10 Relationship between Cell Biology and Biochemistry

The basic unit of a living organism is the cell. In the human, each tissue is composed of similar cell types, which differ from those in other tissues. The diversity of cell types serves the function of the tissue and organs in which they reside, and each cell type has unique structural features that reflect its role. In spite of their diversity in structure, human cell types have certain architectural features in common, such as the plasma membrane, membranes around the nucleus and organelles, and a cytoskeleton (Fig. 10.1). In this chapter, we review some of the chemical characteristics of these common features, the functions of organelles, and the transport systems for compounds into cells and between organelles.

Plasma membrane*. The cell membrane is a* ***lipid bilayer*** *that serves as a selective barrier; it restricts the entry and exit of compounds. Within the plasma membrane, different* ***integral proteins*** *facilitate the* ***transport*** *of specific compounds by*

The cells of humans and other animals are eukaryotes (eu, good; karyon, nucleus) because the genetic material is organized into a membrane-enclosed nucleus. In contrast, bacteria are prokaryotes (pro, before; karyon, nucleus); they do not contain nuclei or other organelles found in eukaryotic cells.

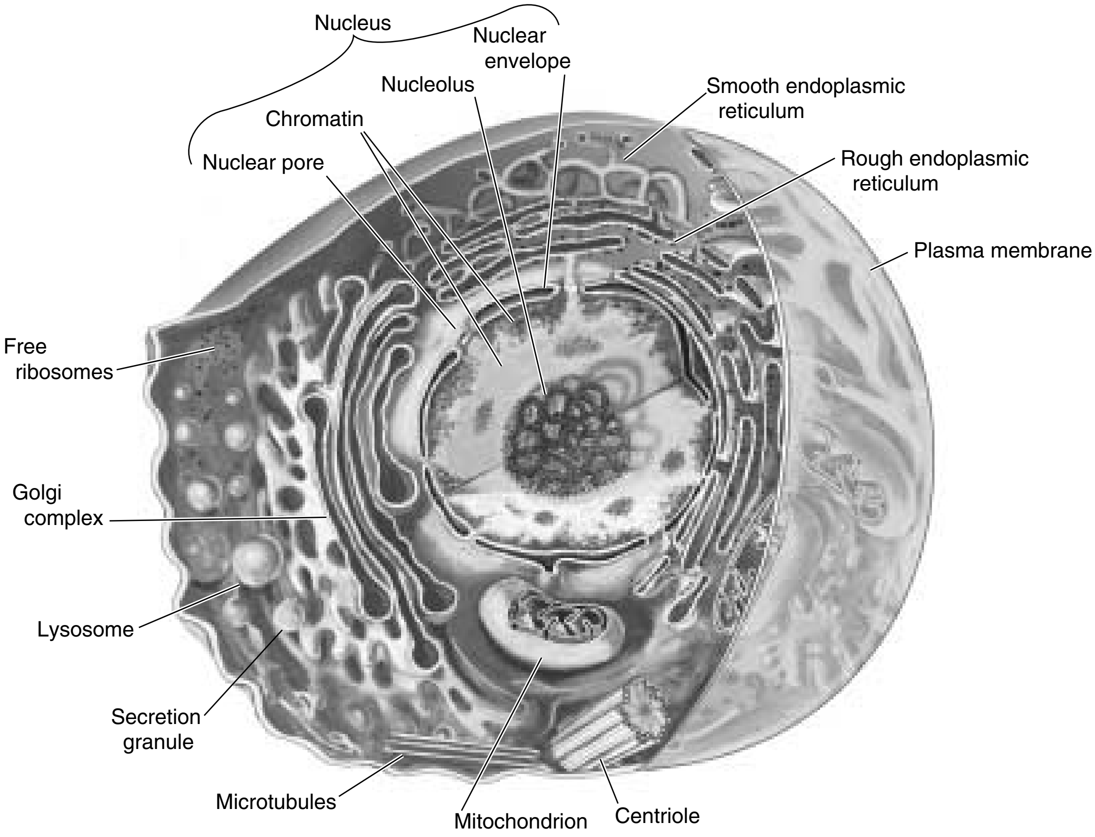


Fig. 10.1. Common components of human cells.

energy-requiring active transport, ***facilitated diffusion***, *or by forming* ***pores*** *or* ***gated-channels***. *The plasma membrane is supported by a* ***membrane skeleton*** *composed of proteins.*

The cytoplasm of the cell is the portion of the cell between the cell membrane and the nucleus. Mitochondria, lysososmes and peroxisomes are referred to as cytoplasmic organelles. The Golgi and the endoplasmic reticulum are referred to as cytoplasmic membrane systems. The plasma membrane can be gently disrupted by detergents or shear stress without damage to the other membrane systems. When a suspension that has been treated this way is centrifuged for a long period of time (100,000*g* for 1 hour), the organelles and membrane systems will collect at the bottom of the tube. The remaining clear liquid of soluble enzymes, cofactors, and metabolites is the cytosol.

Organelles *and* ***cytoplasmic membrane systems***. *Most organelles within the cell are compartments surrounded by a membrane system that restricts exchange of compounds and information with other compartments (see Fig. 10.1). In general, each organelle has unique functions that are served by the enzymes and other compounds it contains, or the environment it maintains.* ***Lysosomes*** *contain* ***hydrolytic enzymes*** *that degrade proteins and other large molecules. The* ***nucleus*** *contains the genetic material and carries out* ***gene replication*** *and* ***transcription*** *of* ***DNA***, *the first step of protein synthesis. The last phase of protein synthesis occurs on* ***ribosomes***. *For certain proteins, the ribosomes become attached to the complex membrane system called the* ***endoplasmic reticulum***; *for other proteins, synthesis is completed on ribosomes that remain in the cytoplasm. The* ***endoplasmic reticulum*** *is also involved in lipid synthesis and transport of molecules to the Golgi. The* ***Golgi*** *forms vesicles for transport of molecules to the plasma membrane and other membrane systems, and for secretion.* ***Mitochondria*** *are organelles of* ***fuel oxidation*** *and* ***ATP generation. Peroxisomes*** *contain many enzymes that use or produce* ***hydrogen peroxide.*** *The* ***cytosol*** *is the intracellular compartment free of organelles and membrane systems.*

Cytoskeleton. *The* ***cytoskeleton*** *is a flexible fibrous protein support system that maintains the geometry of the cell, fixes the position of organelles, and moves compounds within the cell or the cell itself. It is composed principally of* ***actin microfilaments***, ***intermediate filaments***, ***tubulin microtubules***, *and their attached proteins.*

THE WAITING ROOM

Al Martini had been drinking heavily when he drove his car off the road and was taken to the hospital emergency room (see Chapters 8 and 9). Although he suffered only minor injuries, his driving license was suspended.

Two years after **Dennis "the Menace" Veere** successfully recovered from his malathion poisoning, he visited his grandfather, **Percy Veere**. Mr. Veere took Dennis with him to a picnic at the shore, where they ate steamed crabs. Later that night, Dennis experienced episodes of vomiting and watery diarrhea, and Mr. Veere rushed him to the hospital emergency room. Dennis's hands and feet were cold, he appeared severely dehydrated, and he was approaching hypovolemic shock (a severe drop in blood pressure). He was diagnosed with cholera, caused by the bacteria *Vibrio cholerae*.

V. cholerae epidemics are associated with unsanitary conditions affecting the drinking water supply and are rare in the United States. However, these bacteria grow well under the alkaline conditions found in seawater and attach to chitin in shellfish. Thus, sporadic cases occur in the southeast United States associated with the ingestion of contaminated shellfish.

Before **Lotta Topaigne** was treated with allopurinol (see Chapter 8), her physician administered colchicine (acetyltrimethylcolchicinic acid) for the acute attack of gout affecting her great toe. After taking a high dose of colchicine divided over several-hour intervals, the throbbing pain in her toe had abated significantly. The redness and swelling also seemed to have lessened slightly.

Cell lysis, the breaking of the cell membrane and release of cell contents, occurs when the continuity of the cell membrane is disrupted.

I. COMPARTMENTATION IN CELLS

Membranes are lipid structures that separate the contents of the compartment they surround from its environment. An outer plasma membrane separates the cell from the external aqueous environment. Organelles (such as the nucleus, mitochondria, lysosomes, and peroxisosmes) are also surrounded by a membrane system that separates the internal compartment of the organelle from the cytosol. The function of these membranes is to collect or concentrate enzymes and other molecules serving a common function into a compartment with a localized environment. The transporters and receptors in each membrane system control this localized environment and communication of the cell or organelle with the surrounding milieu.

The following sections describe various organelles and membrane systems found in most human cells and outline the relationship between their properties and function. Each organelle has different enzymes and carries out different general functions. For example, the nucleus contains the enzymes for DNA and RNA synthesis.

Not all cells in the human are alike. Different cell types differ quantitatively in their organelle content, or their organelles may contain vastly different amounts of a particular enzyme, ***consistent with the function of the cell.*** For example, liver mitochondria contain a key enzyme for synthesizing ketone bodies, but they lack a key enzyme for their use. The reverse is true in muscle mitochondria. Thus, the enzymic content of the organelles varies somewhat from cell type to cell type.

II. PLASMA MEMBRANE

A. Structure of the Plasma Membrane

All mammalian cells are enclosed by a plasma membrane composed of a lipid bilayer (two layers) containing embedded proteins (Fig. 10.2). The membranes are continuous and sealed so that the hydrophobic lipid bilayer selectively restricts the exchange of polar compounds between the external fluid and the intracellular compartment. The membrane is referred to as a fluid mosaic because it consists of a mosaic of proteins and lipid molecules that can, for the most part, move laterally in the plane of the membrane. The proteins are classified as integral proteins, which span the cell membrane, or peripheral proteins, which are attached to the membrane surface through electrostatic bonds to lipids or integral proteins. Many of the proteins and lipids on the external leaflet contain covalently bound carbohydrate chains and therefore are glycoproteins and glycolipids. This layer of carbohydrate on the outer surface of the cell is called the glycocalyx.

1. LIPIDS IN THE PLASMA MEMBRANE

Each layer of the plasma membrane lipid bilayer is formed primarily by phospholipids, which are arranged with their hydrophilic head groups facing the aqueous medium and their fatty acyl tails forming a hydrophobic membrane core (see Fig. 10.2). The principle phospholipids in the membrane are the glycerol lipids phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine and the sphingolipid sphingomyelin (Fig. 10.3). The lipid composition varies among different cell types, with phosphatidylcholine being the major plasma membrane lipid in most cell types and sphingolipids the most variable.

The lipid composition of the bilayer is asymmetric, with a higher content of phosphatidylcholine and sphingomyelin in the outer leaflet and a higher content of phosphatidylserine and phosphatidylethanolamine in the inner leaflet. Phosphatidylserine contains a net negative charge that contributes to the membrane potential and might be important for binding positively charged molecules within the cell. Phosphatidylinositol, which is found only in the inner membrane, functions in the transfer of information from hormones and neurotransmitters across the cell membrane into the cell (Fig. 10.4).

Bacteria are single cells surrounded by a cell membrane and a cell wall exterior to the membrane. They are prokaryotes, which do not contain nuclei or other organelles (i.e. membrane-surrounded subcellular structures) found in eukaryotic cells. Nonetheless, bacteria carry out many similar metabolic pathways, with the enzymes located in either the intracellular compartment or the cell membrane.

The *Vibrio cholerae* responsible for **Dennis Veere's** cholera are gram-negative bacteria. Their plasma membrane is surrounded by a thin cell wall composed of a protein–polysaccharide structure called peptidoglycan and an outer membrane. In contrast, gram-positive bacteria have a plasma membrane and a thick peptidoglycan cell wall that retains the Gram stain. *Vibrio* grow best under aerobic conditions, but also can grow under low oxygen conditions. They possess enzymes similar to those in human cells for glycolysis, the TCA cycle, and oxidative phosphorylation. They have a low tolerance for acid, which partially accounts for their presence in slightly basic seawater and shellfish.

The variable carbohydrate components of the glycolipids on the cell surface function as cell recognition markers. For example, the A, B, or O blood groups are determined by the carbohydrate composition of the glycolipids. Cell surface glycolipids may also serve as binding sites for viruses and bacterial toxins before penetrating the cell. For example, the cholera AB toxin binds to GM_1-gangliosides on the surface of the intestinal epithelial cells. The toxin is then endocytosed in caveolae (invaginations or "caves" that can form in specific regions of the membrane).

One of the bacterial toxins secreted by *Clostridium perefringens,* the bacteria that cause gas gangrene, is a lipase that hydrolyzes phosphocholine from phosphatidylcholine and from sphingomyelin. The resulting lysis of the cell membrane releases intracellular contents that provide the bacteria with nutrients for rapid growth. These bacteria are strict anaerobes and grow only in the absence of oxygen. As their toxins lyse membranes in the endothelial cells of blood vessels, the capillaries are destroyed, and the bacteria are protected from oxygen transported by the red blood cells. They are also protected from antibiotics and components of the immune system carried in the blood.

