arises from the tail-to-tail packing of the phospholipid molecules that make up the membrane (see chapter 5).



The proteins of the plasma membrane are generally responsible for a cell's ability to interact with the environment. *Transport proteins* help molecules and ions move across the plasma membrane, either from the environment to the interior of the cell or vice versa. *Receptor proteins* induce changes within the cell when they come in contact with specific molecules in the environment, such as hormones, or with molecules on the surface of neighboring cells. These molecules can function as *markers* that identify the cell as a particular type. This interaction between cell surface molecules is especially important in multicellular organisms, whose cells must be able to recognize one another as they form tissues.

We'll examine the structure and function of cell membranes more thoroughly in chapter 5.

Learning Outcomes Review 4.1

All organisms are single cells or aggregates of cells, and all cells arise from preexisting cells. Cell size is limited primarily by the efficiency of diffusion across the plasma membrane. As a cell becomes larger, its volume increases more quickly than its surface area. Past a certain point, diffusion cannot support the cell's needs. All cells are bounded by a plasma membrane and filled with cytoplasm. The genetic material is found in the central portion of the cell; and in eukaryotic cells, it is contained in a membrane-bounded nucleus.

- ***Would finding life on Mars change our view of cell theory?***

4.2 Prokaryotic Cells

Learning Outcomes

1. ***Describe the organization of prokaryotic cells.***
2. ***Distinguish between bacterial and archaeal cell types.***

When cells were visualized with microscopes, two basic cellular architectures were recognized: eukaryotic and prokaryotic. These terms refer to the presence or absence, respectively, of a membrane-bounded nucleus that contains genetic material. We have already mentioned that in addition to lacking a nucleus, prokaryotic cells do not have an internal membrane system or numerous membrane-bounded organelles.

Prokaryote cells have relatively simple organization

Prokaryotes are the simplest organisms. Prokaryotic cells are small. They consist of cytoplasm surrounded by a plasma membrane and are encased within a rigid **cell wall.** They have no distinct interior compartments (figure 4.3). A prokaryotic cell is like a one-room cabin in which eating, sleeping, and watching TV all occur.

Prokaryotes are very important in the ecology of living organisms. Some harvest light by photosynthesis, others break down dead organisms and recycle their components. Still others cause disease or have uses in many important industrial processes. There are two main domains of prokaryotes: archaea and bacteria. Chapter 28 covers prokaryotic diversity in more detail.

Although prokaryotic cells do contain organelles like **ribosomes,** which carry out protein synthesis, most lack the membrane-bounded organelles characteristic of eukaryotic cells. It was long thought that prokaryotes also lack the elaborate cytoskeleton found in eukaryotes, but we have now found they have molecules related to both actin and tubulin, which form two of the cytoskeletal elements described later in the chapter. The actin-like proteins form supporting fibrils near the surface of the cell, but the cytoplasm of a prokaryotic cell does not appear to have an extensive internal support structure. Consequently, the strength of the cell comes primarily from its rigid cell wall (see figure 4.3).

The plasma membrane of a prokaryotic cell carries out some of the functions organelles perform in eukaryotic cells. For example, some photosynthetic bacteria, such as the

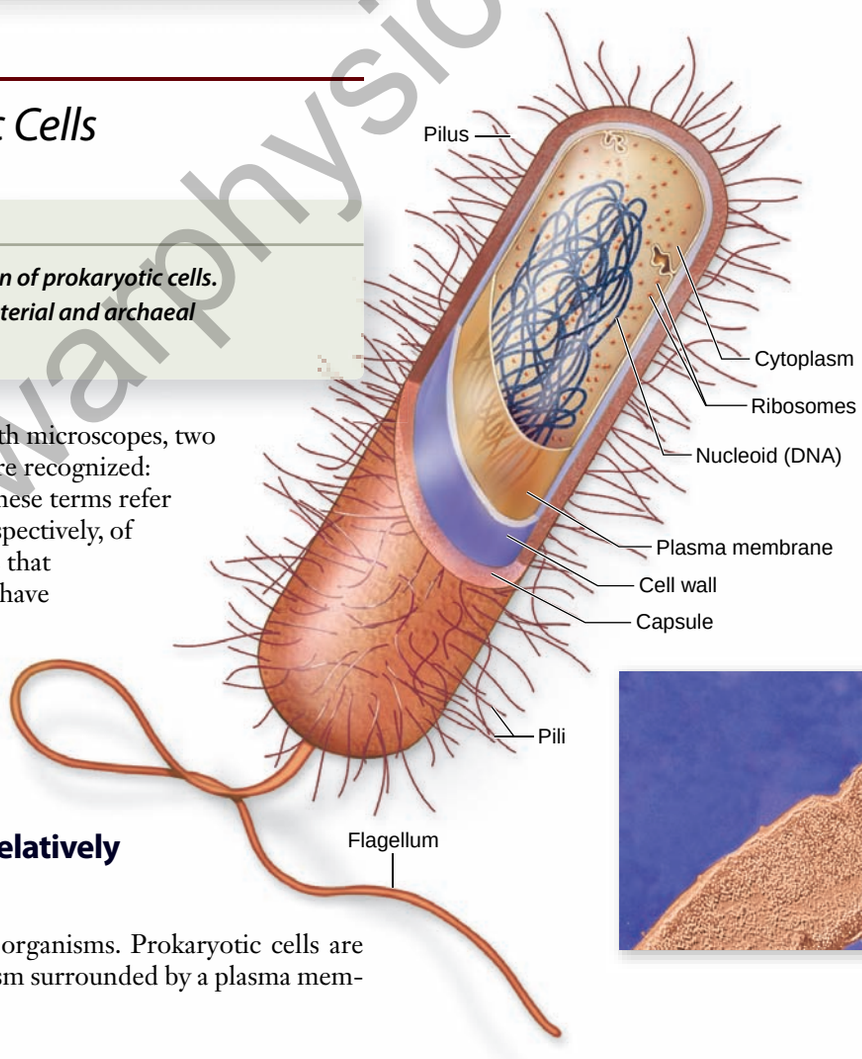


Figure 4.3
Structure of a prokaryotic cell. Generalized cell organization of a prokaryote. The nucleoid is visible as a dense central region segregated from the cytoplasm. Some prokaryotes have hairlike growths (called pili [singular, pilus]) on the outside of the cell.

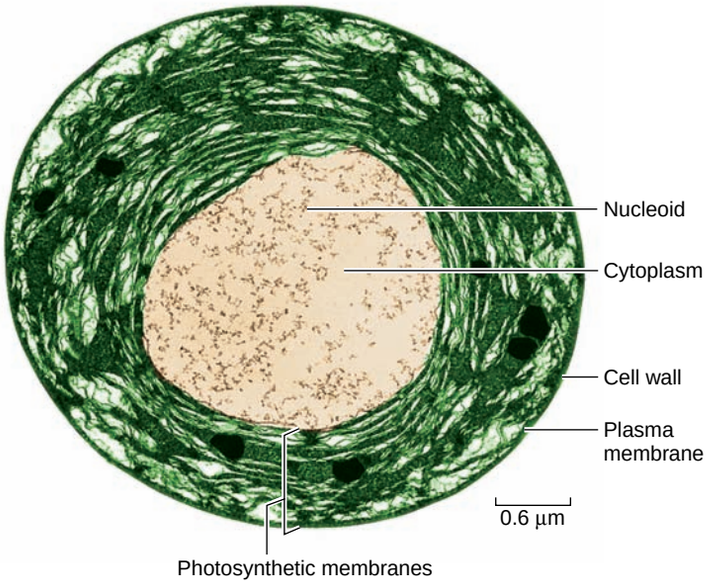


Figure 4.4 Electron micrograph of a photosynthetic bacterial cell. Extensive folded photosynthetic membranes are shown in green in this false color electron micrograph of a *Prochloron* cell.

Inquiry question

What modifications would you include if you wanted to make a cell as large as possible?

cyanobacterium *Prochloron* (figure 4.4), have an extensively folded plasma membrane, with the folds extending into the cell's interior. These membrane folds contain the bacterial pigments connected with photosynthesis. In eukaryotic plant cells, photosynthetic pigments are found in the inner membrane of the chloroplast.

Because a prokaryotic cell contains no membrane-bounded organelles, the DNA, enzymes, and other cytoplasmic constituents have access to all parts of the cell. Reactions are not compartmentalized as they are in eukaryotic cells, and the whole prokaryote operates as a single unit.

Bacterial cell walls consist of peptidoglycan

Most bacterial cells are encased by a strong cell wall. This cell wall is composed of *peptidoglycan*, which consists of a carbohydrate matrix (polymers of sugars) that is cross-linked by short polypeptide units. Details about the structure of this cell wall are discussed in chapter 28. Cell walls protect the cell, maintain its shape, and prevent excessive uptake or loss of water. The exception is the class Mollicutes, which includes the common genus *Mycoplasma*, which lack a cell wall. Plants, fungi, and most protists also have cell walls but with a chemical structure different from peptidoglycan.

The susceptibility of bacteria to antibiotics often depends on the structure of their cell walls. The drugs penicillin and vancomycin, for example, interfere with the ability of bacteria to cross-link the peptides in their peptidoglycan cell wall. Like removing all the nails from a wooden house, this destroys the integrity of the structural matrix, which can no longer prevent water from rushing in and swelling the cell to bursting.

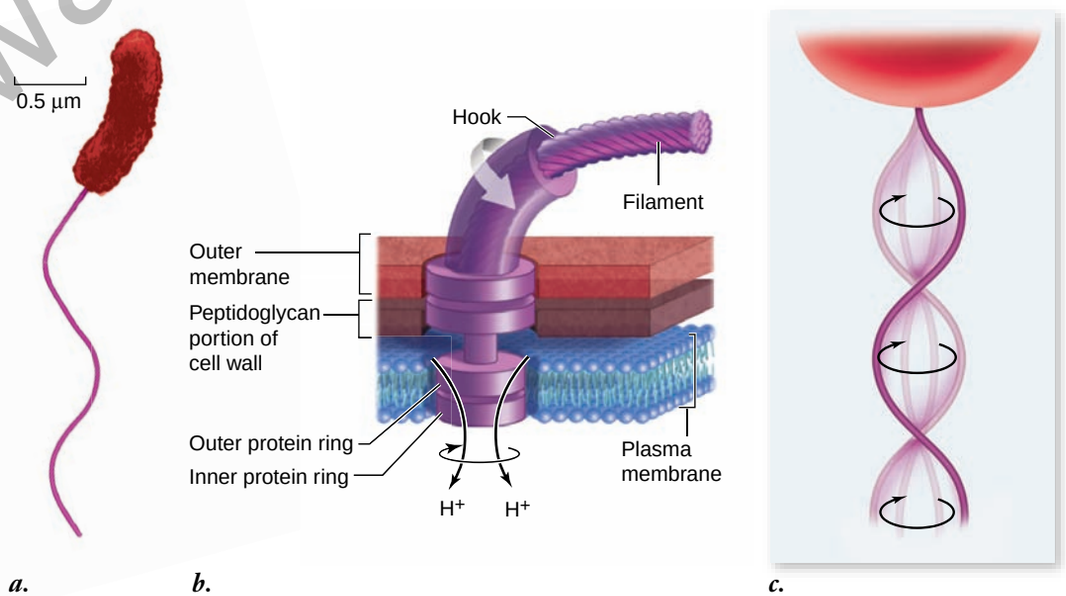
Some bacteria also secrete a jelly-like protective capsule of polysaccharide around the cell. Many disease-causing bacteria have such a capsule, which enables them to adhere to teeth, skin, food—or to practically any surface that will support their growth.

