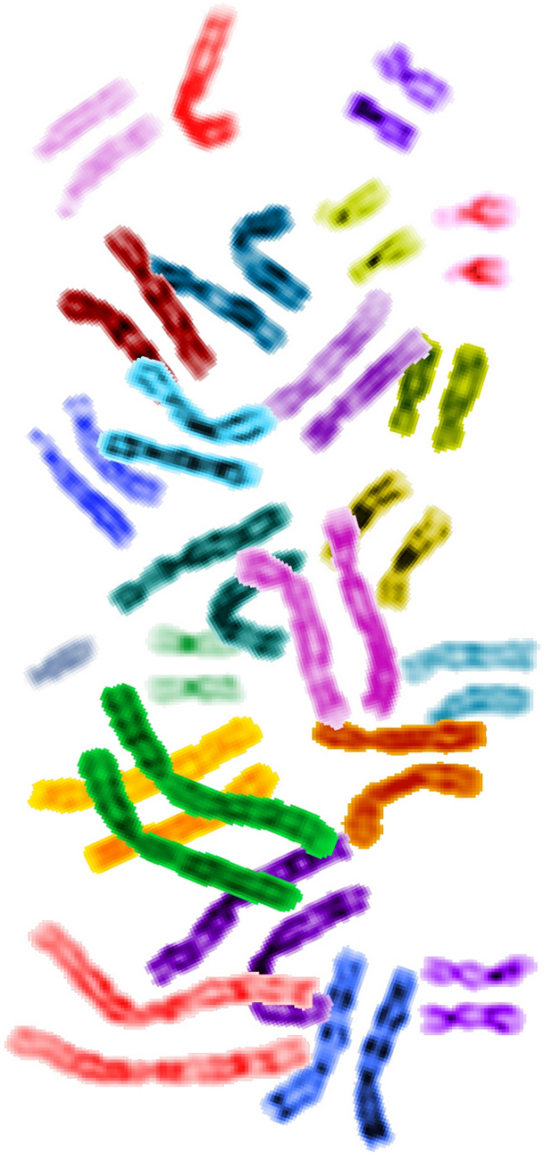

FMS 502 Genes and Proteins

volume 1

THE CELL, DNA, AND NUCLEOTIDE METABOLISM

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Washington State University

Contents

1

Diagram and describe the cell, including organelles, cellular membranes, and the cytoskeleton

THE FOLLOWING is taken from:

Cell Structure. In: Carroll KC, Hobden JA, Miller S, Morse SA, Mietzner TA, Detrick B, Mitchell TG, McKerrow JH, Sakanari JA. eds. Jawetz, Melnick, & Adelberg's Medical Microbiology, 27e New York, NY: McGraw-Hill; . http://accessmedicine.mhmedical.com/content.aspx?bookid=1551&sectionid=94105126.

Accessed November 21, 2017.

THE NUCLEUS

The **nucleus** contains the cell's genome. It is bounded by a membrane that consists of a pair of unit membranes separated by a space of variable thickness. The inner membrane is usually a simple sac, but the outermost membrane is, in many places, continuous with the endoplasmic reticulum (ER). The **nuclear membrane** exhibits selective permeability because of pores, which consist of a complex of several proteins whose function is to import substances into and export substances out of the nucleus. The chromosomes of eukaryotic cells contain linear DNA macromolecules arranged as a double helix. They are only visible with a light microscope when the cell is undergoing division and the DNA is in a highly condensed form; at other times, the chromosomes are not condensed and appear as in Figure 1.1. Eukaryotic DNA