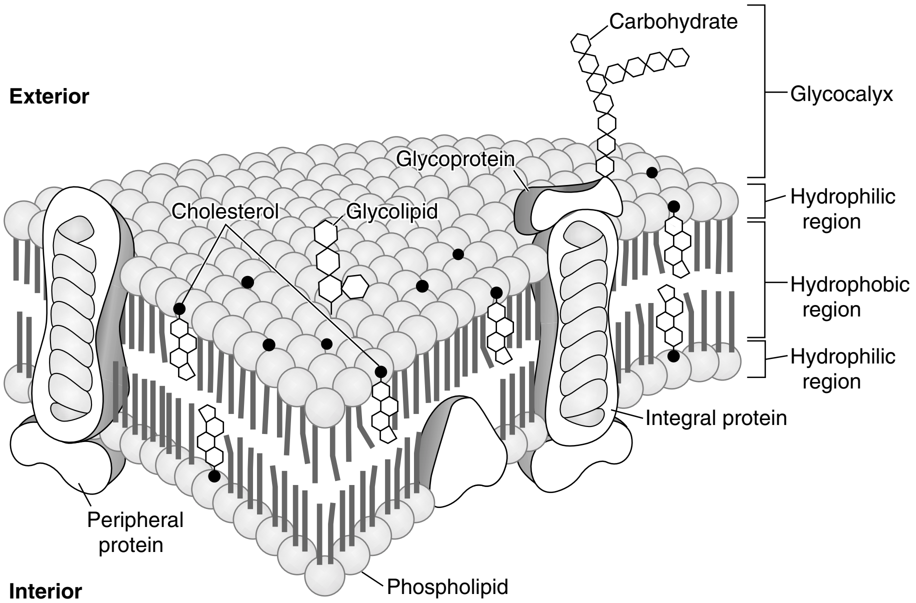


Fig. 10.2. Basic structure of an animal cell membrane.

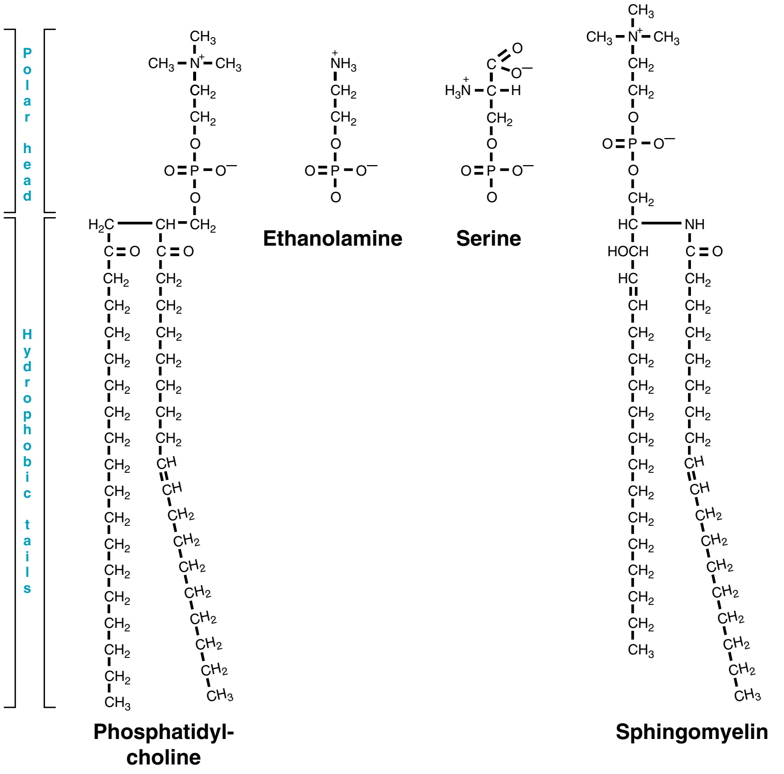


Fig. 10.3. Common phospholipids in the mammalian cell membrane. The polar head groups shown for ethanolamine and serine replace the choline in phosphatidylcholine to form phosphatidylethanolamine and phosphatidylserine, respectively. Phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine are phosphoacylglycerols. In contrast, sphingomyelin does not contain the glycerol backbone but has a sphingosine backbone and is a sphingolipid.

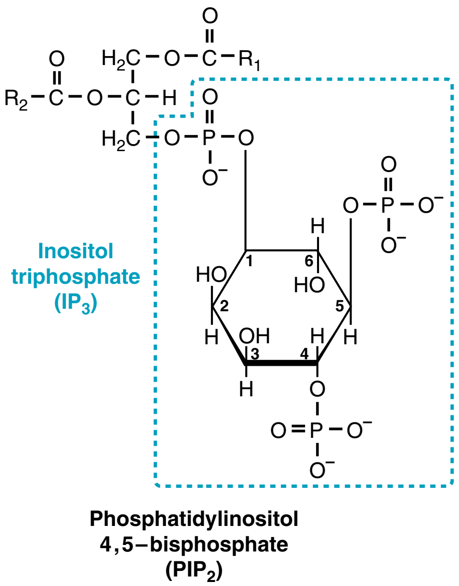


Fig. 10.4. Phosphatidylinositol bisphosphate (PIP_2). R1 and R2 are fatty acyl chains. The portion of PIP_2 that becomes inositol triphosphate, the polar head group extending into the cytosol, is shown in blue.

Cholesterol, which is interspersed between the phospholipids, maintains membrane fluidity. In the phosphoacylglycerols, unsaturated fatty acid chains bent into the *cis* conformation form a pocket for cholesterol, which binds with its hydroxyl group in the external hydrophilic region of the membrane and its hydrophobic steroid nucleus in the hydrophobic membrane core (Fig. 10.5). The presence of cholesterol and the *cis* unsaturated fatty acids in the membrane prevent the hydrophobic chains from packing too closely together. As a consequence, lipid and protein molecules that are not bound to external or internal structural proteins can rotate and move laterally in the plane of the leaflet. This movement enables the plasma membrane to partition between daughter cells during cell division, to

Al Martini is suffering from both short-term and long-term effects of ethanol on his central nervous system. Data support the theory that the short-term effects of ethanol on the brain partially arise from an increase in membrane fluidity caused when ethanol intercalates between the membrane lipids. The changes in membrane fluidity may affect proteins that span the membrane (integral proteins), such as ion channels and receptors for neurotransmitters involved in conducting the nerve impulse.

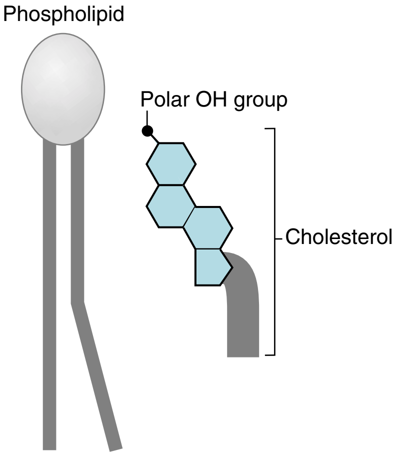


Fig. 10.5. Cholesterol in the plasma membrane. The polar hydroxyl group of cholesterol is oriented toward the surface. The hydrocarbon tail and the steroid nucleus (blue) lie in the hydrophobic core. A *cis* double bond in the fatty acyl chain of a phospholipid bends the chain to create a hydrophobic binding site for cholesterol.

deform as cells pass through capillaries, and to form and fuse with vesicle membranes. The fluidity of the membrane is partially determined by the unsaturated fatty acid content of the diet.

The composition of the membrane is dynamic. Sections of membrane form buds that pinch off into vesicles and membrane vesicles formed in the Golgi and elsewhere bring new and recycled components back to the membrane. Individual fatty acyl chains turn over as they are hydrolyzed from the lipids and replaced, and enzymes called flipases transfer lipids between leaflets.

2. PROTEINS IN THE PLASMA MEMBRANE

The integral proteins contain transmembrane domains with hydrophobic amino acid side chains that interact with the hydrophobic portions of the lipids to seal the membrane (see Fig. 10.2). Hydrophilic regions of the proteins protrude into the aqueous medium on both sides of the membrane. Many of these proteins function as either channels or transporters for the movement of compounds across the membrane, as receptors for the binding of hormones and neurotransmitters, or as structural proteins (Fig. 10.6).

Peripheral membrane proteins, which were originally defined as those proteins that can be released from the membrane by ionic solvents, are bound through weak electrostatic interactions with the polar head groups of lipids or with integral proteins. One of the best-characterized classes of peripheral proteins is the spectrin family of proteins, which are bound to the intracellular membrane surface and provide mechanical support for the membrane. Spectrin is bound to actin, which together form a structure that is called the inner membrane skeleton or the cortical skeleton (see Fig. 10.6).

A third classification of membrane proteins consists of lipid-anchored proteins bound to the inner or outer surface of the membrane. The glycophosphatidylinositolglycan (GPI) anchor is a covalently attached lipid that anchors proteins to the

Two of the prominent integral proteins in the red blood cell membrane are glycophorin, which provides an external negative charge that repels other cells, and band 3, which is a channel for bicarbonate and chloride exchange. The transport of bicarbonate into the red blood cell in exchange for chloride helps to carry the bicarbonate to the lungs, where it is expired as CO_2.

All cells contain an inner membrane skeleton of spectrin-like proteins. Red blood cell spectrin was the first member of the spectrin family described. The protein dystrophin present in skeletal muscle cells is a member of the spectrin family. Genetic defects in the dystrophin gene are responsible for Duchenne's and Becker's muscular dystrophies.

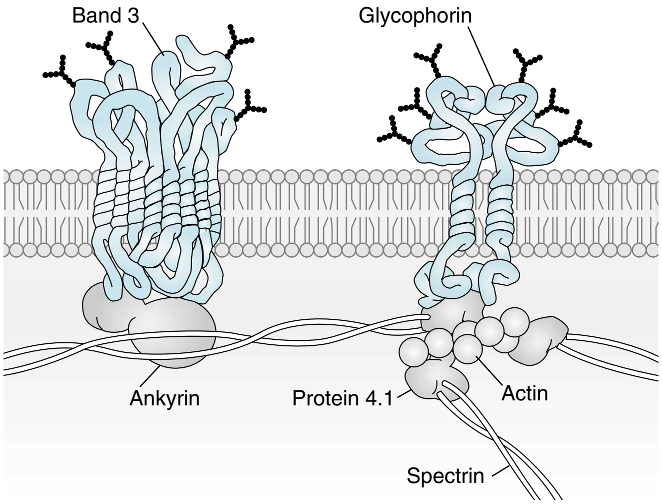


Fig. 10.6. Proteins in the red blood cell membrane. The proteins named Band 3 (the bicarbonate-chloride exchange transporter) and glycophorin contain nonpolar α-helical segments spanning the lipid bilayer. These proteins contain a large number of polar and charged hydrophilic amino acids in the intracellular and extracellular domains. On the inside of the cell, they are attached to peripheral proteins constituting the inner membrane skeleton. Band 3 is connected to spectrin filaments via the protein ankyrin. Glycophorin is connected to short actin filaments and spectrin via protein 4.1.

external surface of the membrane (Fig.10.7). A number of proteins involved in hormonal regulation are anchored to the internal surface of the membrane through palmityl (C16) or myristyl (C14) fatty acyl groups or through geranylgeranyl (C20) or farnesyl (C15) isoprenyl groups (see Ras, Chapter 9, Fig. 9.14, or Chapter 6, Fig. 6.14). However, many integral proteins also contain attached lipid groups to increase their stability in the membrane.

The prion protein, present in neuronal membranes, provides an example of a protein attached to the membrane through a GPI anchor. This is the protein that develops an altered pathogenic conformation in both mad cow disease and Creutzfeldt-Jakob disease (see Chapter 7, Biochemical Comments).

3. THE GLYCOCALYX OF THE PLASMA MEMBRANE

Some of the proteins and lipids on the external surface of the membrane contain short chains of carbohydrates (oligosaccharides) that extend into the aqueous medium. Carbohydrates therefore constitute 2 to10% of the weight of plasma membranes. This hydrophilic carbohydrate layer, called the glycocalyx, protects the cell against digestion and restricts the uptake of hydrophobic compounds.

The glycoproteins generally contain branched oligosaccharide chains of approximately 15 sugar residues that are attached through N-glycosidic bonds to the amide nitrogen of an asparagine side chain (N-glycosidic linkage), or through a glycosidic bond to the oxygen of serine (O-glycoproteins). The membrane glycolipids are usually galactosides or cerebrosides. Specific carbohydrate chains on the glycolipids serve as cell recognition molecules (see Chapter 5 for structures of classes of compounds).

B. Transport of Molecules across the Plasma Membrane

Membranes form hydrophobic barriers around cells to control the internal environment by restricting the entry and exit of molecules. As a consequence, cells require transport systems to permit entry of small polar compounds that they need (e.g., glucose) to concentrate compounds inside the cell (e.g., K^+) and to expel other

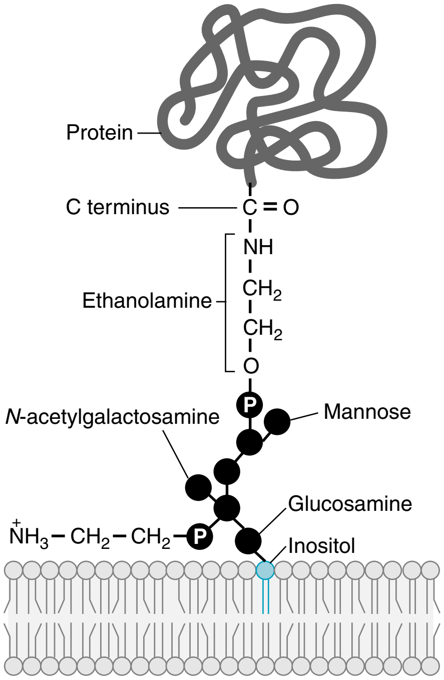


Fig. 10.7. The glycosylphosphatidylinositol glycan anchor (GPI). The carboxy terminus of the protein is attached to phosphoethanolamine, which is bound to a branched oligosaccharide that is attached to the inositol portion of phosphatidylinositol. The hydrophobic fatty acyl chains of the phosphatidylinositol portion are bound in the hydrophobic core of the membrane.

compounds (e.g., Ca^{2+} and Na^+). The transport systems for small organic molecules and inorganic ions fall into four categories: simple diffusion through the lipid bilayer or through a large pore; facilitative diffusion; gated channels; and active transport pumps (Fig. 10.8). These transport mechanisms are classified as passive if energy is not required, or active if energy is required. The energy is often provided by the hydrolysis of ATP.