Archaea lack peptidoglycan

We are still learning about the physiology and structure of archaea. Many of these organisms are difficult to culture in the laboratory, and so this group has not yet been studied in detail. More is known about their genetic makeup than about any other feature.

The cell walls of archaea are composed of various chemical compounds, including polysaccharides and proteins, and possibly even inorganic components. A common feature

Figure 4.5 Some prokaryotes move by rotating their flagella. ***a.*** The photograph shows *Vibrio cholerae*, the microbe that causes the serious disease cholera. ***b.*** The bacterial flagellum is a complex structure. The motor proteins, powered by a proton gradient, are anchored in the plasma membrane. Two rings are found in the cell wall. The motor proteins cause the entire structure to rotate. ***c.*** As the flagellum rotates it creates a spiral wave down the structure. This powers the cell forward.



distinguishing archaea from bacteria is the nature of their membrane lipids. The chemical structure of archaeal lipids is distinctly different from that of lipids in bacteria and can include saturated hydrocarbons that are covalently attached to glycerol at both ends, such that their membrane is a monolayer. These features seem to confer greater thermal stability to archaeal membranes, although the tradeoff seems to be an inability to alter the degree of saturation of the hydrocarbons—meaning that archaea with this characteristic cannot adapt to changing environmental temperatures.

The cellular machinery that replicates DNA and synthesized proteins in archaea is more closely related to eukaryotic systems than to bacterial systems. Even though they share a similar overall cellular architecture with prokaryotes, archaea appear to be more closely related on a molecular basis to eukaryotes.

Some prokaryotes move by means of rotating flagella

Flagella (singular, *flagellum*) are long, threadlike structures protruding from the surface of a cell that are used in locomotion. Prokaryotic flagella are protein fibers that extend out from the cell. There may be one or more per cell, or none, depending on the species. Bacteria can swim at speeds of up to 70 cell lengths per second by rotating their flagella like screws (figure 4.5). The rotary motor uses the energy stored in a gradient that transfers protons across the plasma membrane to power the movement of the flagellum. Interestingly, the same principle, in which a proton gradient powers the rotation of a molecule, is used in eukaryotic mitochondria and chloroplasts by an enzyme that synthesizes ATP (see chapters 7 and 8).

Learning Outcomes Review 4.2

Prokaryotes are small cells that lack complex interior organization. The two domains of prokaryotes are archaea and bacteria. The cell wall of bacteria is composed of peptidoglycan, which is not found in archaea. Archaea have cell walls made from a variety of polysaccharides and peptides, as well as membranes containing unusual lipids. Some bacteria move using a rotating flagellum.

- ***What features do bacteria and archaea share?***

4.3 Eukaryotic Cells

Learning Outcomes

1. ***Compare the organization of eukaryotic and prokaryotic cells.***
2. ***Discuss the role of the nucleus in eukaryotic cells.***
3. ***Describe the role of ribosomes in protein synthesis.***

Eukaryotic cells (figures 4.6 and 4.7) are far more complex than prokaryotic cells. The hallmark of the eukaryotic cell is compartmentalization. This is achieved through a combination of an extensive **endomembrane system** that weaves through the cell interior and by numerous *organelles.* These organelles include membrane-bounded structures that form 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 wound tightly around proteins and packaged into compact units called **chromosomes.**

All eukaryotic cells are supported by an internal protein scaffold, the **cytoskeleton.** Although 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. Through the rest of this chapter, we will examine the internal components of eukaryotic cells in more detail.

The nucleus acts as the information center

The largest and most easily seen organelle within a eukaryotic cell is the **nucleus** (Latin, "kernel" or "nut"), first described by the botanist Robert Brown in 1831. Nuclei are roughly spherical in shape, and in animal cells, they are typically located in the central region of the cell (figure 4.8*a*). In some cells, a network of fine cytoplasmic filaments seems to cradle the nucleus in this position.

The nucleus is the repository of the genetic information that enables the synthesis of nearly all proteins of a living eukaryotic cell. Most eukaryotic cells possess a single nucleus, although the cells of fungi and some other groups may have several to many nuclei. Mammalian erythrocytes (red blood cells) lose their nuclei when they mature. Many nuclei exhibit a dark-staining zone called the **nucleolus,** which is a region where intensive synthesis of ribosomal RNA is taking place.

The nuclear envelope

The surface of the nucleus is bounded by *two* phospholipid bilayer membranes, which together make up the **nuclear envelope** (see figure 4.8). The outer membrane of the nuclear envelope is continuous with the cytoplasm's interior membrane system, called the *endoplasmic reticulum* (described later).

Scattered over the surface of the nuclear envelope are what appear as shallow depressions in the electron micrograph but are in fact structures called **nuclear pores** (see figure 4.8*b*, *c*). These pores form 50 to 80 nm apart at locations where the two membrane layers of the nuclear envelope pinch together. They have a complex structure with a cytoplasmic face, a nuclear face, and a central ring embedded in the membrane. The proteins that make up this nuclear pore complex are arranged in a circle with a large central hole. The complex allows small molecules to diffuse freely between nucleoplasm and cytoplasm while controlling the passage of proteins and RNA–protein complexes. Passage is restricted primarily to two kinds

Figure 4.6 Structure of an animal cell. In this generalized diagram of an animal cell, the plasma membrane encases the cell, which contains the cytoskeleton and various cell organelles and interior structures suspended in a semifluid matrix called the cytoplasm. Some kinds of animal cells possess finger-like projections called microvilli. Other types of eukaryotic cells—for example, many protist cells—may possess flagella, which aid in movement, or cilia, which can have many different functions.

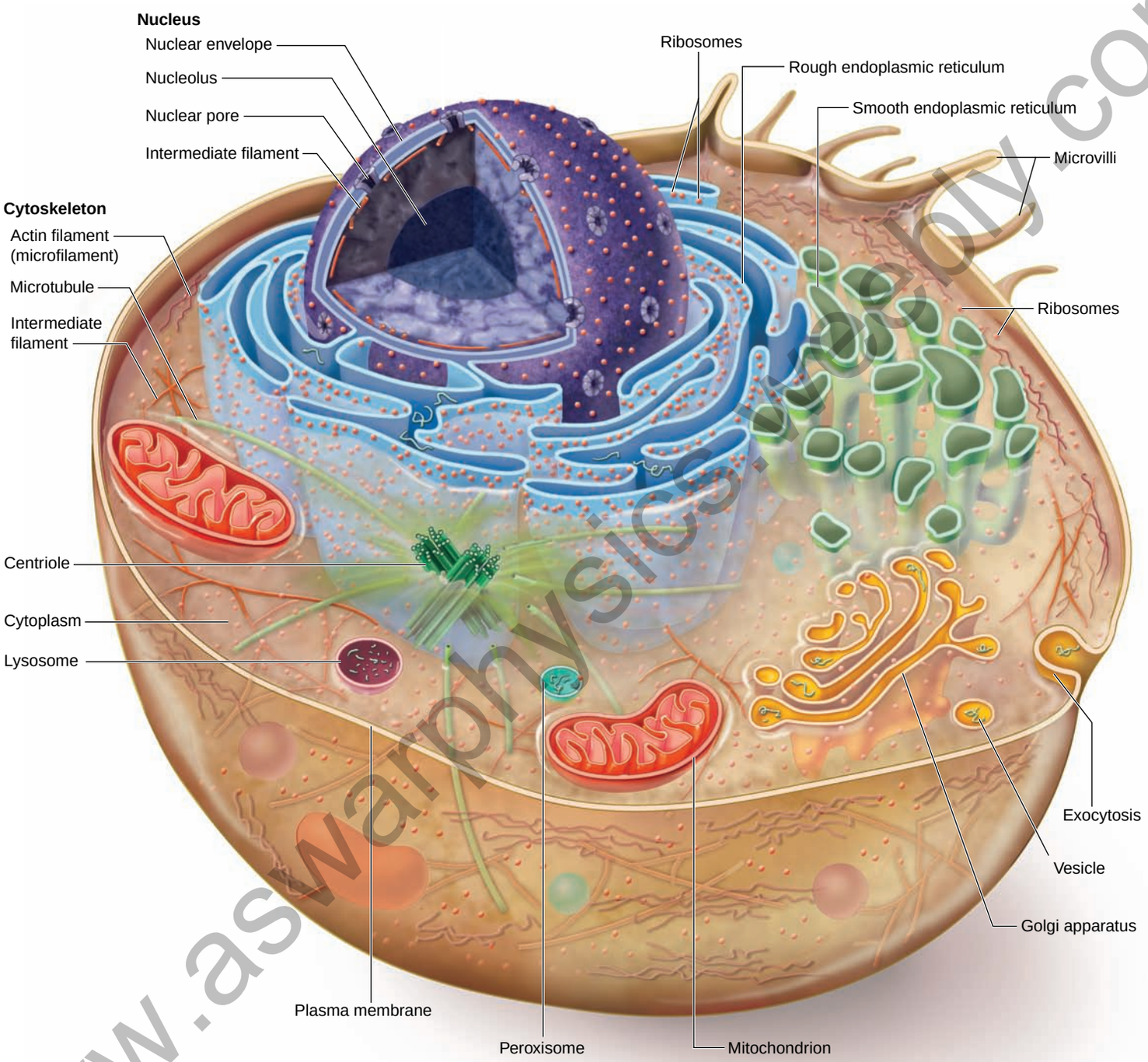
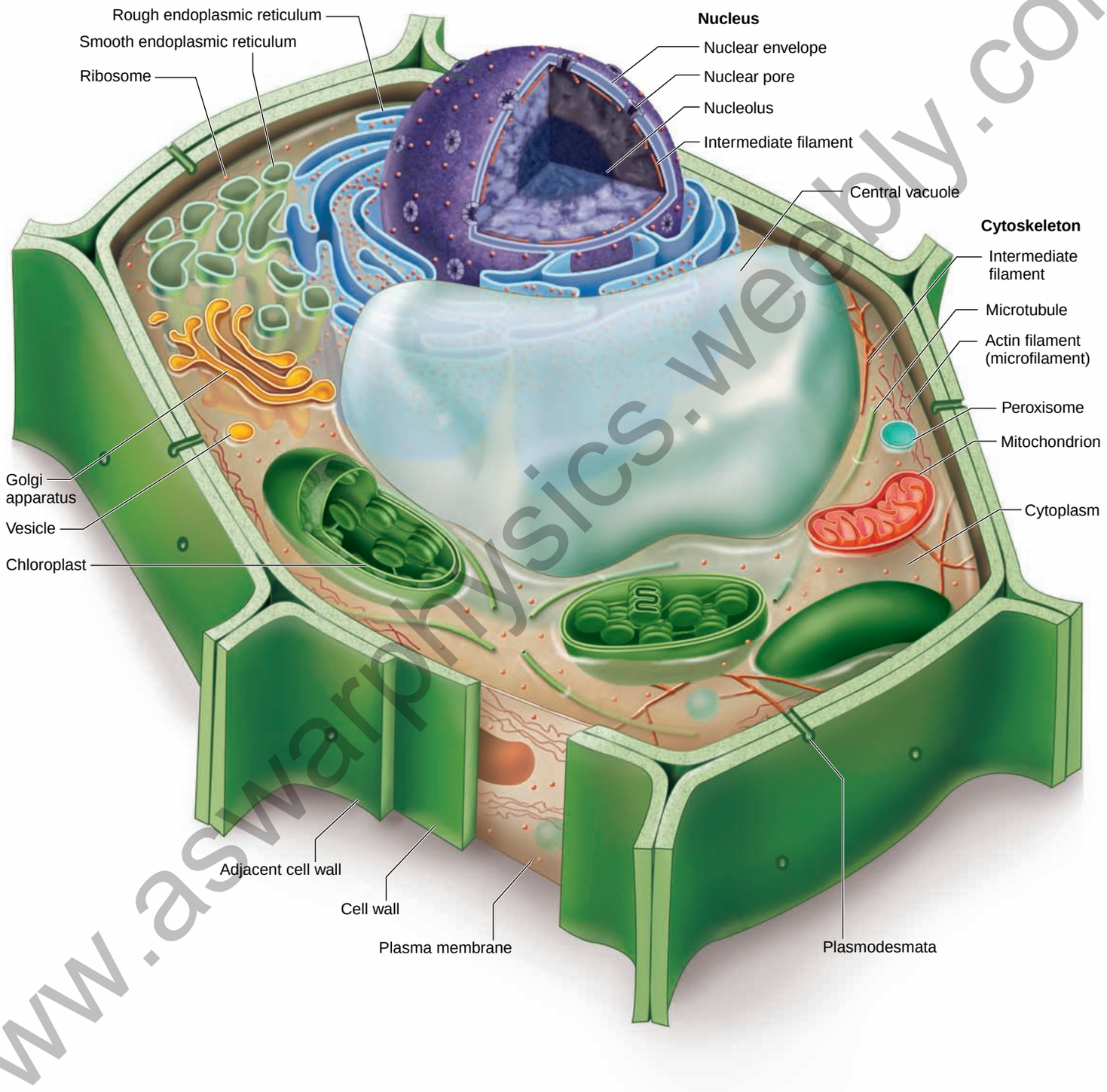