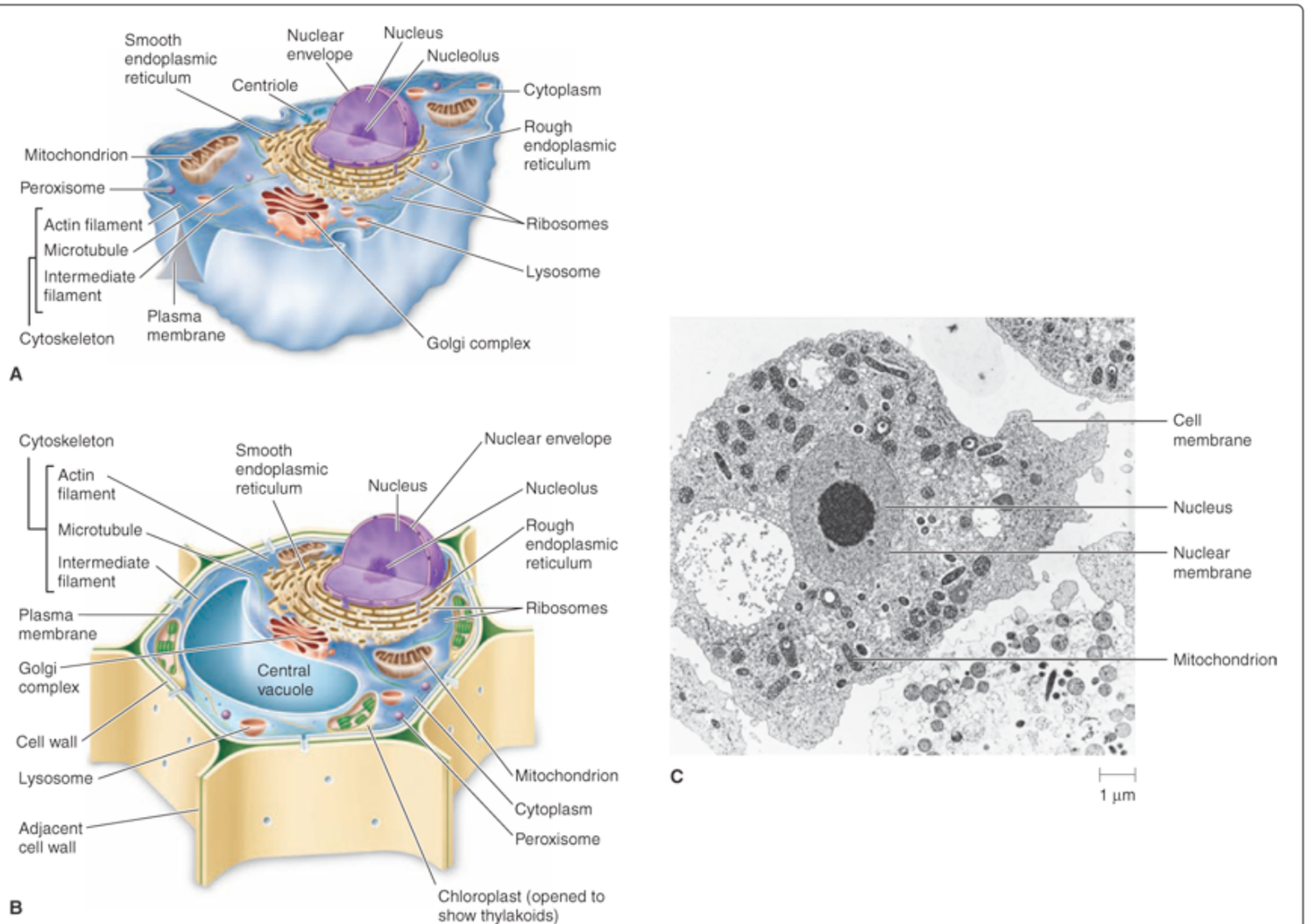


Figure 1.1 Eukaryotic cells. A: Diagrammatic representation of an animal cell. B: Diagrammatic representation of a plant cell. C: Micrograph of an animal cell shows several membrane-bound structures, including mitochondria and a nucleus. (Fig. 2-3(A) and (B) Reproduced with permission from Nester EW, Anderson DG, Roberts CE, Nester MT: Microbiology: A Human Perspective, 6th ed. McGraw-Hill, 2009. Fig. 2-3(C) Reproduced with permission from Thomas Fritsche, MD, PhD.) *(tap for full screen; pinch to zoom)*

macromolecules are associated with basic proteins called **histones** that bind to the DNA by ionic interactions.

A structure often visible within the nucleus is the **nucleolus**, an area rich in RNA that is the site of ribosomal RNA synthesis (see Figure 1.1). Ribosomal proteins synthesized in the cytoplasm are transported into the nucleolus and combine with ribosomal RNA to form the small and large subunits of the eukaryotic ribosome. These are then exported to the cytoplasm, where they associate to form an intact ribosome that can function in protein synthesis.

CYTOPLASMIC STRUCTURES

The cytoplasm of eukaryotic cells is characterized by the presence of an ER, vacuoles, self-reproducing plastids, and an elaborate cytoskeleton composed of microtubules, microfilaments, and intermediate filaments.

The **endoplasmic reticulum (ER)** is a network of membrane-bound channels continuous with the nuclear membrane. Two types of ER are recognized: **rough**, which contains attached 80S ribosomes, and **smooth**, which does not (see Figure 1.1).

Rough ER is a major producer of glycoproteins and produces new membrane material that is transported throughout the cell; smooth ER participates in the synthesis of lipids and in some aspects of carbohydrate metabolism. The **Golgi complex** consists of a stack of membranes that function in concert with the ER to chemically modify and sort products of the ER into those destined to be secreted and those that function in other membranous structures of the cell.