In addition to these mechanisms for the transport of small individual molecules, cells engage in endocytosis. The plasma membrane extends or invaginates to surround a particle, a foreign cell, or extracellular fluid, which then closes into a vesicle that is released into the cytoplasm (see Fig. 10.8).

1. SIMPLE DIFFUSION

Gases such as O_2 and CO_2 and lipid-soluble substances (such as steroid hormones) can cross membranes by simple diffusion (see Fig. 10.8). In simple diffusion (free diffusion), molecules move by engaging in random collisions with other like molecules. There is a net movement from a region of high concentration to a region of low concentration because molecules keep bumping into each other where their concentration is highest. Energy is not required for diffusion, and compounds that are uncharged eventually reach the same concentrations on both sides of the membrane.

Water is considered to diffuse through membranes by unspecific movement through ion channels, pores, or around proteins embedded in the lipids. Certain cells (e.g., renal tubule cells) also contain large protein pores, called aquaporins, which permit a high rate of water flow from a region of a high water concentration (low solute concentration) to one of low water concentration (high solute concentration).

2. FACILITATIVE DIFFUSION THROUGH BINDING TO TRANSPORTER PROTEINS

Facilitative diffusion requires that the transported molecule bind to a specific carrier or transport protein in the membrane (Fig. 10.9). The transporter protein

Dennis Veere has become dehydrated because he has lost so much water through vomiting and diarrhea (see Chapter 4). Cholera toxin increases the efflux of sodium and chloride ions from his intestinal mucosal cells into the intestinal lumen. The increase of water in his stools results from the passive transfer of water from inside the cell and body fluids, where it is in high concentration (i.e., intracellular Na^+ and Cl^- concentrations are low), to the intestinal lumen and bowel, where water is in lower concentration (relative to high Na^+ and Cl^-). The watery diarrhea is also high in K^+ ions and bicarbonate. All of the signs and symptoms of cholera generally derive from this fluid loss.

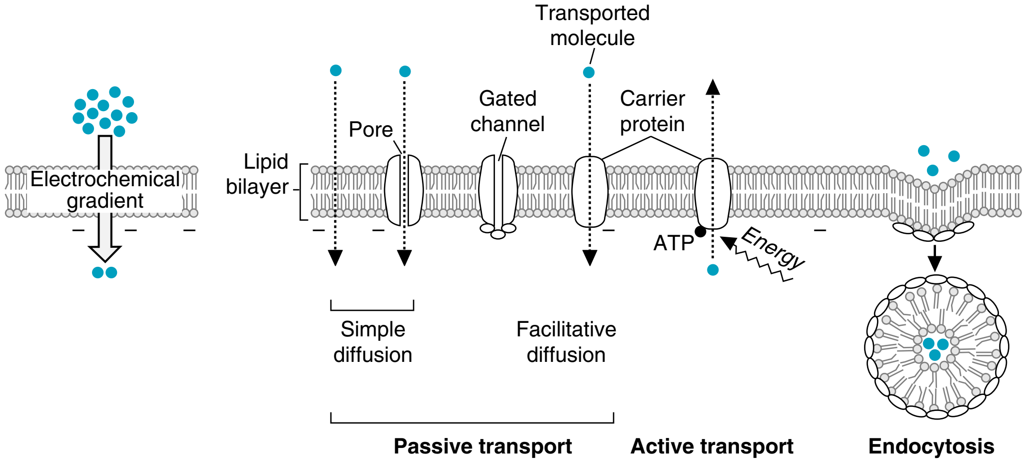


Fig. 10.8. Common types of transport mechanisms for human cells. The electrochemical gradient consists of the concentration gradient of the compound and the distribution of charge on the membrane, which affects the transport of charged ions such as Cl^-. Both protein amino acid residues and lipid polar head groups contribute to the net negative charge on the inside of the membrane. Generally, the diffusion of uncharged molecules (passive transport) is net movement from a region of high concentration to a low concentration, and active transport (energy-requiring) is net movement from a region of low concentration to one of high concentration.

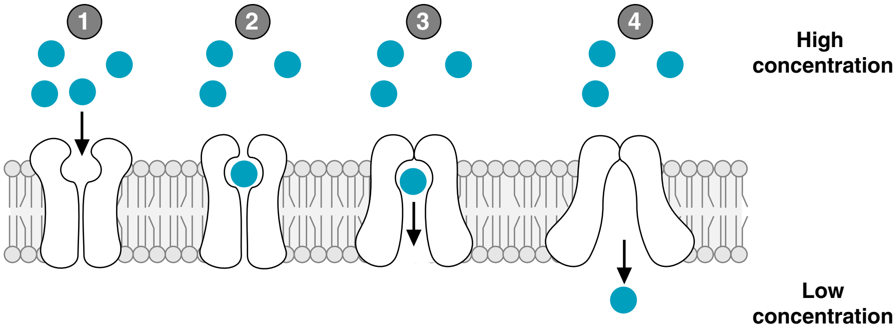


Fig. 10.9. Facilitative transport. Although the molecule being transported must bind to the protein transporter, the mechanism is passive diffusion, and the molecule moves from a region of high concentration to one of low concentration. "Passive" refers to the lack of an energy requirement for the transport.

All of the cells in the body have facilitative glucose transporters that transport glucose across the plasma membrane down an electrochemical (concentration) gradient as it is rapidly metabolized in the cell. In muscle and adipose tissue, insulin increases the content of facilitative glucose transporters in the cell membrane, thus increasing the ability of these tissues to take up glucose. Patients with type 1 diabetes mellitus, who do not produce insulin (e.g., **Di Abietes,** see Chapter 7), have a decreased ability to transport glucose into these tissues, thereby contributing to hyperglycemia (high blood glucose).

then undergoes a conformational change that allows the transported molecule to be released on the other side of the membrane. Although the transported molecules are bound to proteins, the transport process is still classified as diffusion because energy is not required, and the compound equilibrates (achieves a balance of concentration and charge) on both sides of the membrane.

Transporter proteins, like enzymes, exhibit saturation kinetics; when all the binding sites on all of the transporter proteins in the membrane are occupied, the system is saturated and the rate of transport reaches a plateau (the maximum velocity). By analogy to enzymes, the concentration of a transported compound required to reach ½ the maximum velocity is often called the K_m (Fig. 10.10). Facilitative transporters are similar to enzymes with respect to two additional features: they are relatively specific for the compounds they bind and they can be inhibited by compounds that block their binding sites or change their conformation.

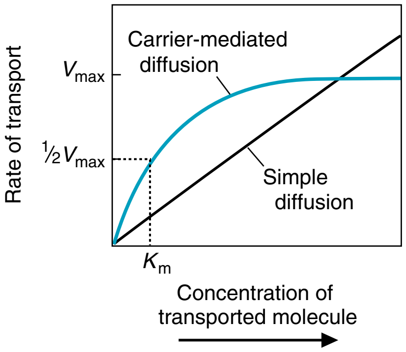


Fig. 10.10. Saturation kinetics of transporter proteins. When a compound must bind to a protein to be transported across a membrane, the velocity of transport depends on the amount of compound bound. It reaches a maximum rate when the compound's concentration is raised so high that all of the transporter binding sites are occupied. The curve is a rectangular hyperbola that approaches V_{max} at infinite substrate concentration, identical to that of Michaelis-Menten enzymes. The K_m of transport is the concentration of compound required for ½ V_{max}. In contrast, simple diffusion of a compound does not require its binding to a protein, and the rate of transport increases linearly with increasing concentration of the compound.

3. GATED CHANNELS IN PLASMA MEMBRANES

In gated channels, transmembrane proteins form a pore for ions that is either opened or closed in response to a stimulus: voltage changes across the membrane (voltage-gated channels), the binding of a compound (ligand-gated channels), or a regulatory change in the intracellular domain (phosphorylation-gated and pressure-gated channels). For example, the conduction of a nerve impulse along the axon depends on the passive flux of Na^+ ions through a voltage-gated channel that is opened by depolarization of the membrane. CFTR (**c**ystic **f**ibrosis **t**ransmembrane conductance **r**egulator) is a Cl^- channel that provides an example of a ligand-gated channel regulated through phosphorylation (phosphorylation-gated) (Fig. 10.11). CFTR is a member of the ABC (adenine nucleotide binding cassette, or ATP binding cassette) superfamily of transport proteins. It has two transmembrane domains that form a closed channel, each connected to an ATP binding site, and a regulatory domain that sits in front of the channel. When the regulatory domain is phosphorylated by a kinase, its conformation changes and it moves away from the ATP binding domains. As ATP binds and is hydrolyzed, the transmembrane domains change conformation and open the channel, and chloride ions diffuse through. As the conformation reverts back to its original form, the channel closes.

Transport through a ligand-gated channel is considered diffusion, although ATP is involved, because only a few ATP molecules are being used to open and close the channel through which many, many chloride ions diffuse. However, the distinction between ligand-gated channels and facilitative transporters is not always as clear. Many gated channels show saturation kinetics at very high concentrations of the compounds being transported.

The cystic fibrosis transmembrane conductance regulator (CFTR) was named for its role in cystic fibrosis. A mutation in the gene encoding its transmembrane subunits results in dried mucus accumulation in the airways and pancreatic ducts.

The CFTR is also involved in the dehydration experienced by cholera patients such as **Dennis Veere**. In intestinal mucosal cells, cholera A toxin indirectly activates phosphorylation of the regulatory domain of CFTR by protein kinase A. Thus, the channel stays open and Cl^- and H_2O flow from the cell into the intestinal lumen, resulting in dehydration.

4. ACTIVE TRANSPORT REQUIRES ENERGY AND TRANSPORTER PROTEINS

Both active transport and facilitative transport are mediated by protein transporters (carriers) in the membrane. However, in facilitative transport, the compound is transported down an electrochemical gradient (the balance of concentration and charge across a membrane), usually from a high concentration to a low concentration, to equilibrate between the two sides of the membrane. In active transport, energy is used to concentrate the compound on one side of the membrane. If energy is directly applied to the transporter (e.g., ATP hydrolysis by Na^+,K^+-ATPase), the transport is called primary active transport; if energy is used to establish an ion gradient (e.g., the Na^+ gradient), and the gradient is used to concentrate another compound, the transport is called secondary active transport.

The Na^+,K^+-ATPase spans the plasma membrane, much like a gated pore, with a binding site for 3 Na^+ ions open to the intracellular side (Fig. 10.12). Energy from

Protein-mediated transport systems, whether facilitative or active, are classified as antiports if they specifically exchange compounds of similar charge across a membrane; they are called symports or cotransporters if they simultaneously transport two molecules across the membrane in the same direction. Band 3 in the red blood cell membrane, which exchanges chloride ion for bicarbonate, provides an example of an antiport.

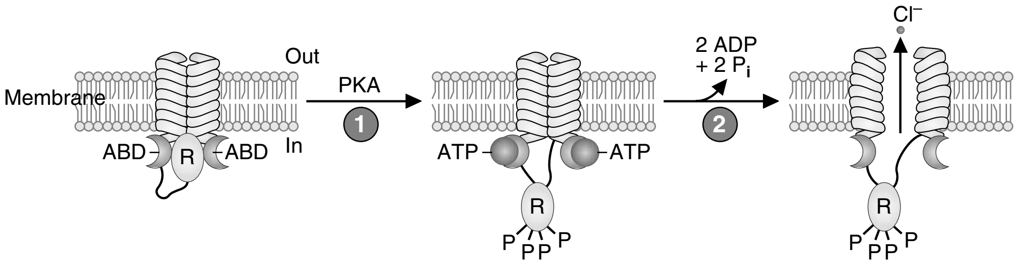


Fig. 10.11. CFTR, a ligand-gated channel controlled by phosphorylation. Two intracellular binding domains control opening of the channel, an adenine nucleotide binding domain (ABD) and a regulatory domain (R). **1** Phosphorylation of the regulatory subunit by protein kinase A causes a conformational change that allows ATP to bind to the adenine nucleotide binding domain (ABD). **2** Hydrolysis of bound ATP opens the channel so that chloride ions can diffuse through.