Figure 4.7 Structure of a plant cell. Most mature plant cells contain a large central vacuole, which occupies a major portion of the internal volume of the cell, and organelles called chloroplasts, within which photosynthesis takes place. The cells of plants, fungi, and some protists have cell walls, although the composition of the walls varies among the groups. Plant cells have cytoplasmic connections to one another through openings in the cell wall called plasmodesmata. Flagella occur in sperm of a few plant species, but are otherwise absent from plant and fungal cells. Centrioles are also usually absent.



of molecules: (1) proteins moving into the nucleus to be incorporated into nuclear structures or to catalyze nuclear activities and (2) RNA and RNA–protein complexes formed in the nucleus and exported to the cytoplasm.

The inner surface of the nuclear envelope is covered with a network of fibers that make up the nuclear lamina (see figure 4.8*d*). This is composed of intermediate filament fibers called *nuclear lamins*. This structure gives the nucleus its shape and is also involved in the deconstruction and reconstruction of the nuclear envelope that accompanies cell division.

Chromatin: DNA packaging

In both prokaryotes and eukaryotes, DNA contains the hereditary information specifying cell structure and function. In most prokaryotes, the DNA is organized into a single circular chromosome. In eukaryotes, the DNA is divided into multiple linear chromosomes. The DNA in these chromosomes is organized with proteins into a complex structure called **chromatin.**

Chromatin is usually in a more extended form that allows regulatory proteins to attach to specific nucleotide sequences along the DNA and regulate gene expression. Without this access, DNA could not direct the day-to-day activities of the cell. When cells divide, the chromatin must be further compacted into a more highly condensed form.

The nucleolus: Ribosomal subunit manufacturing

Before cells can synthesize proteins in large quantity, they must first construct a large number of ribosomes to carry out this synthesis. Hundreds of copies of the genes encoding the ribosomal RNAs are clustered together on the chromosome, facilitating ribsosome construction. By transcribing RNA molecules from this cluster, the cell rapidly generates large numbers of the molecules needed to produce ribosomes.

The clusters of ribosomal RNA genes, the RNAs they produce, and the ribosomal proteins all come together within the nucleus during ribosome production. These ribosomal assembly areas are easily visible within the nucleus as one or more dark-staining regions called nucleoli (singular, nucleolus). Nucleoli can be seen under the light microscope even when the chromosomes are uncoiled.

Ribosomes are the cell's protein synthesis machinery

Although the DNA in a cell's nucleus encodes the amino acid sequence of each protein in the cell, the proteins are not assembled there. A simple experiment demonstrates this: If a brief pulse of radioactive amino acid is administered to a cell, the radioactivity shows up associated with newly made protein in the cytoplasm, not in the nucleus. When investigators first carried out these experiments, they found that protein synthesis is associated with large RNA–protein complexes (called ribosomes) outside the nucleus.

Ribosomes are among the most complex molecular assemblies found in cells. Each ribosome is composed of two

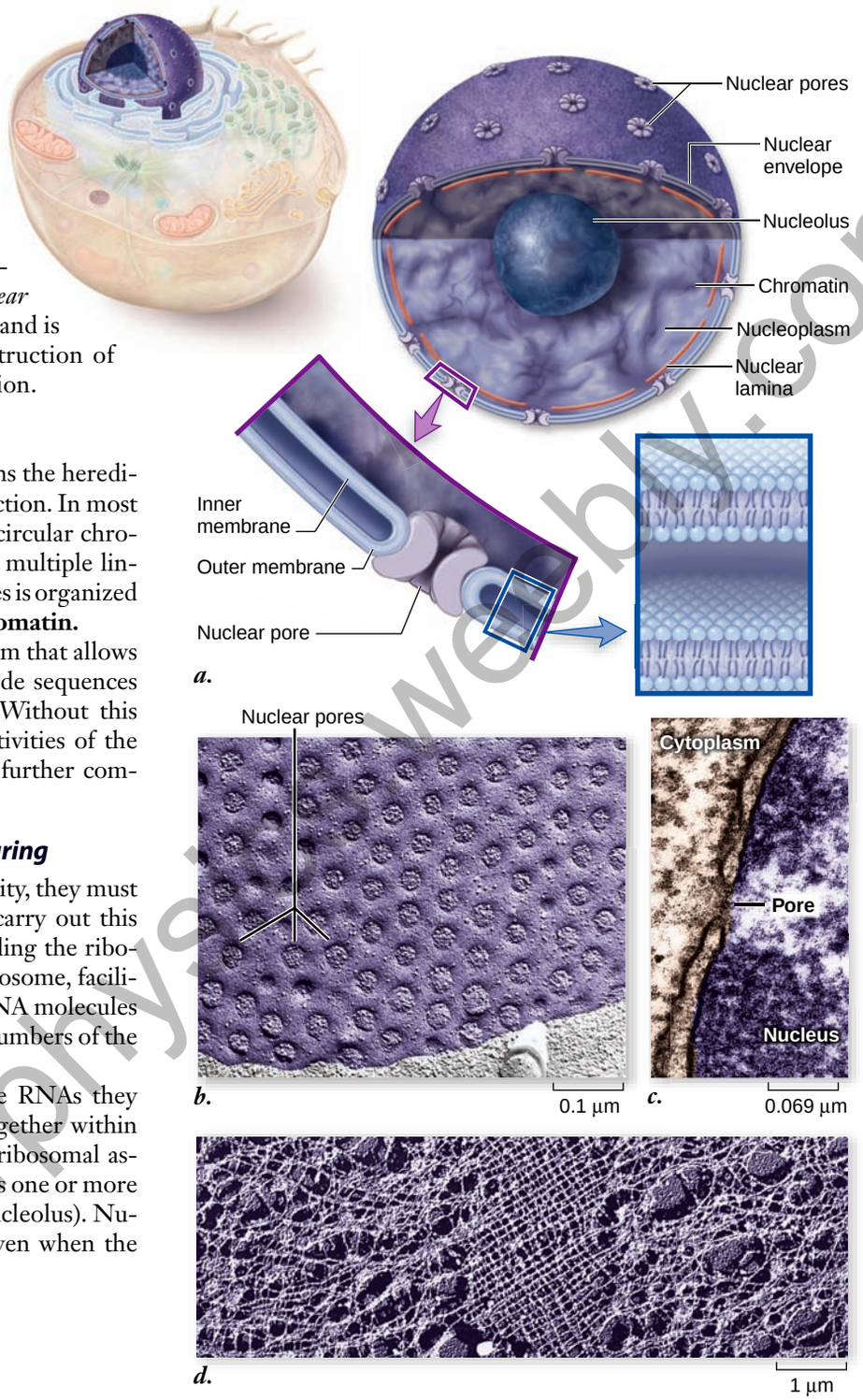


Figure 4.8 The nucleus. ***a.*** The nucleus is composed of a double membrane called the nuclear envelope, enclosing a fluid-filled interior containing chromatin. The individual nuclear pores extend through the two membrane layers of the envelope. ***b.*** A freeze-fracture electron micrograph (see figure 5.3) of a cell nucleus, showing many nuclear pores. ***c.*** A transmission electron micrograph of the nuclear membrane showing a single nuclear pore. The dark material within the pore is protein, which acts to control access through the pore. ***d.*** The nuclear lamina is visible as a dense network of fibers made of intermediate filaments. The nucleus has been colored purple in the micrographs.

(b): © Dr. Richard Kessel & Dr. Gene Shih/Visuals Unlimited

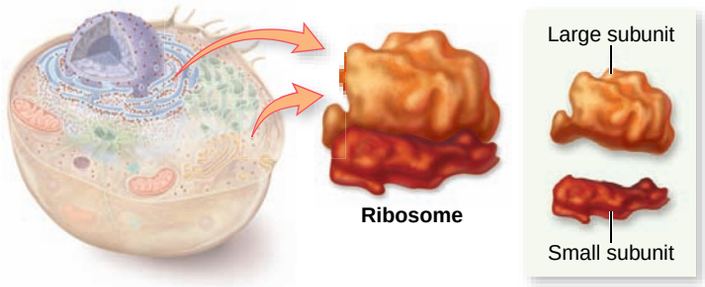


Figure 4.9 A ribosome. Ribosomes consist of a large and a small subunit composed of rRNA and protein. The individual subunits are synthesized in the nucleolus and then move through the nuclear pores to the cytoplasm, where they assemble to translate mRNA. Ribosomes serve as sites of protein synthesis.

subunits (figure 4.9), and each subunit is composed of a combination of RNA, called **ribosomal RNA (rRNA),** and proteins. The subunits join to form a functional ribosome only when they are actively synthesizing proteins. This complicated process requires the two other main forms of RNA: **messenger RNA (mRNA),** which carries coding information from DNA, and **transfer RNA (tRNA),** which carries amino acids. Ribosomes use the information in mRNA to direct the synthesis of a protein. This process will be described in more detail in chapter 15.