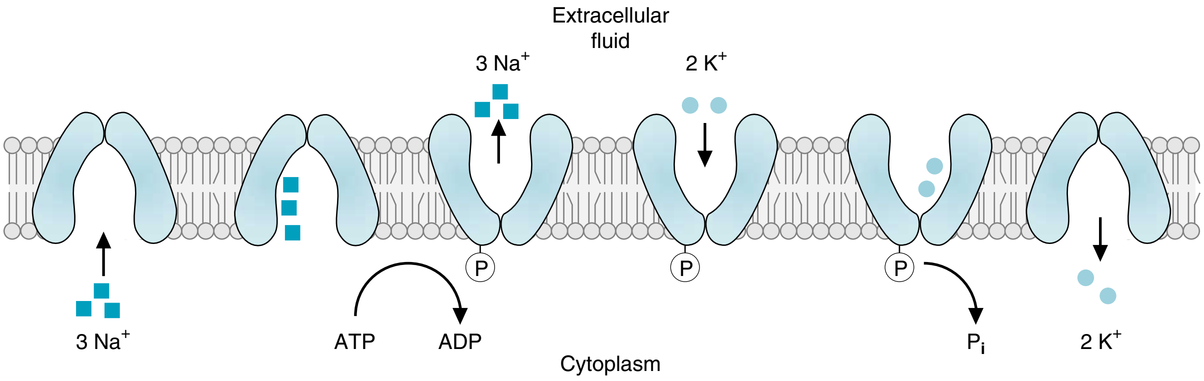


Fig. 10.12. Active transport by Na^+,K^+-ATPase. Three sodium ions bind to the transporter protein on the cytoplasmic side of the membrane. When ATP is hydrolyzed to ADP, the carrier protein is phosphorylated and undergoes a change in conformation that causes the sodium ions to be released into the extracellular fluid. Two potassium ions then bind on the extracellular side. Dephosphorylation of the carrier protein produces another conformational change, and the potassium ions are released on the inside of the cell membrane. The transporter protein then resumes its original conformation, ready to bind more sodium ions.

ATP hydrolysis is used to phosphorylate an internal domain and change the transporters' conformation so that bound Na^+ ions are released to the outside, and two external K^+ ions bind. K^+ binding triggers hydrolysis of the bound phosphate group and a return to the original conformation, accompanied by release of K^+ ions inside the cell. As a consequence, cells are able to maintain a much lower intracellular Na^+ concentration and much higher intracellular K^+ ion concentration than present in the external fluid.

The Na^+ gradient, which is maintained by primary active transport, is used to power the transport of glucose, amino acids, and many other compounds into the cell through secondary active transport. An example is provided by the transport of glucose into cells of the intestinal epithelium in conjunction with Na^+ ions (Fig. 10.13).

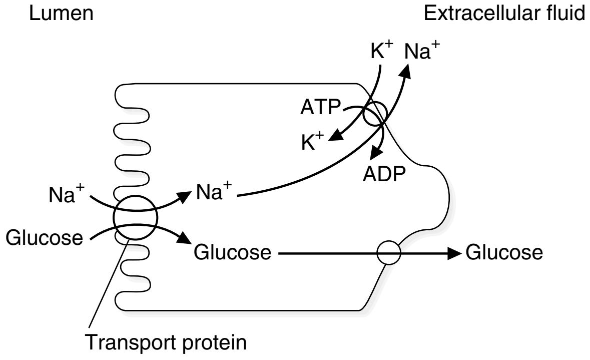


Fig. 10.13. Secondary active transport of glucose by the Na^+-glucose cotransporter. One sodium ion binds to the carrier protein in the luminal membrane, stimulating the binding of glucose. After a conformational change, the protein releases Na^+ and glucose into the cell and returns to its original conformation. Na^+,K^+-ATPase in the basolateral membrane pumps Na^+ against its concentration gradient into the extracellular fluid. Thus, the Na^+ concentration in the cell is low, and Na^+ moves from the lumen down its concentration gradient into the cell and is pumped against its gradient into the extracellular fluid. Glucose, consequently, moves against its concentration gradient from the lumen into the cell by traveling on the same carrier as Na^+. Glucose then passes down its concentration gradient into the extracellular fluid on a passive transporter protein.

The Ca^{2+}-ATPase, a calcium pump, uses a mechanism similar to that of Na^+,K^+-ATPase to maintain intracellular Ca^{2+} concentration below 10^{-7} M in spite of the high extracellular concentration of 10^{-3} M. This transporter is inhibited by binding of the regulatory protein calmodulin. When the intracellular Ca^{2+} concentration increases, Ca^{2+} binds to calmodulin, which dissociates from the transporter, thereby activating it to pump Ca^{2+} out of the cell (see Chapter 9 for the structure of calmodulin). High levels of intracellular Ca^{2+} are associated with irreversible progression from cell injury to cell death.

The dehydration of cholera is often treated with an oral rehydration solution containing Na^+, K^+, and glucose or a diet of rice (which contains glucose and amino acids). Glucose is absorbed from the intestinal lumen via the Na^+-dependent glucose cotransporters, which cotransport Na^+ into the cells together with glucose. Many amino acids are also absorbed by Na^+-dependent cotransport. With the return of Na^+ to the cytoplasm, water efflux from the cell into the intestinal lumen decreases.

The vitamin folate provides an example of a compound transported into cells by caveolae, which form around the occupied folate receptor. In contrast, endocytosis of many compounds such as membrane hormone receptors occurs through clathrin-coated pits. The receptors are targeted for these pits by adaptor proteins that bind to a specific amino acid sequence in the receptor.

These cells create a gradient in Na^+ and then use this gradient to drive the transport of glucose from the intestinal lumen into the cell against its concentration gradient.

D. Vesicular Transport across the Plasma Membrane

Vesicular transport occurs when a membrane completely surrounds a compound, particle, or cell and encloses it into a vesicle. When the vesicle fuses with another membrane system, the entrapped compounds are released. Endocytosis refers to vesicular transport into the cell, and exocytosis to transport out of the cell. Endocytosis is further classified as phagocytosis if the vesicle forms around particulate matter (such as whole bacterial cells or metals and dyes from a tattoo), and pinocytosis if the vesicle forms around fluid containing dispersed molecules. Receptor-mediated endocytosis is the name given to the formation of clathrin-coated vesicles that mediate the internalization of membrane-bound receptors in vesicles coated on the intracellular side with subunits of the protein clathrin (Fig. 10.14). Potocytosis is the name given to endocytosis that occurs via caveolae (small invaginations or "caves"), which are regions of the cell membrane with a unique lipid and protein composition (including the protein caveolin-1).

III. LYSOSOMES

Lysosomes are the intracellular organelles of digestion enclosed by a single membrane that prevents the release of its digestive enzymes into the cytosol. They are central to a wide variety of body functions that involve elimination of unwanted material and recycling their components, including destruction of

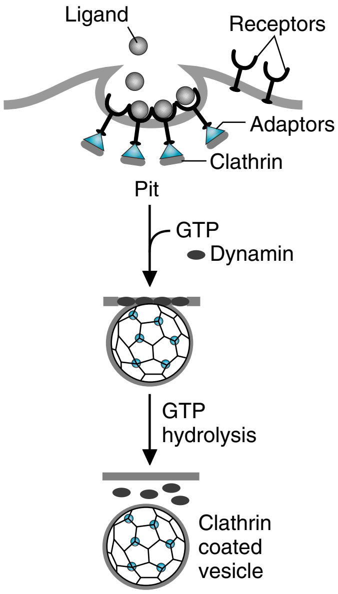


Fig. 10.14. Formation of a clathrin-coated vesicle. Ligands entering the cell through receptor-mediated endocytosis bind to receptors that cluster in an area of the membrane. Adaptor proteins bind to the receptor tails and to the clathrin molecules to enclose the budding membrane in a cage-like clathrin coat.
Molecules of a monomeric G protein called dynamin (from the Rab family) constrict the neck of the vesicle and pinch it off from the membrane as GTP is hydrolyzed.

infectious bacteria and yeast, recovery from injury, tissue remodeling, involution of tissues during development, and normal turnover of cells and organelles.

A. Lysosomal Hydrolases

The lysosomal digestive enzymes include nucleases, phosphatases, glycosidases, esterases, and proteases called cathepsins (Fig. 10.15). These enzymes are all hydrolases, enzymes that cleave amide, ester, and other bonds through the addition of water. Many of the products of lysosomal digestion, such as the amino acids, return to the cytosol. Lysosomes are therefore involved in recycling compounds.

Most of these lysosomal hydrolases have their highest activity near a pH of approximately 5.5 (the pH optimum). The intralysosomal pH is maintained near 5.5 principally by *v*-ATPases (vesicular ATPases), which actively pump protons into the lysosome. The cytosol and other cellular compartments have a pH nearer 7.2 and are therefore protected from escaped lysosomal hydrolases.

B. Endocytosis, Phagocytosis, and Autophagy

Lysosomes are formed from digestive vesicles called endosomes, which are involved in receptor-mediated endocytosis. They also participate in digestion of foreign cells acquired through phagocytosis and the digestion of internal contents in the process of autophagocytosis.

1. RECEPTOR-MEDIATED ENDOCYTOSIS

Lysosomes are involved in the digestion of compounds brought into the cells in endocytotic clathrin-coated vesicles formed by the plasma membrane (Fig. 10.16). These vesicles fuse to form multivesicular bodies called early endosomes. The early endosomes mature into late endosomes as they recycle clathrin, lipids, and other

Lysosomal storage diseases. Genetic defects in lysosomal enzymes, or in proteins such as the mannose 6-phosphate receptors required for targeting the enzymes to the lysosome, lead to an abnormal accumulation of undigested material in lysosomes that may be converted to residual bodies. The accumulation may be so extensive that normal cellular function is compromised, particularly in neuronal cells. Genetic diseases such as the Tay-Sachs disease (an accumulation of partially digested gangliosides in lysosomes), and Pompe's disease (an accumulation of glycogen particles in lysosomes) are caused by the absence or deficiency of specific lysosomal enzymes.

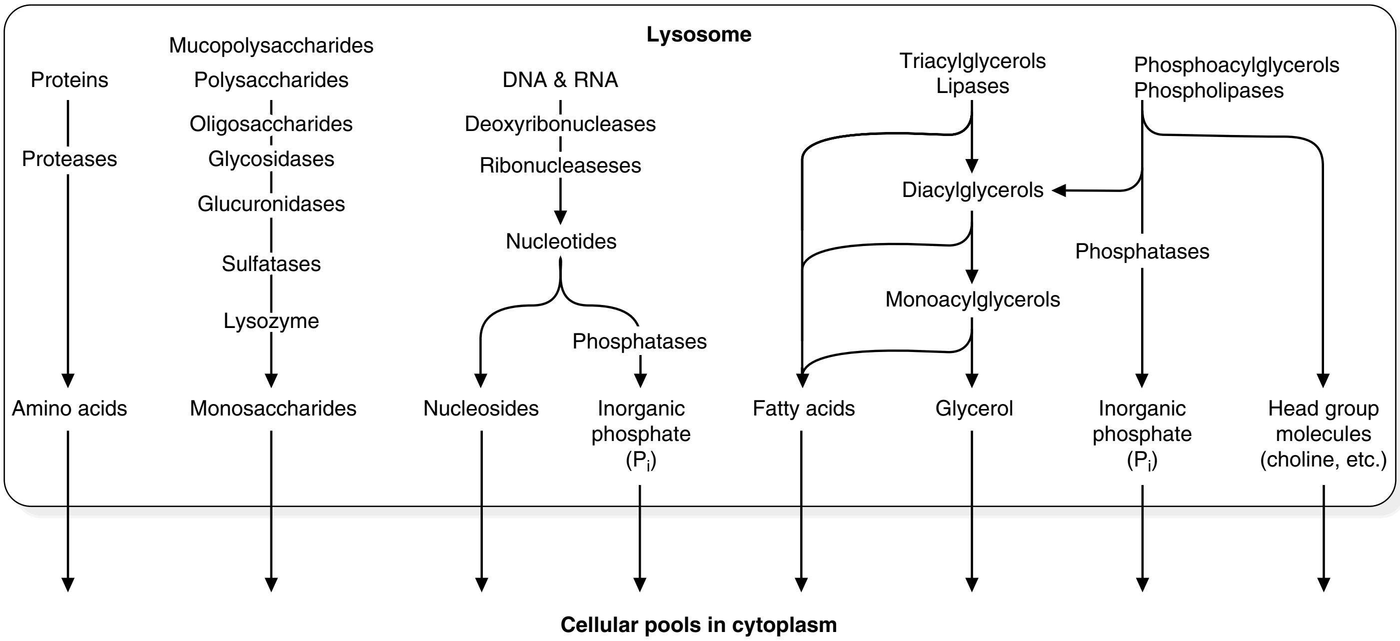


Fig. 10.15. Lysosomal reactions. Most lysosomal enzymes are hydrolases, which cleave peptide, ester, and glycosidic bonds by adding the components of water across the bond. These enzymes are active at the acidic pH of the lysosome and inactive if accidentally released into the cytosol.