Ribosomes are found either free in the cytoplasm or associated with internal membranes, as described in the following section. Free ribosomes synthesize proteins that are found in the cytoplasm, nuclear proteins, mitochondrial proteins, and proteins found in other organelles not derived from the endomembrane system. Membrane-associated ribosomes synthesize membrane proteins, proteins found in the endomembrane system, and proteins destined for export from the cell.

Ribosomes can be thought of as "universal organelles" because they are found in all cell types from all three domains of life. As we build a picture of the minimal essential functions for cellular life, ribosomes will be on the short list. Life is protein-based, and ribosomes are the factories that make proteins.

Learning Outcomes Review 4.3

In contrast to prokaryotic cells, eukaryotic cells exhibit compartmentalization. Eukaryotic cells contain an endomembrane system and organelles that carry out specialized functions. The nucleus, composed of a double membrane connected to the endomembrane system, contains the cell's genetic information. Material moves between the nucleus and cytoplasm through nuclear pores. Ribosomes translate mRNA, which is transcribed from DNA in the nucleus, into polypeptides that make up proteins. Ribosomes are a universal organelle found in all known cells.

- ***Would you expect cells in different organs in complex animals to have the same structure?***

4.4 The Endomembrane System

Learning Outcomes

1. ***Identify the different parts of the endomembrane system.***
2. ***Contrast the different functions of internal membranes and compartments.***
3. ***Evaluate the importance of each step in the protein processing pathway.***

The interior of a eukaryotic cell is packed with membranes so thin that they are invisible under the low resolving power of light microscopes. This endomembrane system fills the cell, dividing it into compartments, channeling the passage of molecules through the interior of the cell, and providing surfaces for the synthesis of lipids and some proteins. The presence of these membranes in eukaryotic cells marks one of the fundamental distinctions between eukaryotes and prokaryotes.

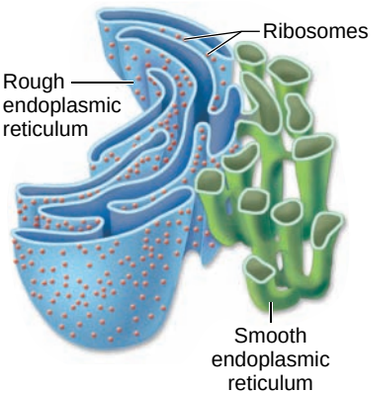
The largest of the internal membranes is called the **endoplasmic reticulum (ER).** *Endoplasmic* means "within the cytoplasm," and *reticulum* is Latin for "a little net." Like the plasma membrane, the ER is composed of a phospholipid bilayer embedded with proteins. It weaves in sheets through the interior of the cell, creating a series of channels between its folds (figure 4.10). Of the many compartments in eukaryotic cells, the two largest are the inner region of the ER, called the **cisternal space** or **lumen,** and the region exterior to it, the cytosol, which is the fluid component of the cytoplasm containing dissolved organic molecules such as proteins and ions.

The rough ER is a site of protein synthesis

The **rough ER (RER)** gets its name from its surface appearance, which is pebbly due to the presence of ribosomes. The RER is not easily visible with a light microscope, but it can be seen using the electron microscope. It appears to be composed of flattened sacs, the surfaces of which are bumpy with ribosomes (see figure 4.10).

The proteins synthesized on the surface of the RER are destined to be exported from the cell, sent to lysosomes or vacuoles (described in a later section), or embedded in the plasma membrane. These proteins enter the cisternal space as a first step in the pathway that will sort proteins to their eventual destinations. This pathway also involves vesicles and the Golgi apparatus, described later. The sequence of the protein being synthesized determines whether the ribosome will become associated with the ER or remain a cytoplasmic ribosome.

In the ER, newly synthesized proteins can be modified by the addition of short-chain carbohydrates to form **glycoproteins.** Those proteins destined for secretion are separated from other products and later packaged into vesicles. The ER also manufactures membranes by producing membrane proteins and phospholipid molecules. The membrane



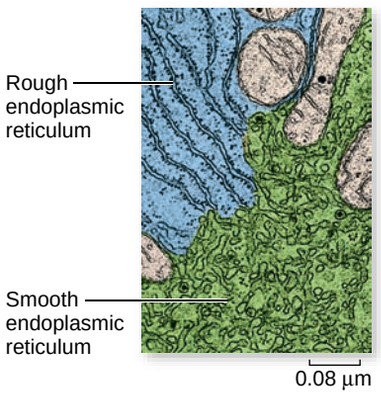


Figure 4.10 The endoplasmic reticulum. Rough ER (RER), blue in the drawing, is composed more of flattened sacs and forms a compartment throughout the cytoplasm. Ribosomes associated with the cytoplasmic face of the RER extrude newly made proteins into the interior, or lumen. The smooth ER (SER), green in the drawing, is a more tubelike structure connected to the RER. The micrograph has been colored to match the drawing.

proteins are inserted into the ER's own membrane, which can then expand and pinch off in the form of vesicles to be transferred to other locations.

The smooth ER has multiple roles

Regions of the ER with relatively few bound ribosomes are referred to as **smooth ER (SER).** The SER appears more like a network of tubules than the flattened sacs of the RER. The membranes of the SER contain many embedded enzymes. Enzymes anchored within the ER, for example, catalyze the synthesis of a variety of carbohydrates and lipids. Steroid hormones are synthesized in the SER as well. The majority of membrane lipids are assembled in the SER and then sent to whatever parts of the cell need membrane components.

The SER is used to store Ca^{2+} in cells. This keeps the cytoplasmic level low, allowing Ca^{2+} to be used as a signaling molecule. In muscle cells, for example, Ca^{2+} is used to trigger muscle contraction. In other cells, Ca^{2+} release from SER stores is involved in diverse signaling pathways.

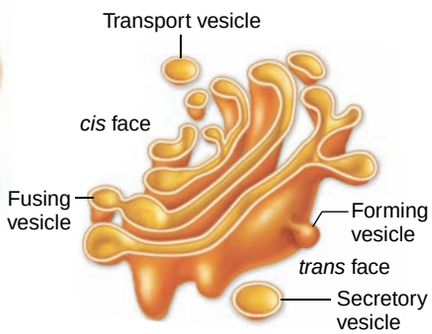
The ratio of SER to RER depends on a cell's function. In multicellular animals such as ourselves, great variation exists in this ratio. Cells that carry out extensive lipid synthesis, such as those in the testes, intestine, and brain, have abundant SER. Cells that synthesize proteins that are secreted, such as antibodies, have much more extensive RER.

Another role of the SER is the modification of foreign substances to make them less toxic. In the liver, the enzymes of the SER carry out this detoxification. This action can include neutralizing substances that we have taken for a therapeutic reason, such as penicillin. Thus, relatively high doses are prescribed for some drugs to offset our body's efforts to remove them. Liver cells have extensive SER as well as enzymes that can process a variety of substances by chemically modifying them.

The Golgi apparatus sorts and packages proteins

Flattened stacks of membranes, often interconnected with one another, form a complex called the **Golgi body.** These structures are named for Camillo Golgi, the 19th-century physician who first identified them. The number of stacked membranes within the Golgi body ranges from 1 or a few in protists, to 20 or more in animal cells and to several hundred in plant cells. They are especially abundant in glandular cells, which manufacture and secrete substances. The Golgi body is often referred to as the **Golgi apparatus** (figure 4.11).

The Golgi apparatus functions in the collection, packaging, and distribution of molecules synthesized at one location and used at another within the cell or even outside of it. A Golgi body has a front and a back, with distinctly different membrane compositions at these opposite ends. The front, or receiving end, is called the *cis* face and is usually located near ER. Materials move to the *cis* face in transport vesicles that bud off the ER. These vesicles fuse with the *cis* face, emptying their contents into the interior, or lumen, of the Golgi apparatus. The ER-synthesized molecules then pass through the channels of the Golgi apparatus until they reach the back, or discharging end,



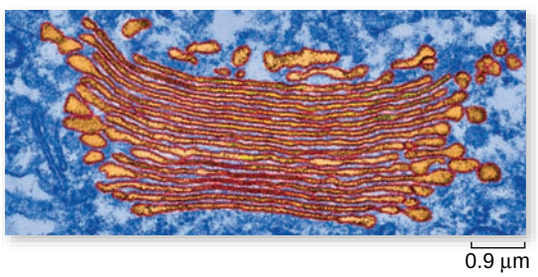


Figure 4.11 The Golgi apparatus. The Golgi apparatus is a smooth, concave, membranous structure. It receives material for processing in transport vesicles on the *cis* face and sends the material packaged in transport or secretory vesicles off the *trans* face. The substance in a vesicle could be for export out of the cell or for distribution to another region within the same cell.

called the *trans* face, where they are discharged in secretory vesicles (figure 4.12).

Proteins and lipids manufactured on the rough and smooth ER membranes are transported into the Golgi apparatus and modified as they pass through it. The most common alteration is the addition or modification of short sugar chains, forming glycoproteins and glycolipids. In many instances, enzymes in the Golgi apparatus modify existing glycoproteins and glycolipids made in the ER by cleaving a sugar from a chain or by modifying one or more of the sugars.

The newly formed or altered glycoproteins and glycolipids collect at the ends of the Golgi bodies in flattened, stacked membrane folds called **cisternae** (Latin, "collecting vessels"). Periodically, the membranes of the cisternae push together, pinching off small, membrane-bounded secretory vesicles containing the glycoprotein and glycolipid molecules. These vesicles then diffuse to other locations in the cell, distributing the newly synthesized molecules to their appropriate destinations.

Another function of the Golgi apparatus is the synthesis of cell wall components. Noncellulose polysaccharides that form part of the cell wall of plants are synthesized in the Golgi apparatus and sent to the plasma membrane where they can be added to the cellulose that is assembled on the exterior of the cell. Other polysaccharides secreted by plants are also synthesized in the Golgi apparatus.

Lysosomes contain digestive enzymes

Membrane-bounded digestive vesicles, called **lysosomes**, are also components of the endomembrane system. They arise from the Golgi apparatus. They contain high levels of degrading enzymes, which catalyze the rapid breakdown of proteins, nucleic acids, lipids, and carbohydrates. Throughout the lives of eukaryotic cells, lysosomal enzymes break down old organelles and recycle their component molecules. This makes room for newly formed organelles. For example, mitochondria are replaced in some tissues every 10 days.

The digestive enzymes in the lysosome are optimally active at acid pH. Lysosomes are activated by fusing with a food vesicle produced by *phagocytosis* (a specific type of endocytosis; see chapter 5) or by fusing with an old or worn-out organelle. The fusion event activates proton pumps in the lysosomal membrane, resulting in a lower internal pH. As the interior pH falls, the arsenal of digestive enzymes contained in the lysosome is activated. This leads to the degradation of macromolecules in the food vesicle or the destruction of the old organelle.

A number of human genetic disorders, collectively called lysosomal storage disorders, affect lysosomes. For example, the genetic abnormality called Tay–Sachs disease is caused by the loss of function of a single lysosomal enzyme. This enzyme is necessary to break down a membrane glycolipid found in nerve cells. Accumulation of glycolipid in lysosomes affects nerve cell function, leading to a variety of clinical symptoms such as seizures and muscle rigidity.