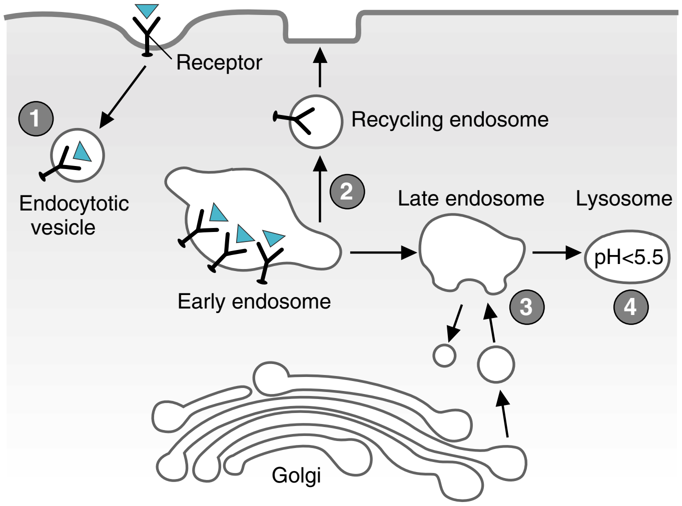


Fig. 10.16. Lysosomes in receptor-mediated endocytosis via clathrin-coated pits. **1** Endocytotic vesicles fuse to form early endosomes. **2** Vesicle contents are sorted, and receptors, clathrin, and lipids are sent back to the plasma membrane. **3** Transport vesicles from the trans-Golgi carry lysosomal hydrolases to the late endosome. **4** Lysosomes containing concentrated hydrolases digest proteins and other components acquired from endocytotic vesicles.

The elevated level of uric acid in **Lotta Topaigne**'s blood led to the deposition of monosodium urate crystals in the joint space (synovial fluid) of her right great toe, resulting in podagra (painful great toe). Neutrophils, the mediators of the acute inflammation that followed, attempted to phagocytose the urate crystals. The engulfed urate crystals were deposited in the late endosomes and lysosomes of the neutrophil. Because urate crystals are particles that cannot be degraded by any of the lysosomal acid hydrolases, their accumulation caused lysis of the lysosomal membranes, followed by cell lysis and release of lysosomal enzymes into the joint space. The urate crystals also resulted in release of chemical mediators of inflammation that recruited other cells into the area. This further amplified the acute inflammatory reaction in the tissues of the joint caspsule (synovitis), leading to the extremely painful swelling of acute gouty arthritis.

Phagocytosis and autophagy are part of the normal turnover of body components, such as degradation of cells that have a shorter lifespan than the whole organism and remodeling of tissues during pregnancy. For example, phagocytes, located mainly in the spleen and liver, remove approximately 3×10^{11} red blood cells from the circulation each day. During pregnancy, breast tissue is remodeled to develop the capacity for lactation; after weaning of an infant, the lactating breast returns to its original state (involution).

membrane components back to the plasma membrane in vesicles called recycling endosomes. The late endosomes mature into lysosomes as they progressively accumulate newly synthesized acid hydrolases and vesicular proton pumps brought to them in clathrin-coated vesicles from the Golgi. Thus, lysosomes do not acquire their full digestive power until after sorting of membrane lipids and proteins for recycling.

Within the Golgi, enzymes are targeted for endosomes (and eventually lysosomes) by addition of mannose 6-phosphate residues that bind to mannose 6-phosphate receptor proteins in the Golgi membrane. The mannose 6-phosphate receptors together with their bound acid hydrolases are incorporated into the clathrin-coated Golgi transport vesicles and released. The transport vesicles lose their clathrin coat and then fuse with the late endosomal membrane. The acidity of the endosome releases the acid hydrolases from the receptors into the vesicle lumen. The receptors are eventually recycled back to the Golgi.

2. PHAGOCYTOSIS AND AUTOPHAGY

One of the major roles of lysosomes is phagocytosis (Fig. 10.17). Neutrophils and macrophages, the major phagocytic cells, devour pathogenic microorganisms and clean up wound debris and dead cells, thus aiding in repair. As bacteria or other particles are enclosed into clathrin-coated pits in the plasma membrane, these vesicles bud off to form intracellular phagosomes. The phagosomes fuse with lysosomes, where the acidity and digestive enzymes destroy the contents. Pinocytotic vescicles also may fuse with lysosomes.

In autophagy (self-eating), intracellular components such as organelles or glycogen particles are surrounded by a membrane derived from ER vesicles, forming an autophagosome. The autophagosome fuses with a lysosome, and the contents of the phagolysosome are digested by lysosomal enzymes. Organelles usually turn over much more rapidly than the cells in which they reside (e.g., approximately four mitochondria in each liver cell are degraded per hour). Cells that are damaged but still viable recover, in part, by using autophagy to eliminate damaged components.

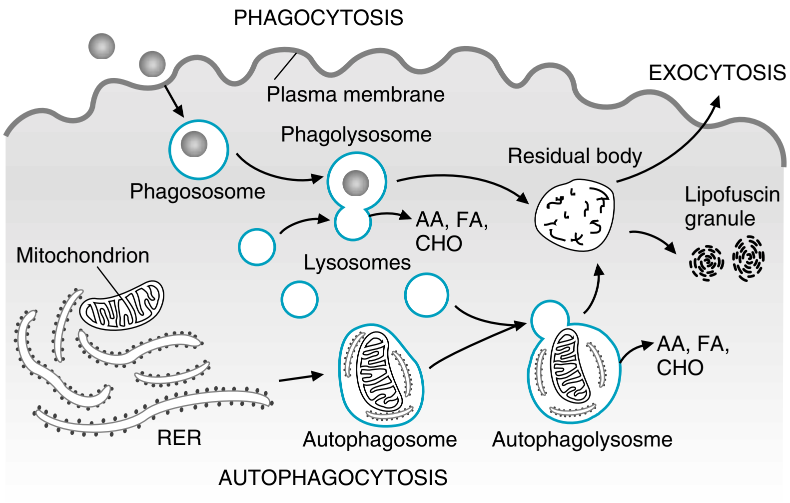


Fig. 10.17. Phagocytosis and autophagy. Cells and large particles are phagocytosed. The phagosomes fuse with lysosomes to form phagolysosomes. Recyclable amino acids (AA), fatty acids (FA), and carbohydrates (CHO) are released into the cytosol. Autophagosomes are formed in the ER as the cell digests mitochondria and its own large particles. These also merge with lysosomes. Undigested material may remain in the lysosomes to form residual bodies, which are either extruded or remain in the cell as lipofuscin granules.

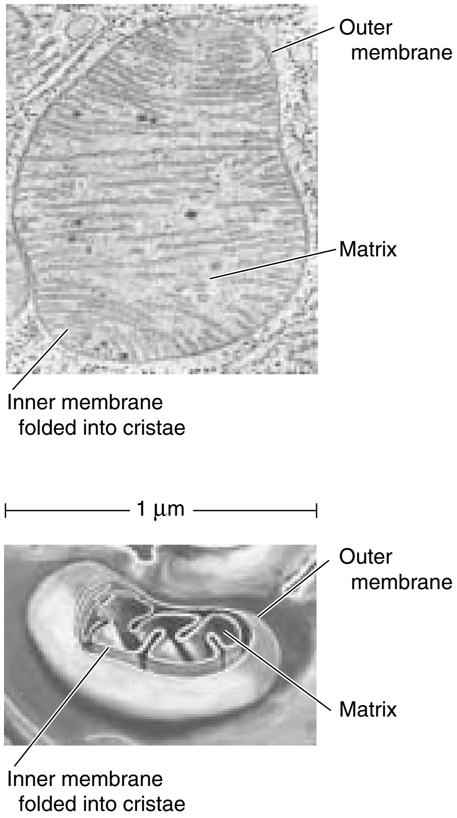


Fig. 10.18. Mitochondrion. Electron micrograph (top); three-dimensional drawing (bottom).

If a significant amount of undigestible material remains within the lysosome after the digestion process is completed, the lysosome is called a residual body. Depending on the cell type, residual bodies may be expelled (exocytosis) or remain indefinitely in the cell as lipofuscin granules that accumulate with age.

IV. MITOCHONDRIA

Mitochondria contain most of the enzymes for the pathways of fuel oxidation and oxidative phosphorylation and thus generate most of the ATP required by mammalian cells. Each mitochondrion is surrounded by two membranes, an outer membrane and an inner membrane, separating the mitochondrial matrix from the cytosol (Fig. 10.18). The inner membrane forms invaginations known as cristae containing the electron transport chain and ATP synthase. Most of the enzymes for the TCA cycle and other pathways for oxidation are located in the mitochondrial matrix, the compartment enclosed by the inner mitochondrial membrane. (The TCA cycle and electron transport chain are described in more detail in Chapters 20 and 21.)

The inner mitochondrial membrane is highly impermeable, and the proton gradient that is built up across this membrane during oxidative phosphorylation is essential for ATP generation from ADP and phosphate. The transport of ions occurs principally through facilitative transporters in a type of secondary active transport powered by the proton gradient established by the electron transport chain. The outer membrane contains pores made from proteins called porins and is permeable to molecules with a molecular weight up to about 1000 g/mole.

Mitochondria can replicate by division; however, most of their proteins must be imported from the cytosol. Mitochondria contain a small amount of DNA, which encodes for only 13 different subunits of proteins involved in oxidative phosphorylation. Most of the enzymes and proteins in mitochondria are encoded by nuclear DNA and synthesized on cytoplasmic ribosomes. They are imported

Mitochondial diseases. Mitochondria contain DNA and can reproduce by replicating their DNA and then dividing in half. Although nuclear DNA encodes most of the enzymes found in mitochondria, mitochondrial DNA encodes some of the subunits of the electron transport chain proteins and ATP synthase. Mutations in mitochondrial DNA result in a number of genetic diseases that affect skeletal muscle, neuronal, and renal tissues. They are implicated in aging.

through membrane pores by a receptor-mediated process involving members of the heat shock family of proteins.

V. PEROXISOMES

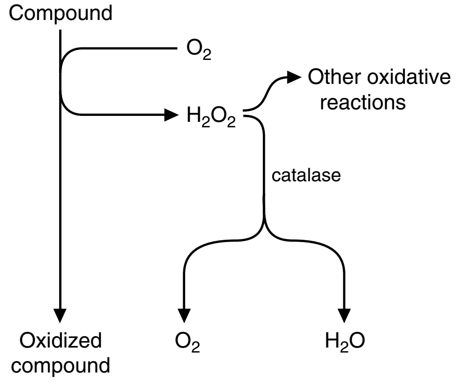


Fig. 10.19. Types of reactions in peroxisomes.

Peroxisomes are cytoplasmic organelles, similar in size to lysosomes, that are involved in oxidative reactions using molecular oxygen (Fig. 10.19). These reactions produce the toxic chemical hydrogen peroxide (H_2O_2), which is subsequently used or degraded within the peroxisome by catalase and other enzymes. Peroxisomes function in the oxidation of very long chain fatty acids (containing 20 or more carbons) to shorter chain fatty acids, the conversion of cholesterol to bile acids, and the synthesis of ether lipids called plasmalogens. They are bounded by a single membrane.

Like mitochondria, peroxisomes can replicate by division. However, they are dependent on the import of proteins to function. They contain no DNA.

Peroxisomal Diseases. Peroxisomal diseases are caused by mutations affecting either the synthesis of functional peroxisomal enzymes or their incorporation into peroxisomes. For example, adrenoleukodystrophy probably involves a mutation that decreases the content of a transporter in the peroxisomal membrane. Zellweger's syndrome is caused by the failure to complete the synthesis of peroxisomes.

VI. NUCLEUS

The largest of the subcellular organelles of animal cells is the nucleus (Fig. 10.20). Most of the genetic material of the cell is located in the chromosomes of the nucleus, which are composed of DNA, an equal weight of small, positively charged proteins called histones, and a variable amount of other proteins. This nucleoprotein

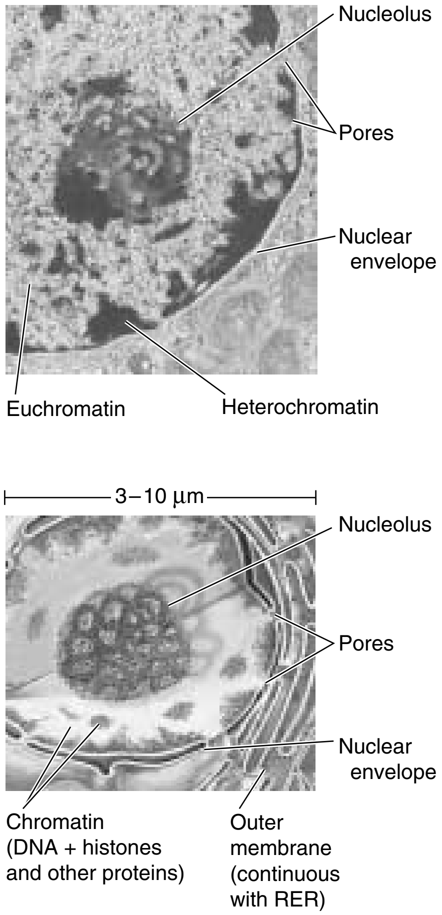


Fig. 10.20. Nucleus. Electron micrograph (top); three-dimensional drawing (bottom).

complex is called chromatin. The nucleolus, a substructure of the nucleus, is the site of rRNA transcription and processing, and of ribosome assembly. Replication, transcription, translation, and the regulation of these processes are the major focus of the molecular biology section of this text (see Section Three).

The nucleus is separated from the rest of the cell (the cytoplasm) by the nuclear envelope, which consists of two membranes joined at nuclear pores. The outer nuclear membrane is continuous with the rough endoplasmic reticulum. To convert the genetic code of the DNA into the primary sequence of a protein, DNA is transcribed into RNA, which is modified and edited into mRNA. The mRNA travels through the nuclear pores into the cytoplasm, where it is translated into the primary sequence of a protein on ribosomes (Fig. 10.21). Ribosomes, which are generated in the nucleolus, also must travel through nuclear pores to the cytoplasm. Conversely, proteins required for replication, transcription, and other processes pass into the nucleus through these pores. Thus, transport through the pore is specific for the molecule and the direction of transport.

Specificity and direction of travel through the nuclear pore (import vs. export) is dictated by binding proteins (importins vs. exportins), by a small GTP protein called Ran, and by the location of the regulatory protein, RanGAP (GTPase activating protein) only on the cytoplasmic side (Fig. 10.22). Proteins transported into the nucleus have a nuclear localization signal that causes them to bind to one of the subunits of cytosolic proteins called importins. The other subunit of the importin molecule binds to cytoplasmic filaments attached to the outer ring of the nuclear pore. As the importin-protein complex enters the nucleus, the small GTP-binding protein Ran binds to an importin subunit, causing release of the transported protein into the nucleus. The Ran-importin complex is returned to the cytosol, where RanGAP (GTPase activating protein) activates hydrolysis of bound GTP to GDP and phosphate. The energy released by GTP hydrolysis changes the conformation of Ran and the complex dissociates. The free importin can then bind another protein.

Table 10.1 Monomeric G Proteins in the Ras Superfamily

G-Protein Family	Function	Some Family Members	Location and Membrane Attachment Site
Ras	Regulator of gene expression and cell growth, found in mutated oncogenic forms in many human tumors	H-Ras, K-Ras, N-Ras, Ral A, Rad, Rap, Rit,	Anchored to plasma membrane by farnesyl, palmitoyl, or other lipid groups
Rho	Controls organization of actin cytoskeleton and gene expression (F-actin bundling, myosin filament assembly)	Rho (A-E), Cdc42, Rac (1-3)	Anchored to plasma membrane by lipids, and translocates to cytosol
Arf/Sar	Assembly of coatomer-coated vesicles (COPI and COPII) for vesicular trafficking pathways originating in the Golgi	Arf (1-6), Sar 1a,1b; Arl (1-7)	Arf is anchored to vesicular membranes by myristyl groups, but Sar is anchored by the protein itself.
Rab	Targeting of vesicles involved in secretory and endocytotic pathways and formation of v-SNARE–t-SNARE complexes	Dynamin, Rab (11-33)	Anchored to lipid membranes with geranylgeranyl (C20 isoprenoid) groups and other lipids
Ran	Transport through nuclear pore complexes.	Ran	Not anchored to lipid membrane. Found in cytosol and nucleus

The Ras family of monomeric G proteins. Ras and Ran belong to a superfamily of proteins called small G proteins (also called small GTP-binding proteins, small GTPases, or monomeric G proteins: see Chapter 9, section III.C.2). These proteins function as timing regulators for a variety of cell functions. They are referred to as "small" because they are composed of a single subunit with a weight of 20 to 40 kDa, and they are called GTPases because they slowly hydrolyze bound GTP. When small G proteins contain bound GTP, they bind to and activate their target proteins. As their bound GTP is hydrolyzed to GDP and Pi, their conformation changes dramatically, and they dissociate from the target protein. They thus serve as "automatic clocks" that shut themselves off. Many of the monomeric GTP binding proteins are regulated by GAPs (GTPase activating proteins), GEFs (GTP exchange proteins which stimulate GDP dissociation and GTP binding), or GDIs (GDP-dissociation inhibitory proteins). (See Chapter 9 for a more complete discussion of these regulators). The function of each of the five major classes of Ras monomeric G proteins in cell biology is summarized in Table 10.1.

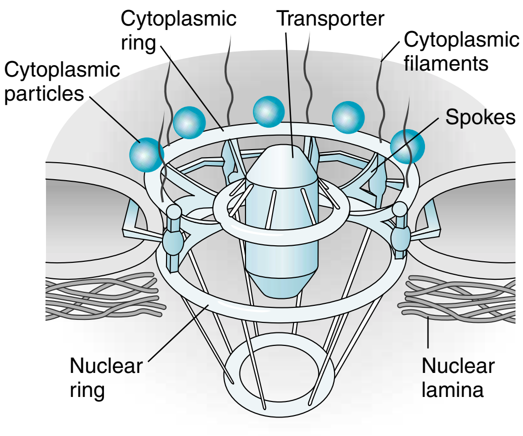


Fig. 10.21. The nuclear pore complex. The approximately 100 different polypeptide chains of the nuclear pore complex form an assembly of 8 spokes attached to two ring structures (a cytoplasmic ring in the outer nuclear membrane and a nuclear ring through the inner membrane) with a transporter "plug" in the center. Small molecules, ions, and proteins with less than a 50-kDa mass passively diffuse through the pore in either direction. However, RNAs and most proteins are too large to diffuse through, and are actively transported in a process that requires energy, is selective for the molecule transported, is unidirectional, and can be regulated.

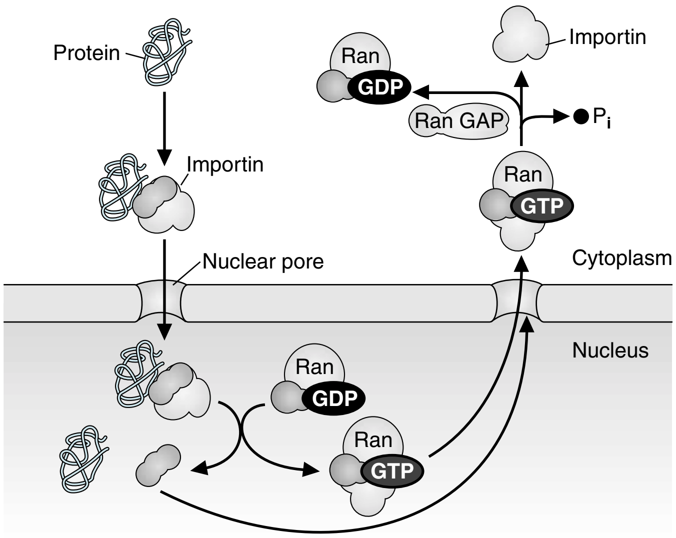


Fig. 10.22. Nuclear import. Proteins with the nuclear localization signal bind to importins, which carry them through the nuclear pore into the nucleus. The monomeric G protein Ran containing bound GTP binds to one of the subunits of importin. This causes dissociation of the importin subunits and release of the imported protein in the nucleus. The Ran-importin complex exits a nuclear pore. On the cytoplasmic side, a RanGAP (GTPase activating protein) activates the hydrolysis of GTP to GDP, which causes dissociation of the complex. RanGDP is subsequently returned to the nucleus, where an accessory protein activates dissociation of GDP and association of GTP.

RNAs are transported from the nucleus to the cytoplasm as ribonucleoproteins, which are targeted for export by a specific amino acid sequence called the nuclear export signal. The nucleoprotein forms a complex with additional proteins called exportins and with Ran. This complex is transported through the pore to the cytoplasm, where RanGAP activates hydrolysis of the bound GTP. In the absence of GTP, the complex dissociates with the release of RNA into the cytoplasm, and the exportins and Ran are transported back to the nucleus.

VII. ENDOPLASMIC RETICULUM

The endoplasmic reticulum (ER) is a network of membranous tubules within the cell consisting of smooth endoplasmic reticulum (SER), which lacks ribosomes, and rough endoplasmic reticulum (RER), which is studded with ribosomes (Fig. 10.23). The SER has a number of functions. It contains enzymes for the synthesis of many lipids, such as triacylglycerols and phospholipids. It also contains the cytochrome P450 oxidative enzymes involved in metabolism of drugs and toxic chemicals such as ethanol and the synthesis of hydrophobic molecules such as steroid hormones. Glycogen is stored in regions of liver cells that are rich in SER.

The RER is involved in the synthesis of certain proteins. Ribosomes attached to the membranes of the RER give them their "rough" appearance. Proteins produced on these ribosomes enter the lumen of the RER, travel to the Golgi complex in vesicles, and are subsequently either secreted from the cell, sequestered within membrane-enclosed organelles such as lysosomes, or embedded in the plasma membrane. Posttranslational modifications of these proteins, such as the initiation of N-linked glycosylation and the addition of GPI anchors, occur in the RER. In contrast, proteins encoded by the nucleus and found in the cytosol, peroxisomes, or mitochondria are synthesized on free ribosomes in the cytosol and are seldom modified by the attachment of oligosaccharides.

Chronic ingestion of ethanol has increased the content of MEOS, the microsomal ethanol oxidizing system, in **Al Martini's** liver. MEOS is a cytochrome P450 enzyme that catalyzes the conversion of ethanol, NADPH and O_2 to acetaldehyde, $NADP^+$, and 2 H_2O (see Chapter 9). The adjective *microsomal* is a term derived from experimental cell biology that is sometimes used for processes occurring in the ER. When cells are lysed in the laboratory, the ER is fragmented into vesicles called *microsomes,* which can be isolated by centrifugation. Microsomes, as such, are not actually present in cells.

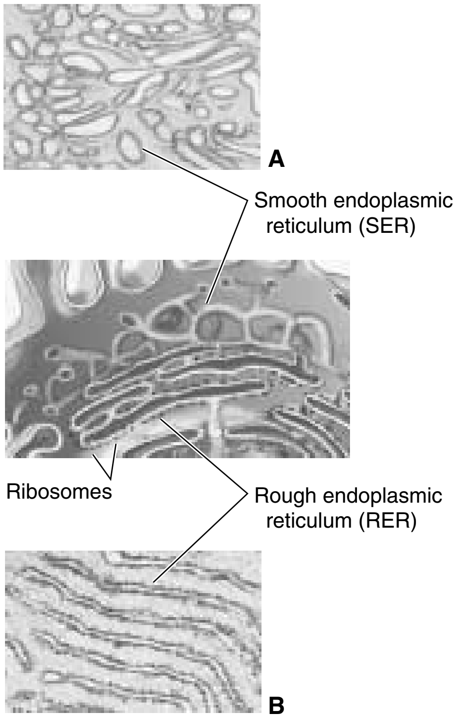


Fig. 10.23 **A.** Smooth endoplasmic reticulum. **B.** Rough endoplasmic reticulum. A and B are electron micrographs. A three-dimensional drawing is in the middle.

VIII. GOLGI COMPLEX

The Golgi complex is involved in modifying proteins produced in the RER and in sorting and distributing these proteins to the lysosomes, secretory vesicles, or the plasma membrane. It consists of a curved stack of flattened vesicles in the cytoplasm that is generally divided into three compartments: the *cis*-Golgi network, which is often convex and faces the nucleus; the *medial* Golgi stacks; and the *trans* Golgi network, which often faces the plasma membrane (Fig. 10.24).

Proteins are transported to and from the Golgi in at least three kinds of vesicles: coatomer-coated COP I vesicles, coatomer-coated COP II vesicles, and clathrin-coated vesicles (see Fig. 10.24). Proteins produced on the RER travel in COP II vesicles to an endoplasmic reticulum-Golgi intermediate compartment (ERGIC), and then to the *cis*-Golgi network, where they enter the lumen. Here N-linked oligosaccharide chains that were added to proteins in the RER are modified, and O-linked oligosaccharides are added. COP I vesicles recycle material from the Golgi back to the ER and possibly transfer material from the Golgi to other sites.

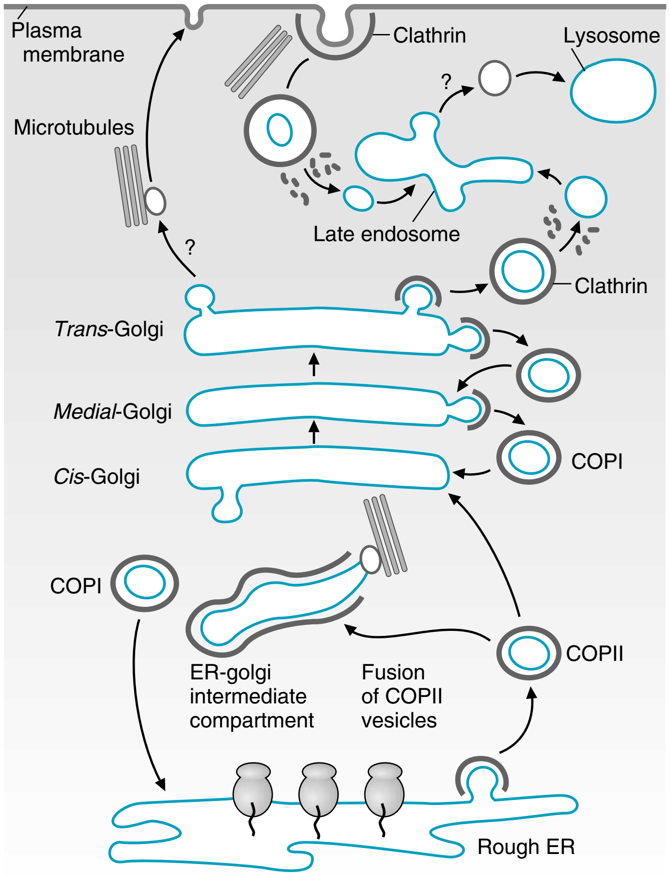


Fig. 10.24. Vesicular transport to and from the Golgi complex. COP II vesicles (coatomer-coated) form in the rough ER and move to the Golgi. COP I vesicles generally go from the trans to the cis Golgi to the ER. Vesicles that go to late endosomes (eventually lysosomes) from the Golgi or the plasma membrane are clathrin-coated. Less is known about exocytotic vesicles. Vesicle transport, as well as transport of organelles and secretory proteins, occurs along microtubules (structures formed from the protein tubulin).