In addition to breaking down organelles and other structures within cells, lysosomes eliminate other cells that

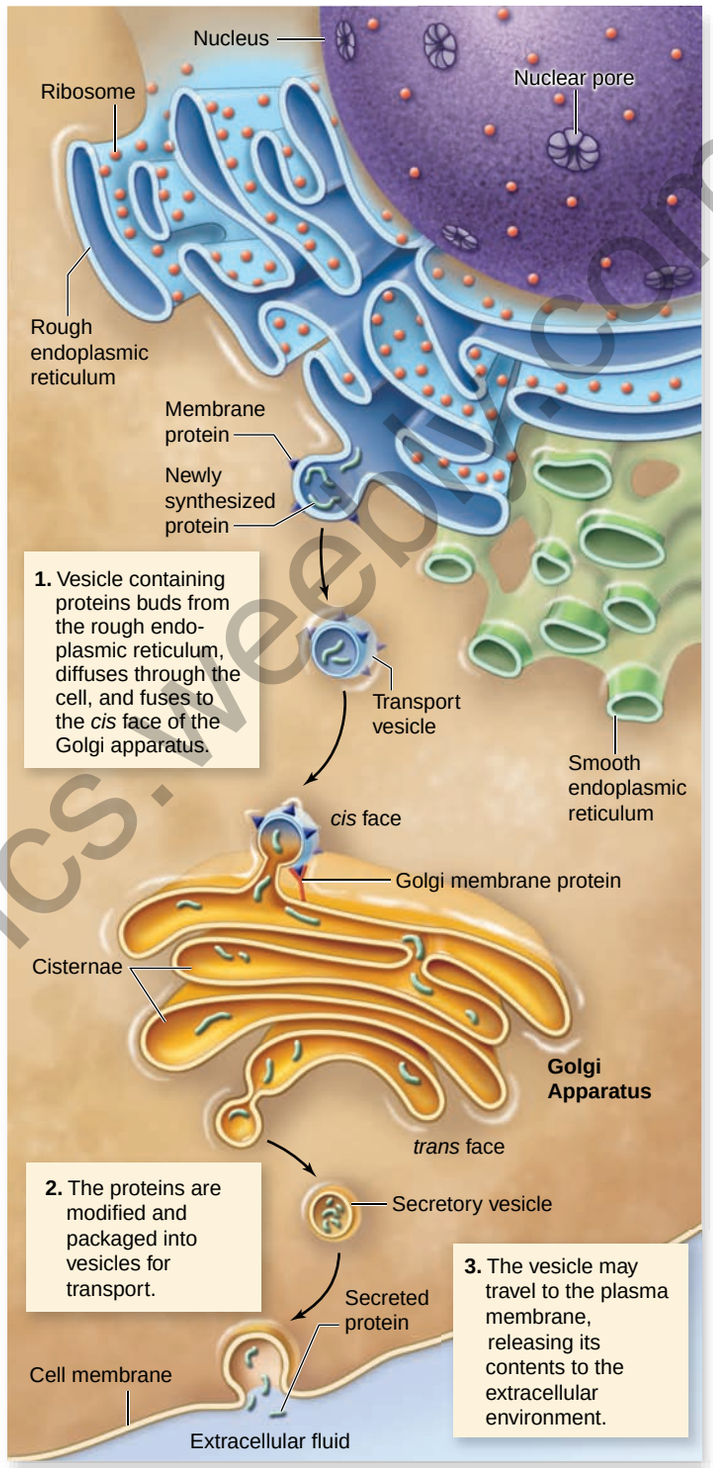


Figure 4.12 Protein transport through the endomembrane system. Proteins synthesized by ribosomes on the RER are translocated into the internal compartment of the ER. These proteins may be used at a distant location within the cell or secreted from the cell. They are transported within vesicles that bud off the rough ER. These transport vesicles travel to the *cis* face of the Golgi apparatus. There they can be modified and packaged into vesicles that bud off the *trans* face of the Golgi apparatus. Vesicles leaving the *trans* face transport proteins to other locations in the cell, or fuse with the plasma membrane, releasing their contents to the extracellular environment.

the cell has engulfed by phagocytosis. 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 (figure 4.13).

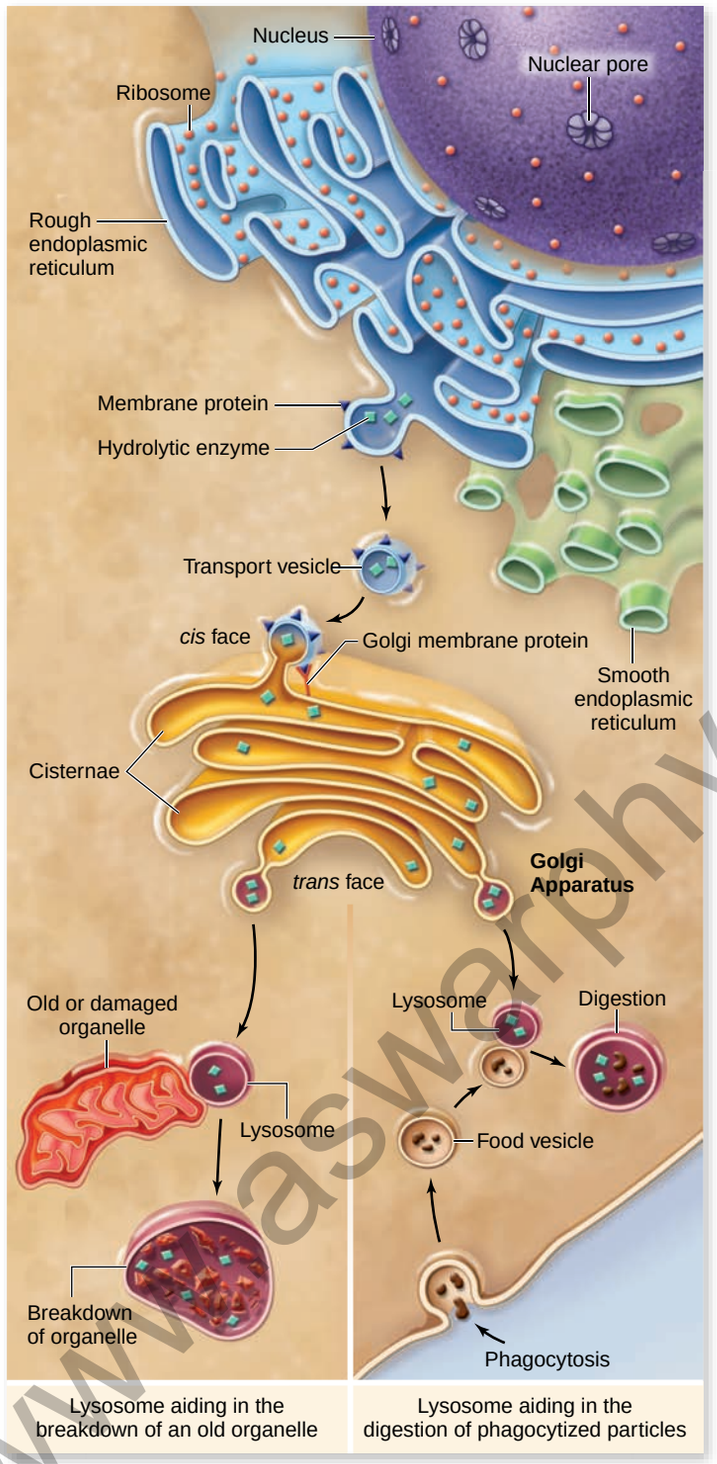


Figure 4.13 Lysosomes. Lysosomes are formed from vesicles budding off the Golgi. They contain hydrolytic enzymes that digest particles or cells taken into the cell by phagocytosis, and break down old organelles.

Microbodies are a diverse category of organelles

Eukaryotic cells contain a variety of enzyme-bearing, membrane-enclosed vesicles called **microbodies.** These are found in the cells of plants, animals, fungi, and protists. The distribution of enzymes into microbodies is one of the principal ways eukaryotic cells organize their metabolism.

Peroxisomes: Peroxide utilization

An important type of microbody is the **peroxisome** (figure 4.14), which contains enzymes involved in the oxidation of fatty acids. If these oxidative enzymes were not isolated within microbodies, they would tend to short-circuit the metabolism of the cytoplasm, which often involves adding hydrogen atoms to oxygen. Because many peroxisomal proteins are synthesized by cytoplasmic ribosomes, the organelles themselves were long thought to form by the addition of lipids and proteins, leading to growth. As they grow larger, they divide to produce new peroxisomes. Although division of peroxisomes still appears to occur, it is now clear that peroxisomes can form from the fusion of ER-derived vesicles. These vesicles then import peroxisomal proteins to form a mature peroxisome. Genetic screens have isolated some 32 genes that encode proteins involved in biogenesis and maintenance of peroxisomes. The human genetic diseases called peroxisome biogenesis disorders (PBDs) appear to be caused by mutations in some of these genes.

Peroxisomes get their name from the hydrogen peroxide produced as a by-product of the activities of oxidative enzymes. Hydrogen peroxide is dangerous to cells because of its violent

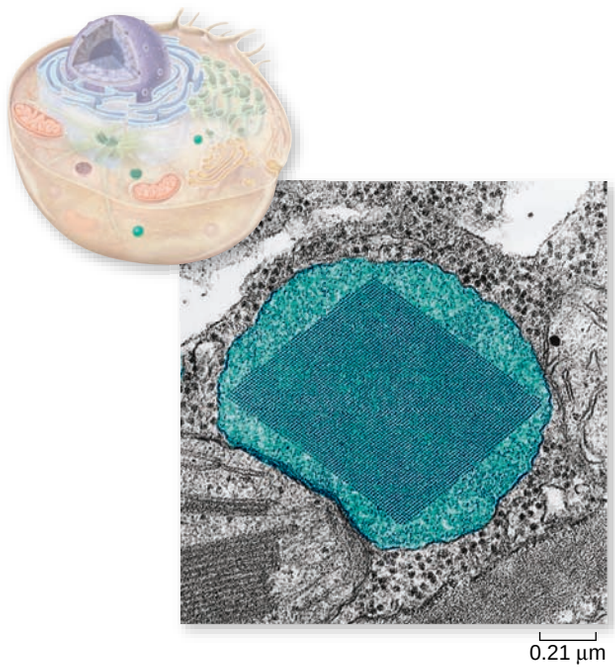


Figure 4.14 A peroxisome. Peroxisomes are spherical organelles that may contain a large crystal structure composed of protein. Peroxisomes contain digestive and detoxifying enzymes that produce hydrogen peroxide as a by-product. A peroxisome has been colored green in the electron micrograph.

chemical reactivity. However, peroxisomes also contain the enzyme catalase, which breaks down hydrogen peroxide into its harmless constituents—water and oxygen.

Plants use vacuoles for storage and water balance

Plant cells have specialized membrane-bounded structures called **vacuoles.** The most conspicuous example is the large central vacuole seen in most plant cells (figure 4.15). In fact, *vacuole* actually means blank space, referring to its appearance in the light microscope. The membrane surrounding this vacuole is called the **tonoplast** because it contains channels for water that are used to help the cell maintain its tonicity, or osmotic balance (see osmosis in chapter 5).

For many years biologists assumed that only one type of vacuole existed and that it served multiple functions. The functions assigned to this vacuole included water balance and storage of both useful molecules (such as sugars, ions and pigments) and waste products. The vacuole was also thought to store enzymes involved in the breakdown of macromolecules and those used in detoxifying foreign substances. Old textbooks of plant physiology referred to vacuoles as the attic of the cell for the variety of substances thought to be stored there.

Studies of tonoplast transporters and the isolation of vacuoles from a variety of cell types have led to a more complex view of vacuoles. These studies have made it clear that different vacuolar types can be found in different cells. These vacuoles are specialized, depending on the function of the cell.

The central vacuole is clearly important for a number of roles in all plant cells. The central vacuole and the water channels of the tonoplast maintain the tonicity of the cell, allowing the cell to expand and contract depending on conditions. The central vacuole is also involved in cell growth by occupying most of the volume of the cell. Plant cells grow by expanding the vacuole, rather than by increasing cytoplasmic volume.

Vacuoles with a variety of functions are also found in some types of fungi and protists. One form is the contractile vacuole, found in some protists, which can pump water and is used to maintain water balance in the cell. Other vacuoles are used for storage or to segregate toxic materials from the rest of the cytoplasm. The number and kind of vacuoles found in a cell depends on the needs of the particular cell type.

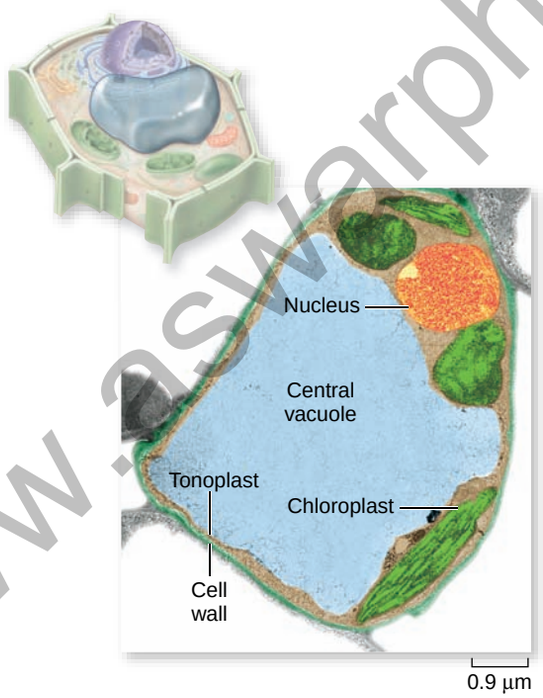


Figure 4.15 The central vacuole. A plant's central vacuole stores dissolved substances and can expand in size to increase the tonicity of a plant cell. Micrograph shown with false color.

Learning Outcomes Review 4.4

The endoplasmic reticulum (ER) is an extensive system of folded membranes that spatially organize the cell's biosynthetic activities. Smooth ER (SER) is the site of lipid and membrane synthesis and is used to store Ca^{2+}. Rough ER (RER) is covered with ribosomes and is a site of protein synthesis. Proteins from the RER are transported by vesicles to the Golgi apparatus where they are modified, packaged, and distributed to their final location. Lysosomes are vesicles that contain digestive enzymes used to degrade materials such as invaders or worn-out components. Peroxisomes carry out oxidative metabolism that generates peroxides. Vacuoles are membrane-bounded structures that have roles ranging from storage to cell growth in plants. They are also found in some fungi and protists.

- ***How do ribosomes on the RER differ from cytoplasmic ribosomes?***

4.5 *Mitochondria and Chloroplasts: Cellular Generators*

Learning Outcomes

1. ***Describe the structure of mitochondria and chloroplasts.***
2. ***Compare the function of mitochondria and chloroplasts.***
3. ***Explain the probable origin of mitochondria and chloroplasts.***

Mitochondria and chloroplasts share structural and functional similarities. Structurally, they are both surrounded by a double membrane, and both contain their own DNA and protein synthesis machinery. Functionally, they are both involved in energy metabolism, as we will explore in detail in later chapters on energy metabolism and photosynthesis.

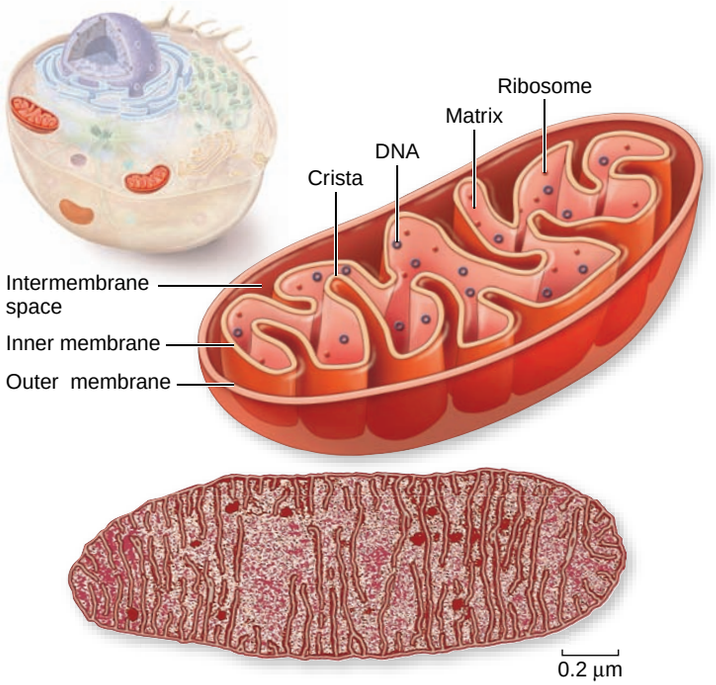


Figure 4.16 Mitochondria. The inner membrane of a mitochondrion is shaped into folds called cristae that greatly increase the surface area for oxidative metabolism. A mitochondrion in cross section and cut lengthwise is shown colored red in the micrograph.

Mitochondria metabolize sugar to generate ATP

Mitochondria (singular, *mitochondrion*) are typically tubular or sausage-shaped organelles about the size of bacteria that are found in all types of eukaryotic cells (figure 4.16). Mitochondria are bounded by two membranes: a smooth outer membrane, and an inner folded membrane with numerous contiguous layers called **cristae** (singular, *crista*).

The cristae partition the mitochondrion into two compartments: a **matrix,** lying inside the inner membrane; and an outer compartment, or **intermembrane space,** lying between the two mitochondrial membranes. On the surface of the inner membrane, and also embedded within it, are proteins that carry out oxidative metabolism, the oxygen-requiring process by which energy in macromolecules is used to produce ATP (chapter 7).

Mitochondria have their own DNA; this DNA contains several genes that produce proteins essential to the mitochondrion's role in oxidative metabolism. Thus, the mitochondrion, in many respects, acts as a cell within a cell, containing its own genetic information specifying proteins for its unique functions. The mitochondria are not fully autonomous, however, because most of the genes that encode the enzymes used in oxidative metabolism are located in the cell nucleus.

A eukaryotic cell does not produce brand-new mitochondria each time the cell divides. Instead, the mitochondria themselves divide in two, doubling in number, and these are partitioned between the new cells. Most of the components required for mitochondrial division are encoded by genes in the nucleus and are translated into proteins by cytoplasmic ribosomes. Mitochondrial replication is, therefore, impossible without nuclear participation, and mitochondria thus cannot be grown in a cell-free culture.

Chloroplasts use light to generate ATP and sugars

Plant cells and cells of other eukaryotic organisms that carry out photosynthesis typically contain from one to several hundred **chloroplasts.** Chloroplasts bestow an obvious advantage on the organisms that possess them: They can manufacture their own food. Chloroplasts contain the photosynthetic pigment chlorophyll that gives most plants their green color.

The chloroplast, like the mitochondrion, is surrounded by two membranes (figure 4.17). However, chloroplasts are larger and more complex than mitochondria. In addition to the outer and inner membranes, which lie in close association with each other, chloroplasts have closed compartments of stacked membranes called **grana** (singular, *granum*), which lie inside the inner membrane.

A chloroplast may contain a hundred or more grana, and each granum may contain from a few to several dozen disk-shaped structures called **thylakoids.** On the surface of the thylakoids are the light-capturing photosynthetic pigments, to be discussed in depth in chapter 8. Surrounding the thylakoid is a fluid matrix called the *stroma*. The enzymes used to synthesize glucose during photosynthesis are found in the stroma.

Like mitochondria, chloroplasts contain DNA, but many of the genes that specify chloroplast components are also located in the nucleus. Some of the elements used in photosynthesis, including the specific protein components necessary to accomplish the reaction, are synthesized entirely within the chloroplast.

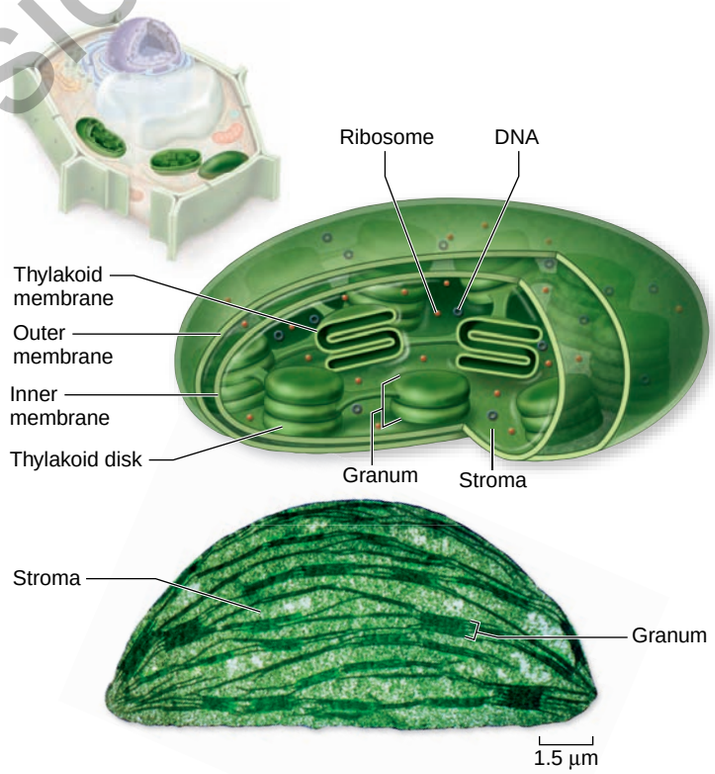


Figure 4.17 Chloroplast structure. The inner membrane of a chloroplast surrounds a membrane system of stacks of closed chlorophyll-containing vesicles called thylakoids, within which photosynthesis occurs. Thylakoids are typically stacked one on top of the other in columns called grana. The chloroplast has been colored green in the micrograph.

Other DNA-containing organelles in plants, called *leucoplasts*, lack pigment and a complex internal structure. In root cells and some other plant cells, leucoplasts may serve as starch storage sites. A leucoplast that stores starch (amylose) is sometimes termed an **amyloplast.** These organelles—chloroplasts, leucoplasts, and amyloplasts—are collectively called **plastids.** All plastids are produced by the division of existing plastids.

Inquiry question

? **Mitochondria and chloroplasts both generate ATP. What structural features do they share?**

Mitochondria and chloroplasts arose by endosymbiosis

Symbiosis is a close relationship between organisms of different species that live together. As noted in chapter 29, the theory of **endosymbiosis** proposes that some of today's eukaryotic organelles evolved by a symbiosis arising between two cells that were each free-living. One cell, a prokaryote, was engulfed by and became part of another cell, which was the precursor of modern eukaryotes (figure 4.18).