Vesicles released from the *trans* face of the Golgi complex travel to endosomes as clathrin-coated vesicles.

COP vesicles are coated with a complex composed of coatomer proteins (COP), an Arf family monomeric G protein that mediates vesicle assembly, and other proteins (Fig. 10.25). COP I vesicles contain the monomeric G protein Arf (**A**DP-**r**ibosylating **f**actor), and COP II vesicles contain the monomeric G protein Sar (another member of the Arf family). In both types of vesicles, hydrolysis of GTP causes dissociation of the G-protein and disassembly of the vesicle coat. The vesicle components are then recycled. Glycoproteins or glycolipids once anchored in the membrane of the vesicle remain in the plasma membrane when the vesicular and plasma membranes fuse.

Vesicles that have lost their coats are ready to fuse with the target membrane. The vesicle membranes contain proteins called v-SNARES (vesicle-SNARES) (see Fig. 10.25). Each type of v-SNARE is able to recognize and bind to its complementary t-SNARE (target SNARE) on the target membrane, thus ensuring that

The monomeric G protein Arf was named for its contribution to the pathogenesis of cholera and not for its normal function in the assembly of COP I vesicles. However, it is also required for the transport of *V. cholerae* A-toxin. The cholera toxin is endocytosed in caveolae vesicles that subsequently merge with lysosomes (or are transformed into lysosomes), where the acidic pH contributes to activation of the toxin. As the toxin is transported through the Golgi and ER, it is further processed and activated. Arf forms a complex with the A-toxin that promotes its travel between compartments. The A-toxin is actually an ADP-ribosylase (an enzyme that cleaves NAD and attaches the ADP portion to a protein) (see Chapter 6, Fig. 6.14), and hence, Arf became known as the ADP-ribosylating factor. The ADP-ribosylation of proteins regulating the CFTR chloride channel leads to **Dennis Veere's** dehydration and diarrhea.

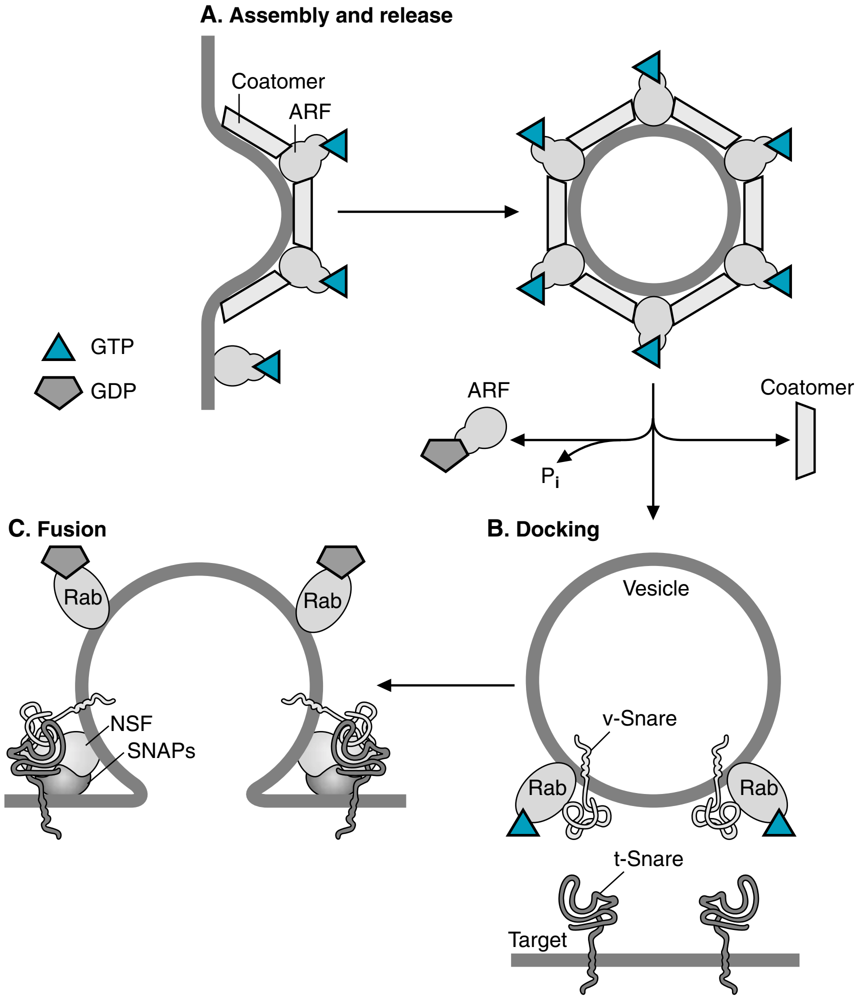


Fig. 10.25. Transport in COP-coated vesicles. **A.** Assembly and release. Arf with bound GTP assembles a region of the trans-Golgi membrane containing receptors for the protein cargo and coatomers. As GTP is hydrolyzed to GDP, the coat is released. **B.** Docking. The small G protein Rab assists in docking. v-Snares in the vesicle membrane recognize complementary t-Snares in the target membrane. **C.** Fusion. NSF and SNAP are fusion proteins.

the vesicle contents are delivered to the right location. Several additional proteins are required for fusion of the vesicle with the target membrane, including Rab (another monomeric G protein) and two additional proteins called SNAP (**s**oluble **N**SF **a**ttachment **p**roteins) and NSF (**N**-ethylmaleimide **s**ensitive **f**actor).

Secretory vesicles. The hormone insulin is synthesized as a prohormone, proinsulin, which is incorporated into secretory vesicles. These vesicles contain a protease that is activated by the acidic pH of the secretory vesicle. It cleaves proinsulin into the A, B, and C chains (see Fig. 6.13).

Exocytotic vesicles release proteins into the extracellular space after fusion of the vesicular and plasma cell membranes. Exocytotic vesicles containing hormones also may contain proteases that cleave the prohormone at a specific site and *v*-ATPases that acidify the vesicle and activate the protease (similar to lysosomes).

IX. CYTOSKELETON

The structure of the cell, the shape of the cell surface, and the arrangement of subcellular organelles is organized by three major protein components: microtubules composed of tubulin, which move and position organelles and vesicles; thin filaments composed of actin, which form a cytoskeleton, and intermediate filaments composed of different fibrous proteins. Actin and tubulin, which are involved in cell movement, are dynamic structures composed of continuously associating and dissociating globular subunits. Intermediate filaments, which play a structural role, are composed of stable fibrous proteins that turn over more slowly.

A. Microtubules

Microtubules, cylindrical tubes composed of tubulin subunits, are present in all nucleated cells and the platelets in blood (Fig. 10.26). They are responsible for the positioning of organelles in the cell cytoplasm and the movement of vesicles, including phagocytic vesicles, exocytotic vesicles, and the transport vesicles between the ER, Golgi, and endosomes (see Fig. 10.24). They also form the spindle apparatus for cell division. The microtubule network (the minus end) begins in the nucleus at the centriole and extends outward to the plasma membrane (usually the plus end). Microtubule-associated proteins (MAPs) attach microtubules to other cellular components, and can determine cell shape and polarity.

Lotta Topaigne was given colchicine, a drug that is frequently used to treat gout. One of its actions is to prevent phagocytic activity by binding to dimers of the α and β subunits of tubulin. When the tubulin dimer–colchicine complexes bind to microtubules, further polymerization of the microtubules is inhibited, depolymerization predominates, and the microtubules disassemble. Microtubules are necessary for vesicular movement of urate crystals during phagocytosis and release of mediators that activate the inflammatory response. Thus, colchicine diminishes the inflammatory response, swelling and pain caused by formation of urate crystals.

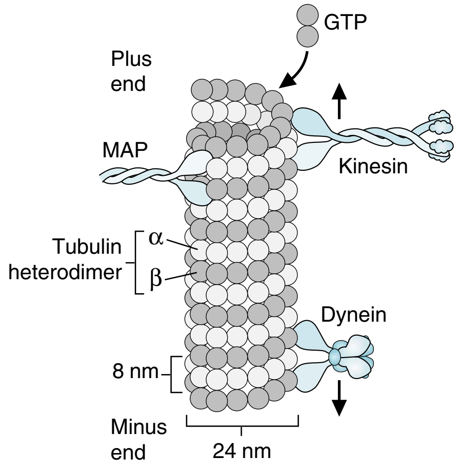


Fig. 10.26. Microtubules composed of αβ tubulin heterodimers. MAP, microtubule-associated protein. These proteins project outward to attach the microtubules to other cellular components. The microtubule grows by the addition of αβ dimers containing bound GTP to the plus end of the polymer. Kinesin and dyneins are motor proteins that transport cargo (e.g., vesicles) along the microtubule.

Motor proteins called kinesins and cytoplasmic dyneins use ATP energy to move cargo along the microtubules. Kinesins moves molecules, vesicles, and organelles toward the plus end of microtubules, usually toward the plasma membrane. Cytoplasmic dyneins are huge proteins that move vesicles and organelles to the minus end, generally toward the nucleus. They are also involved in the positioning of the Golgi complex and the movement of chromosomes during mitosis.

Microtubules consist of polymerized arrays of α and β tubulin dimers that form 13 protofilaments organized around a hollow core (see Fig. 10.26). Three different tubulin polypeptides (α, β, and γ) of similar amino acid composition are encoded by related genes; α and β dimers polymerize to form most microtubules, and γ-tubuluin is found only in the centrosome. Tubulin dimers composed of one α and one β subunit bind GTP, which creates a conformational change in the dimer that favors addition of dimers to the tubulin polymer. The dimers can add to and dissociate from both ends of the tubulin, but the end to which they add more rapidly (the plus end) has a net rate of growth, and the end to which they add more slowly (the minus end) has a net rate of loss. As GTP is hydrolyzed to GDP, the binding of tubulin subunits is weakened, resulting in their dissociation (dynamic instability). Thus, the net rate and direction of growth is dictated by the fastest growing end of the microtubule.

A variety of human cells have cilia and flagella, hairlike projections from the surface that have a stroke-like motion. These projections contain a flexible organized array of microtubules. Fluid or mucus is propelled over the surface of ciliated epithelial cells by the coordinated beating of cilia. A sperm cell swims by means of a flagellum.

B. Actin Filaments

Actin filaments form a network controlling the shape of the cell and movement of the cell surface, thereby allowing cells to move, divide, engulf particles, and contract. Actin is present in all living cells. The actin polymer, called F-actin, is composed of a helical arrangement of globular G-actin subunits (Fig. 10.27). Within the polymer, each G-actin subunit contains a bound ATP or ADP that

Colchicine has a narrow therapeutic index (i.e., the amount of drug that produces the desirable therapeutic effect is not much lower than the amount that produces an adverse effect). Its therapeutic effect depends on inhibiting tubulin synthesis in neutrophils, but it can also prevent tubulin synthesis (and, thus, cell division and other cellular processes) in other cells. Fortunately, neutrophils concentrate colchicine, so they are affected at lower intakes than other cell types. Neutrophils lack the transport protein P-glycoprotein, a member of the ABC cassette family (which includes the CFTR channel). In most other cell types, P-glycoprotein exports chemicals such as colchicine, thus preventing their accumulation.

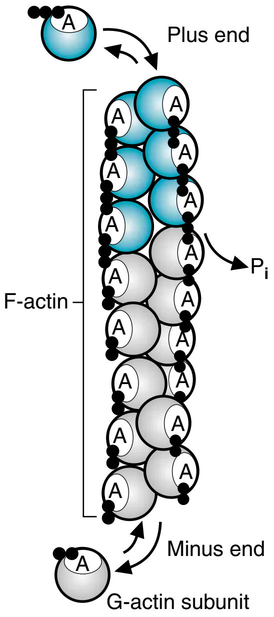


Fig. 10.27. Actin filaments. The polymer F-actin is assembled from G-actin subunits containing bound ATP. While bound, the ATP is slowly hydrolyzed to ADP. The conformational change shifts the equilibrium so that dissociation of the G-actin subunits is favorable at the minus end of the polymer. Once dissociated, the actin subunits exchange ADP for ATP, which may again associate with the actin polymer. At the plus end of the molecule, association is favored over dissociation.

holds the actin fold into a closed conformation (see Chapter 7). The actin polymer is dynamic. New subunits of G-actin containing ATP continuously combine with the assembled F-actin polymer at the plus end. As F-actin elongates, bound ATP is hydrolyzed to ADP, so that most of the polymer contains G-actin-ADP subunits. The conformation of ADP-actin favors dissociation from the minus end of the polymer; thus, the polymer is capable of lengthening from the plus end. This directional growth can account for certain types of cell movement and shape changes: the formation of pseudopodia that surround other cells during phagocytosis, the migration of cells in the developing embryo, or the movement of white blood cells through tissues.