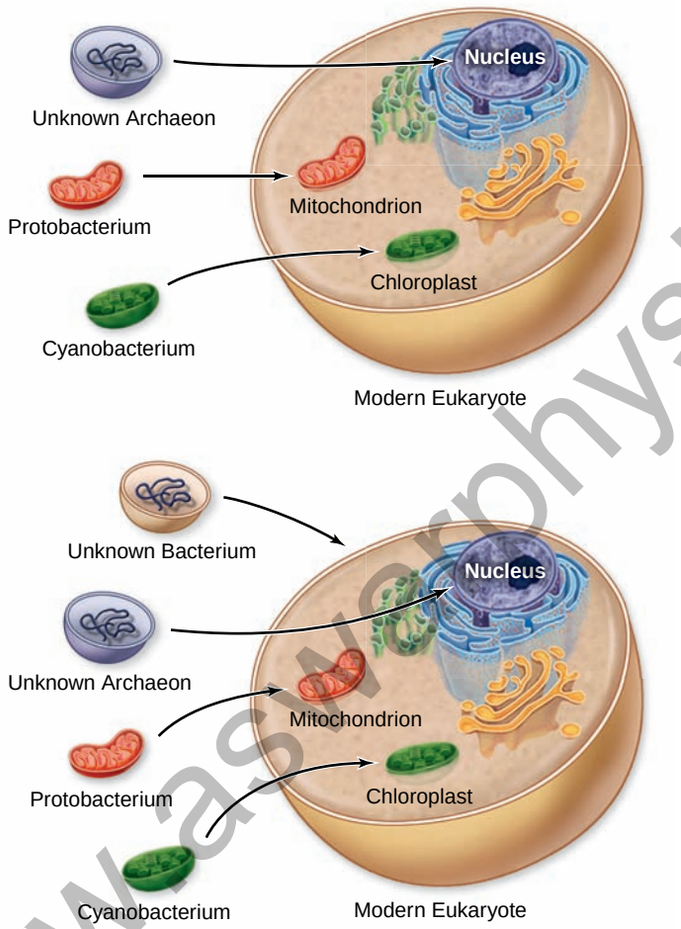


Figure 4.18 Possible origins of eukaryotic cells. Both mitochondria and chloroplasts are thought to have arisen by endosymbiosis where a free-living cell is taken up but not digested. The nature of the engulfing cell is unknown. Two possibilities are The engulfing cell *(top)* is an archaeon that gave rise to the nuclear genome and cytoplasmic contents. The engulfing cell *(bottom)* consists of a nucleus derived from an archaeon in a bacterial cell. This could arise by a fusion event or by engulfment of the archaeon by the bacterium.

According to the endosymbiont theory, the engulfed prokaryotes provided their hosts with certain advantages associated with their special metabolic abilities. Two key eukaryotic organelles are believed to be the descendants of these endosymbiotic prokaryotes: mitochondria, which are thought to have originated as bacteria capable of carrying out oxidative metabolism, and chloroplasts, which apparently arose from photosynthetic bacteria. This is discussed in detail in chapter 29.

Learning Outcomes Review 4.5

Mitochondria and chloroplasts have similar structures, with an outer membrane and an extensive inner membrane compartment. Both mitochondria and chloroplasts have their own DNA, but both also depend on nuclear genes for some functions. Mitochondria and chloroplasts are both involved in energy conversion: Mitochondria metabolize sugar to produce ATP, whereas chloroplasts harness light energy to produce ATP and synthesize sugars. Endosymbiosis theory proposes that both mitochondria and chloroplasts arose as prokaryotic cells engulfed by a eukaryotic precursor.

- ***Many proteins in mitochondria and chloroplasts are encoded by nuclear genes. In light of the endosymbiont hypothesis, how might this come about?***

4.6 The Cytoskeleton

Learning Outcomes

1. ***Contrast the structure and function of different fibers in the cytoskeleton.***
2. ***Illustrate the role of microtubules in intracellular transport.***

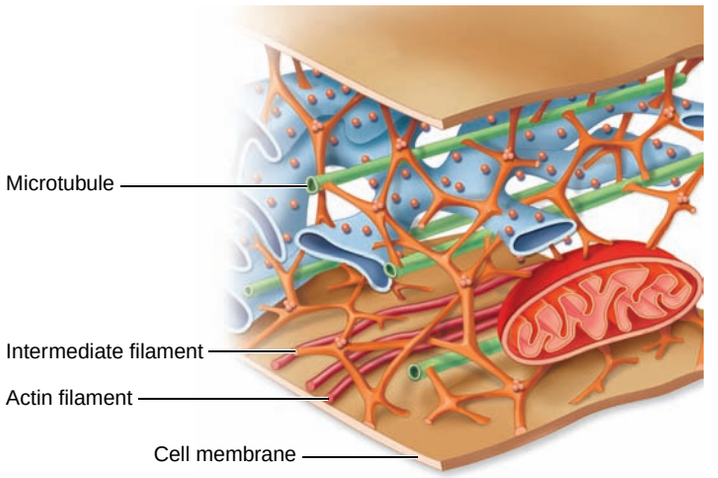
The cytoplasm of all eukaryotic cells is crisscrossed by a network of protein fibers that supports the shape of the cell and anchors organelles to fixed locations. This network, called the cytoskeleton, is a dynamic system, constantly assembling and disassembling. Individual fibers consist of polymers of identical protein subunits that attract one another and spontaneously assemble into long chains. Fibers disassemble in the same way, as one subunit after another breaks away from one end of the chain.

Three types of fibers compose the cytoskeleton

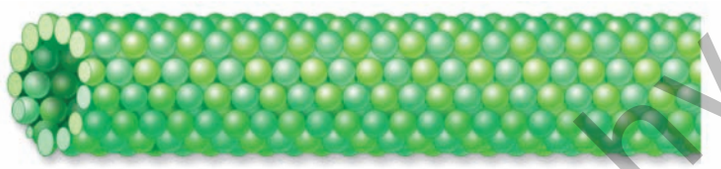
Eukaryotic cells may contain the following three types of cytoskeletal fibers, each formed from a different kind of subunit: (1) actin filaments, sometimes called microfilaments, (2) microtubules, and (3) intermediate filaments.

Actin filaments (microfilaments)

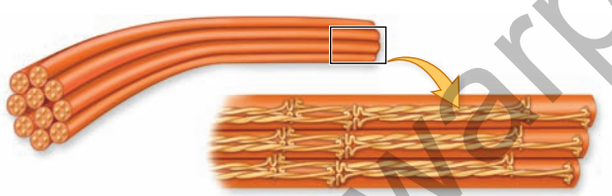
Actin filaments are long fibers about 7 nm in diameter. Each filament is composed of two protein chains loosely twined together like two strands of pearls (figure 4.19). Each "pearl," or subunit, on the chain is the globular protein **actin.** Actin filaments exhibit polarity, that is, they have plus (+) and minus (–) ends. These designate the direction of growth of the filaments.



a. Actin filaments



b. Microtubules



c. Intermediate filament

Figure 4.19 Molecules that make up the cytoskeleton.
a. *Actin filaments:* Actin filaments, also called *microfilaments*, are made of two strands of the globular protein actin twisted together. They are often found in bundles or in a branching network. Actin filaments in many cells are concentrated below the plasma membrane in bundles known as stress fibers, which may have a contractile function. ***b.*** *Microtubules:* Microtubules are composed of α- and β-tubulin protein subunits arranged side by side to form a tube. Microtubules are comparatively stiff cytoskeletal elements and have many functions in the cell including intracellular transport and the separation of chromosomes during mitosis. ***c.*** *Intermediate filaments:* Intermediate filaments are composed of overlapping staggered tetramers of protein. These tetramers are then bundled into cables. This molecular arrangement allows for a ropelike structure that imparts tremendous mechanical strength to the cell.

Actin molecules spontaneously form these filaments, even in a test tube.

Cells regulate the rate of actin polymerization through other proteins that act as switches, turning on polymerization when appropriate. Actin filaments are responsible for cellular movements such as contraction, crawling, "pinching" during division, and formation of cellular extensions.

Microtubule

Microtubules, the largest of the cytoskeletal elements, are hollow tubes about 25 nm in diameter, each composed of a ring of 13 protein protofilaments (see figure 4.19). Globular proteins consisting of dimers of α- and β-*tubulin* subunits polymerize to form the 13 protofilaments. The protofilaments are arrayed side by side around a central core, giving the microtubule its characteristic tube shape.

In many cells, microtubules form from nucleation centers near the center of the cell and radiate toward the periphery. They are in a constant state of flux, continually polymerizing and depolymerizing. The average half-life of a microtubule ranges from as long as 10 minutes in a nondividing animal cell to as short as 20 seconds in a dividing animal cell. The ends of the microtubule are designated as plus (+) (away from the nucleation center) or minus (–) (toward the nucleation center).

Along with facilitating cellular movement, microtubules organize the cytoplasm and are responsible for moving materials within the cell itself, as described shortly.

Intermediate filaments

The most durable element of the cytoskeleton in animal cells is a system of tough, fibrous protein molecules twined together in an overlapping arrangement (see figure 4.19). These *intermediate filaments* are characteristically 8 to 10 nm in diameter—between the size of actin filaments and microtubules. Once formed, intermediate filaments are stable and usually do not break down.

Intermediate filaments constitute a mixed group of cytoskeletal fibers. The most common type, composed of protein subunits called *vimentin*, provides structural stability for many kinds of cells. *Keratin*, another class of intermediate filament, is found in epithelial cells (cells that line organs and body cavities) and associated structures such as hair and fingernails. The intermediate filaments of nerve cells are called *neurofilaments.*

Centrosomes are microtubule-organizing centers

Centrioles are barrel-shaped organelles found in the cells of animals and most protists. They occur in pairs, usually located at right angles to each other near the nuclear membranes (figure 4.20). The region surrounding the pair in almost all animal cells is referred to as a *centrosome.* Surrounding the centrioles in the centrosome is the **pericentriolar material,** which contains ring-shaped structures composed of tubulin. The pericentriolar material can nucleate the assembly of microtubules in animal cells. Structures with this function are called *microtubule-organizing centers.* The centrosome is also responsible for the reorganization of microtubules that occurs during

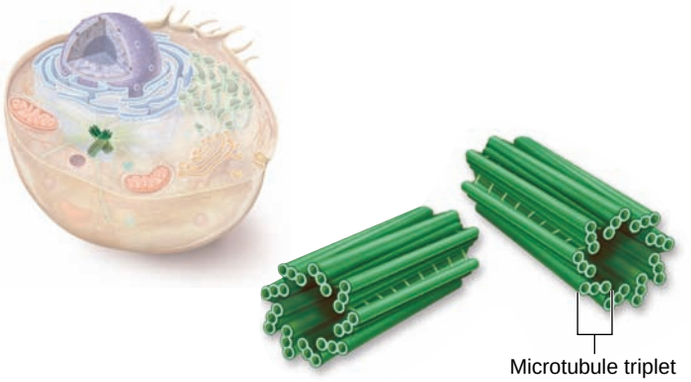


Figure 4.20 Centrioles. Each centriole is composed of nine triplets of microtubules. Centrioles are usually not found in plant cells. In animal cells they help to organize microtubules.

cell division. The centrosomes of plants and fungi lack centrioles, but still contain microtubule-organizing centers. You will learn more about the actions of the centrosomes when we describe the process of cell division in chapter 10.