Actin polymers form the thin filaments (also called microfilaments) in the cell that are organized into compact ordered bundles or loose network arrays by cross-linking proteins. Short actin filaments bind to the cross-linking protein spectrin to form the cortical actin skeleton network (see Fig. 10.6). In muscle cells, long actin filaments combine with thick filaments, composed of the protein myosin, to produce muscle contraction. The assembly of G-actin subunits into polymers, bundling of fibers, and attachments of actin to spectrin and to the plasma membrane proteins and organelles, are mediated by a number of actin-binding proteins and G-proteins from the Rho family.

C. Intermediate Filaments

Intermediate filaments (IF) are composed of fibrous protein polymers that provide structural support to membranes of the cells and scaffolding for attachment of other cellular components. Each IF subunit is composed of a long rod-like α-helical core containing globular spacing domains, and globular N- and C-terminal domains. The α-helical segments of two subunits coil around each other to form a coiled coil, and then combine with another dimer coil to form a tetramer. Depending on the type of filament, the dimers may be either hetero- or homo-dimers. The tetramers join end-to end to form protofilaments and approximately eight proto filaments combine to form filaments (Fig. 10.28). Filament assembly is partially controlled through phosphorylation.

In contrast to actin thin filaments, the 50 or so different types of intermediate filaments are each composed of a different protein having the same general structure described above (Table 10.2). Some of the intermediate filaments, such as the nuclear lamins, are common to all cell types. These filaments provide a lattice-like support network attached to the inner nuclear membrane. Other intermediate filaments are specific for types of cells (e.g., epithelial cells have cytokeratins, and neurons have neurofilaments). These provide an internal network that helps to support the shape and resilience of the cell.

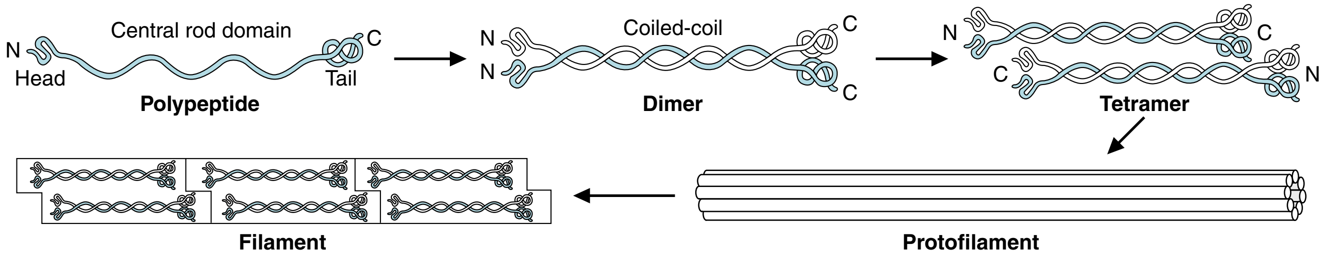
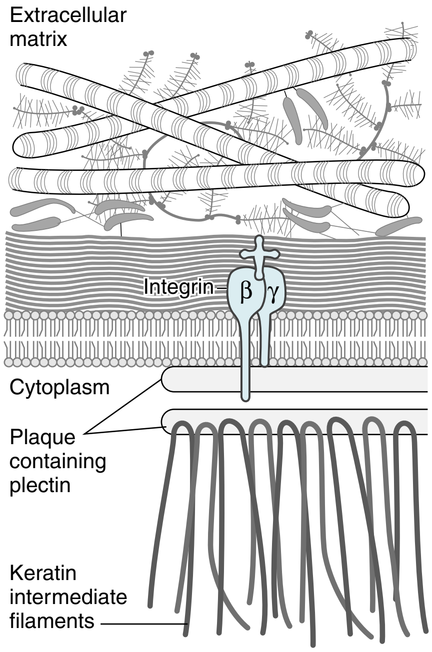


Fig. 10.28. Formation of a cytokeratin filament. The central rod of the keratin monomer is principally α-helical structure. A specific acidic keratin monomer combines with a specific basic keratin monomer to form a heterodimer coil (a coiled coil structure). Two dimers combine in antiparallel fashion to form a tetramer, and the tetramers combine head-to-tail to form protofilaments. Approximately eight protofilaments combine to form a filament. The filament is thicker than actin filaments (called thin filaments or microfilaments) and thinner than microtubules (thick tubes) and is therefore called an intermediate filament.

Table 10.2 Intermediate Filaments

Type	Protein	Location
Type I	Acidic keratins	Different epithelial cells express different keratins.
Type II	Basic keratins	Epithelial cells. Each cell type has a specific acidic and a specific basic keratin.
Type III	Vimentin-like	Cells of mesenchymal origin
	Vimentin Desmin	Muscle
	Glial fibrillary acid protein	Glial cells in the central nervous system
Type IV	Neurofilaments (NF-L, NF-M, NF-H)	Neurons, particularly long thin motor axons
Type V	Nuclear lamins A, B, and C	Forms a mesh-like network in the inner lining of cellular nuclear envelopes.
Type VI	Nestin	Stem cells of central nervous system

In epidermolysis bullosa simplex, the skin blisters in response to a very slight mechanical stress. The familial form of the disease is generally caused by mutations in either of the two forms of keratin that constitute the keratin heterodimers of the basal layer of the epidermis. The weakened keratin cytoskeleton results in cytolysis when stress is applied. The disease also can be caused by mutations in plektin, the protein that attaches keratin to the membrane protein integrin in hemidesmosomes (a structure involved in attaching cells to the extracellular matrix).



CLINICAL COMMENTS

Al Martini. Al Martini has been drinking for 5 years and has begun to exhibit mental and systemic effects of chronic alcohol consumption. In his brain, ethanol has altered the fluidity of neuronal lipids, causing changes in their response to neurotransmitters released from exocytotic vesicles. In his liver, increased levels of MEOS (CYP2E1) located in the smooth ER increased his rate of ethanol oxidation to acetaldehyde, a compound that is toxic to the cell. His liver also continues to oxidize ethanol to acetaldehyde through a cytosolic enzyme, liver alcohol dehydrogenase.

One of the toxic effects of acetaldehyde is inhibition of tubulin polymerization. Tubulin is used in the liver for secretion of very low-density lipoprotein (VLDL) particles containing newly synthesized triacylglycerols. As a result, these triacylglycerols accumulate in the liver, and he has begun to develop a fatty liver. Acetaldehyde may also damage protein components of the inner mitochondrial membrane and affect its ability to pump protons to the cytosol.

Lotta Topaigne had a rapid and gratifying clinical response to the hourly administration of colchicine. This drug diminishes phagocytosis and the subsequent release of the lysosomal enzymes that initiate the inflammatory response in synovial tissue.

The inflammatory response that causes the symptoms of an acute gout attack begins when neutrophils and macrophages ingest urate crystals. In neutrophils, urate activates the conversion of the polyunsaturated fatty acid arachidonic acid (present in membrane phospholipids) to leukotriene B_4. The release of this prostaglandin contributes to the pain. Colchicine, through its effect on tubulin, inhibits phagocytosis, leukotriene B_4 release, and recruitment and cell division of additional cells involved in inflammation. Colchicine also inhibits the tubulin-dependent release of histamine from mast cells. As a result, there was a rapid improvement in the pain and swelling in Lotta's great toe.

After the gout attack subsided, Ms. Topaigne was placed on allopurinol, a drug that inhibits urate production (see Chapter 8). During the next 6 months of allopurinol therapy, Ms. Topaigne's blood urate levels decreased. She did not have another gout attack during this time.

Dennis Veere. Dennis Veere was diagnosed with cholera. He was placed on intravenous rehydration therapy, followed by oral rehydration therapy with high glucose and Na^+-containing fluids (to be continued in Chapter 11).

Vibrio cholerae secrete an A toxin that is processed and transported in the cell in conjunction with the monomeric G protein Arf (ADP-ribosylation factor). The A toxin ADP-ribosylates the G_α subunit of a heterotrimeric G protein (a process discussed in Chapter 11.) The net result is activation of protein kinase A, which then phosphorylates the CFTR (cystic fibrosis transmembrane conductance regulator) chloride channel so that it remains permanently open. The subsequent efflux of chloride, sodium, and water into the bowel lumen is responsible for Dennis Veere's diarrhea and subsequent dehydration.

BIOCHEMICAL COMMENTS

Differences between bacteria and human cells. Different species of bacteria have some common structural features that distinguish them from animal cells. They are single-cell organisms that are prokaryotes ("before nucleus"). Their genetic material (DNA) is concentrated in the central region of the cell called a nucleoid, rather than a nucleus, because it is not separated from the rest of the cellular contents by a membrane. Likewise, bacteria contain no cytoplasmic organelles defined by membranes. They do have a plasma membrane that encloses the cytoplasm. External to this membrane is a peptidoglycan cell wall composed of extensively cross-linked polysaccharides that form a protective shield on the surface of the cell.

The mitochondria of eukaryotic cells have many features in common with prokaryotic cells and, in fact, may have originated when primordial anaerobic eukaryotes engulfed ancient aerobic prokaryotes, establishing a symbiotic relationship. These prokaryotes provided eukaryotes with a more efficient mechanism for oxidizing fuels to obtain energy.

Bacterial cells obtain nutrients from the medium on which they grow. Many of their metabolic pathways for fuel oxidation are similar to those in eukaryotes and generate NADH and ATP; however, individual steps in these pathways may use different coenzymes or very different enzymes for catalysis than do human cells. Like human cells, bacteria use intermediates of glycolysis and other basic degradative pathways to serve as precurors for biosynthetic pathways, and energy acquired from catabolic pathways is used in anabolic pathways. Aerobic bacteria, such as *Escherichia coli*, contain enzymes of the tricarboxylic acid (TCA) cycle and the components of the electron transport chain, which are located in the cell membrane. However, many bacteria are anaerobes and can function in the absence of oxygen.

Many of the metabolic differences between human cells and bacteria are related to their interactions with their environment. Some bacteria, such as *E. coli*, can adapt to adverse or changing conditions (high versus low O_2 tension or a single supply of nutrients from which to synthesize everything) by dramatic shifts in the genes that are transcribed. Other bacteria find a unique environmental niche where they do not have to compete with other bacteria for nutrients (e.g., *Lactobaccili* in yogurt are adapted to an acidic pH). In contrast, the human cells are adapted to interacting with blood and interstitial fluid, which provides a well-controlled pH, a constant nutrient supply, and a medium for communication between very distant cells. As a consequence of their constant environment, adult human cells seldom need to adapt (or can adapt) to widely fluctuating conditions through large variations in the genes transcribed. As a consequence of being organized into a multicellular organism, human cell types have been able to specialize in function, structure, and enzyme content.

Suggested References

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Takai Y, Sasaki T, Matozaki T. Small GTP-binding proteins. Physiol Rev 2001;81:153–208. Although this is a lengthy review for medical students, it is very useful in identifying the roles of different G proteins.

A number of extremely good cell and molecular biology textbooks have recently been published. Some of the most recent textbooks that we found helpful include:

Karp G. Cell and Molecular Biology: Concepts and Experiments. 3rd Ed. New York: John Wiley and Sons, 2002.

Cooper GM. The Cell: A Molecular Approach. 2nd Ed. Washington, DC: ASM Press, Sinauer Assoc., 2000.
Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. Molecular Cell Biology. 4th Ed. New York: W.H. Freeman, 2000. The section on cell structure is particularly good.
Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell. 4th Ed. New York: Garland Science, 2002.

REVIEW QUESTIONS—CHAPTER 10

1. Which of the following is a characteristic of the plasma membrane?

   (A) It is composed principally of triacylglycerols and cholesterol.
   (B) It contains principally nonpolar lipids.
   (C) It contains phospholipids with their acyl groups extending into the cytosol.
   (D) It contains more phosphatidylserine in the inner than the outer leaflet.
   (E) It contains oligosaccharides sandwiched between the inner and outer leaflets.

2. Transmembrane proteins

   (A) can usually be dissociated from membranes without disrupting the lipid bilayer.
   (B) are classified as peripheral membrane proteins.
   (C) contain hydrophobic amino acid residues at their carboxy terminus.
   (D) contain hydrophilic amino acid residues extending into the lipid bilayer.
   (E) contain membrane-spanning regions that are α-helices.

Use the following case history for questions 3 and 4.

A patient had a sudden heart attack caused by inadequate blood flow through the vessels of the heart. As a consequence, there was an inadequate supply of oxygen to generate ATP in his cardiomyocytes.

3. The compartment of the cardiomyocyte most directly involved in ATP generation is the

   (A) mitochondrion.
   (B) peroxisome.
   (C) lyosome.
   (D) nucleus.
   (E) Golgi.

4. The transport process most directly affected by his decreased rate of ATP generation is

   (A) active transport of Na^+ out of the cell.
   (B) facilitative transport of glucose into the cell.
   (C) passive diffusion of H_2O into the cell.
   (D) ion flux through ligand-gated channels.
   (E) receptor-mediated endocytosis.

Use the following case history for questions 5 and 6.

A patient in a nursing home who developed severe diarrhea was diagnosed with a *Clostridium difficile* infection. The severe diarrhea associated with *C. difficile* is caused principally by two toxins that are UDP-glucosyltransferases. These toxins modify a monomeric G protein, thereby disrupting cellular attachments associated with the actin skeleton.

5. On the basis of its effect, which of the following G protein families is the most likely target of this modification?

   (A) Ras
   (B) Ran
   (C) Rho
   (D) Rab
   (E) Arf