The cytoskeleton helps move materials within cells

Actin filaments and microtubules often orchestrate their activities to affect cellular processes. For example, during cell reproduction (see chapter 10), newly replicated chromosomes move to opposite sides of a dividing cell because they are attached to shortening microtubules. Then, in animal cells, a belt of actin pinches the cell in two by contracting like a purse string.

Muscle cells also use actin filaments, which slide along filaments of the motor protein myosin when a muscle contracts. The fluttering of an eyelash, the flight of an eagle, and the awkward crawling of a baby all depend on these cytoskeletal movements within muscle cells.

Not only is the cytoskeleton responsible for the cell's shape and movement, but it also provides a scaffold that holds certain enzymes and other macromolecules in defined areas of the cytoplasm. For example, many of the enzymes involved in cell metabolism bind to actin filaments, as do ribosomes. By moving and anchoring particular enzymes near one another, the cytoskeleton, like the endoplasmic reticulum, helps organize the cell's activities.

Molecular motors

All eukaryotic cells must move materials from one place to another in the cytoplasm. One way cells do this is by using the channels of the endoplasmic reticulum as an intracellular highway. Material can also be moved using vesicles loaded with cargo that can move along the cytoskeleton like a railroad track. For example, in a nerve cell with an axon that may extend far from the cell body, vesicles can be moved along tracks of microtubules from the cell body to the end of the axon.

Four components are required to move material along microtubules: (1) a vesicle or organelle that is to be transported, (2) a motor protein that provides the energy-driven motion, (3) a connector molecule that connects the vesicle to the motor molecule, and (4) microtubules on which the vesicle will ride like a train on a rail (figure 4.21).

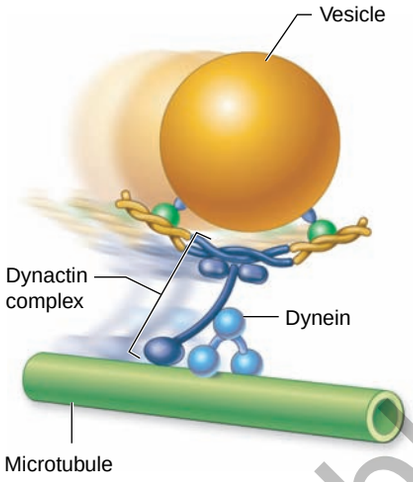


Figure 4.21 Molecular motors. Vesicles can be transported along microtubules using motor proteins that use ATP to generate force. The vesicles are attached to motor proteins by connector molecules, such as the dynactin complex shown here. The motor protein dynein moves the connected vesicle along microtubules.

The direction a vesicle is moved depends on the type of motor protein involved and the fact that microtubules are organized with their plus ends toward the periphery of the cell. In one case, a protein called kinectin binds vesicles to the motor protein *kinesin*. Kinesin uses ATP to power its movement toward the cell periphery, dragging the vesicle with it as it travels along the microtubule toward the plus end (figure 4.22). As nature's tiniest motors, these proteins pull the transport vesicles along the microtubular tracks. Another set of vesicle proteins, called the dynactin complex, binds vesicles to the motor protein *dynein* (see figure 4.22), which directs movement in the opposite

SCIENTIFIC THINKING

Hypothesis: *Kinesin molecules can act as molecular motors and move along microtubules using energy from ATP.*

Test: *A microscope slide is covered with purified kinesin. Purified microtubules are added in a buffer containing ATP. The microtubules are monitored under a microscope using a video recorder to capture any movement.*

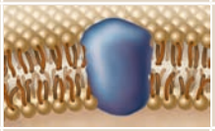
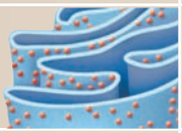
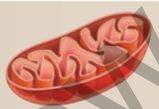
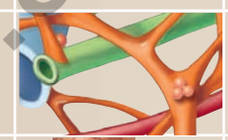
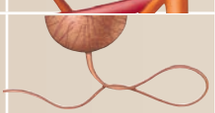
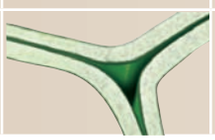
Result: *Over time, the movement of individual microtubules can be observed in the microscope. This is shown schematically in the figure by the movement of specific microtubules shown in color.*

Conclusion: *Kinesin acts as a molecular motor moving along (in this case actually moving) microtubules.*

Further Experiments: *Are there any further controls that are not shown in this experiment? What additional conclusions could be drawn by varying the amount of kinesin sticking to the slide?*

Figure 4.22 Demonstration of kinesin as molecular motor. Microtubules can be observed moving over a slide coated with kinesin.

TABLE 4.2 Eukaryotic Cell Structures and their Functions

Structure		Description	Function
Plasma membrane		Phospholipid bilayer with embedded proteins	Regulates what passes into and out of cell; cell-to-cell recognition; connection and adhesion; cell communication
Nucleus		Structure (usually spherical) that contains chromosomes and is surrounded by double membrane	Instructions for protein synthesis and cell reproduction; contains genetic information
Chromosomes		Long threads of DNA that form a complex with protein	Contain hereditary information used to direct synthesis of proteins
Nucleolus		Site of genes for rRNA synthesis	Synthesis of rRNA and ribosome assembly
Ribosomes		Small, complex assemblies of protein and RNA, often bound to ER	Sites of protein synthesis
Endoplasmic reticulum (ER)		Network of internal membranes	Intracellular compartment forms transport vesicles; participates in lipid synthesis and synthesis of membrane or secreted proteins
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; digest material taken up by endocytosis
Microbodies		Vesicles that are formed from incorporation of lipids and proteins and that contain 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 double membrane surrounding a third, thylakoid membrane containing chlorophyll, a photosynthetic pigment	Sites of photosynthesis
Cytoskeleton		Network of protein filaments	Structural support; cell movement; movement of vesicles within cells
Flagella (cilia)		Cellular extensions with 9 + 2 arrangement of pairs of microtubles	Motility or moving fluids over surfaces
Cell wall		Outer layer of cellulose or chitin; or absent	Protection; support

direction along microtubules toward the minus end, inward toward the cell's center. (Dynein is also involved in the movement of eukaryotic flagella, as discussed later.) The destination of a particular transport vesicle and its content is thus determined by the nature of the linking protein embedded within the vesicle's membrane.

The major eukaryotic cell structures and their respective functions are summarized in table 4.2.

Learning Outcomes Review 4.6

The three principal fibers of the cytoskeleton are actin filaments (microfilaments), microtubules, and intermediate filaments. These fibers interact to modulate cell shape and permit cell movement. They also act to move materials within the cytoplasm. Material is also moved in large cells using vesicles and molecular motors. The motor proteins move vesicles along tracks of microtubules.

- ***What advantage does the cytoskeleton give to large eukaryotic cells?***

4.7 Extracellular Structures and Cell Movement

Learning Outcomes

1. ***Describe how cells move.***
2. ***Identify the different cytoskeletal elements involved in cell movement.***
3. ***Classify the elements of extracellular matrix in animal cells.***

Essentially all cell motion is tied to the movement of actin filaments, microtubules, or both. Intermediate filaments act as intracellular tendons, preventing excessive stretching of cells. Actin filaments play a major role in determining the shape of cells. Because actin filaments can form and dissolve so readily, they enable some cells to change shape quickly.

Some cells crawl

The arrangement of actin filaments within the cell cytoplasm allows cells to crawl, literally! Crawling is a significant cellular phenomenon, essential to such diverse processes as inflammation, clotting, wound healing, and the spread of cancer. White blood cells in particular exhibit this ability. Produced in the bone marrow, these cells are released into the circulatory system and then eventually crawl out of venules and into the tissues to destroy potential pathogens.

At the leading edge of a crawling cell, actin filaments rapidly polymerize, and their extension forces the edge of the cell forward. This extended region is stabilized when microtubules polymerize into the newly formed region. Overall forward movement of the cell is then achieved through the action of the protein **myosin,** which is best known for its role in muscle contraction. Myosin motors along the actin filaments contract, pulling the contents of the cell toward the newly extended front edge.

Cells crawl when these steps occur continuously, with a leading edge extending and stabilizing, and then motors contracting to pull the remaining cell contents along. Receptors on the cell surface can detect molecules outside the cell and stimulate extension in specific directions, allowing cells to move toward particular targets.

Flagella and cilia aid movement

Earlier in this chapter, we described the structure of prokaryotic flagella. Eukaryotic cells have a completely different kind of flagellum, consisting of a circle of nine microtubule pairs surrounding two central microtubules. This arrangement is referred to as the *9 + 2 structure* (figure 4.23).

As pairs of microtubules move past each other using arms composed of the motor protein dynein, the eukaryotic flagellum *undulates*, rather than rotates. When examined carefully, each flagellum proves to be an outward projection of the cell's interior, containing cytoplasm and enclosed by the plasma membrane. The microtubules of the flagellum are derived from a **basal body,** situated just below the point where the flagellum protrudes from the surface of the cell.

The flagellum's complex microtubular apparatus evolved early in the history of eukaryotes. Today the cells of many

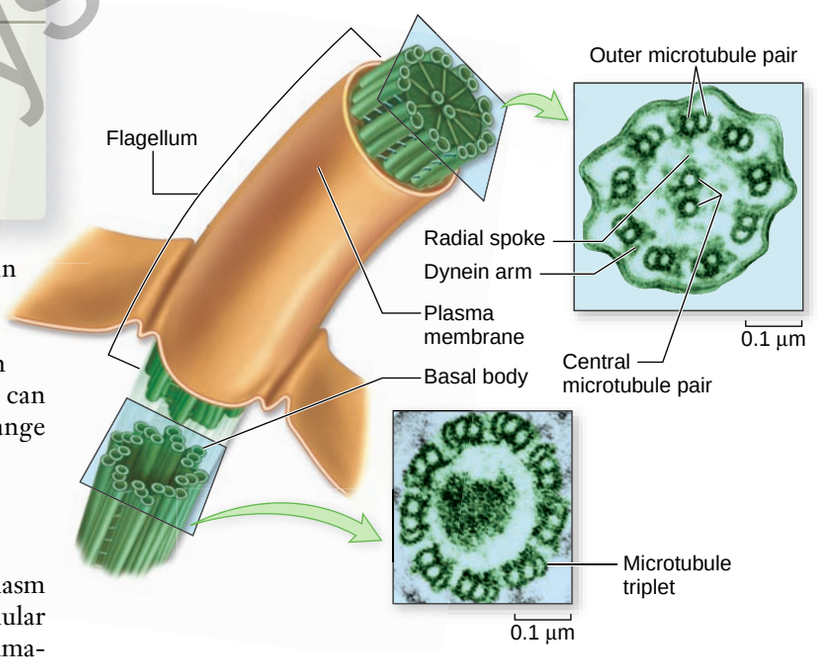


Figure 4.23 Flagella and cilia. A eukaryotic flagellum originates directly from a basal body. The flagellum has two microtubules in its core connected by radial spokes to an outer ring of nine paired microtubules with dynein arms (9 + 2 structure). The basal body consists of nine microtubule triplets connected by short protein segments. The structure of cilia is similar to that of flagella, but cilia are usually shorter.