Several lines of evidence suggest that mitochondria were descendants of ancient prokaryotic organisms and arose from the engulfment of a prokaryotic cell by a larger cell **(endosymbiosis)**. Mitochondria are of prokaryotic size, and its membrane, which lacks sterols, is much less rigid than the eukaryotic cell's cytoplasmic membrane, which does contain sterols. Mitochondria contain two sets of membranes. The outermost membrane is rather permeable, having numerous minute channels that allow passage of ions and small molecules (eg, adenosine triphosphate [ATP]). Invagination of the outer membrane forms a system of inner folded membranes called **cristae**. The cristae are the sites of enzymes involved in respiration and ATP production. Cristae also

contain specific transport proteins that regulate passage of metabolites into and out of the mitochondrial **matrix**. The matrix contains a number of enzymes, particularly those of the citric acid cycle. Mitochondria contain their own DNA, which exists in a covalently closed circular form and codes for some (not all) of their constituent proteins and transfer RNAs. Mitochondria and chloroplasts also contain 70S ribosomes, the same as those of prokaryotes.

Lysosomes are membrane-enclosed sacs that contain various digestive enzymes that the cell uses to digest macromolecules such as proteins, fats, and polysaccharides. The lysosome allows these enzymes to be partitioned away from the cytoplasm proper, where they could destroy key cellular macromolecules if not contained. After the hydrolysis of macromolecules in the lysosome, the resulting monomers pass from the lysosome into the cytoplasm, where they serve as nutrients.

The **peroxisome** is a membrane-enclosed structure whose function is to produce H_2O_2 from the reduction of O_2 by various hydrogen donors. The H_2O_2 produced in the peroxisome is subsequently degraded to H_2O and O_2 by the enzyme **catalase**.

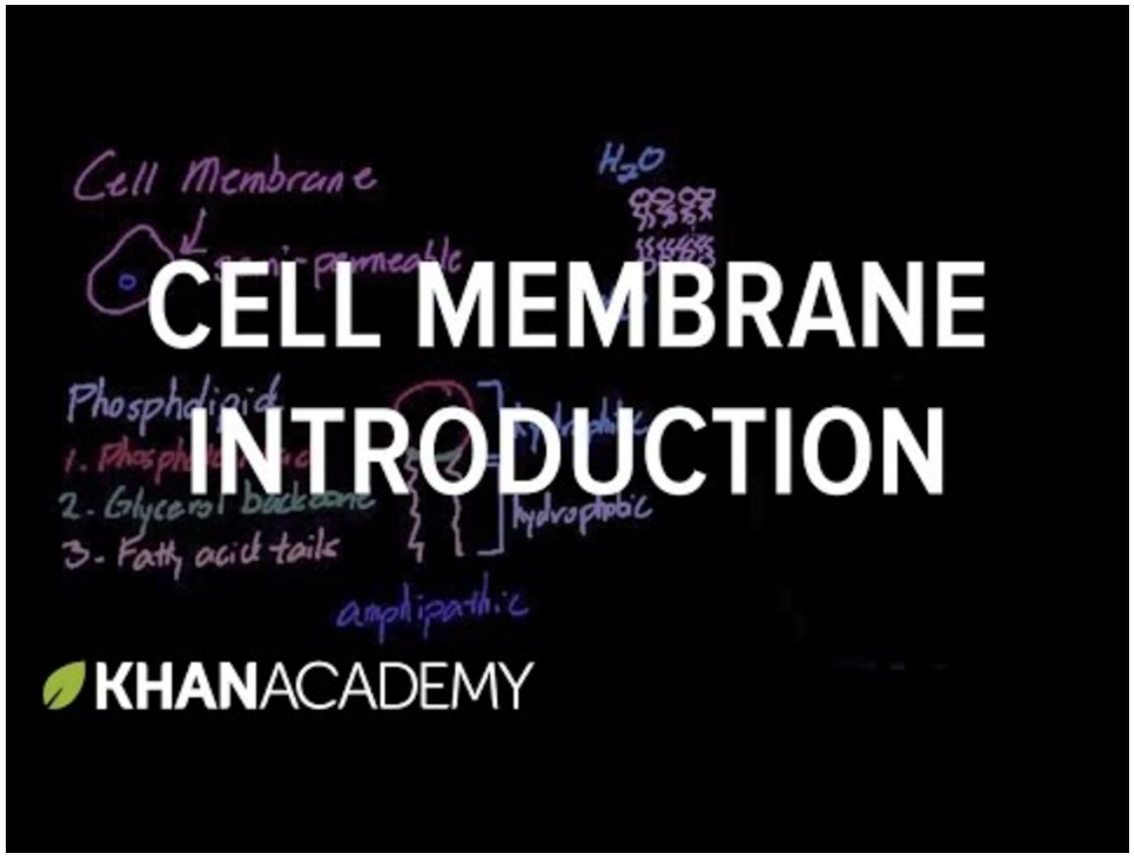
The **cytoskeleton** is a three-dimensional structure that fills the cytoplasm. The primary types of fibers comprising the cytoskeleton are **microfilaments**, **intermediate filaments**, and **microtubules**.

2

Diagram and describe the cell, including organelles, cellular membranes, and the cytoskeleton

THE FOLLOWING is taken from:

Murray RK, Weil P. Membranes: Structure & Function. In: Rodwell VW, Bender DA, Botham KM, Kennelly PJ, Weil P. eds. Harper's Illustrated Biochemistry, 30e New York, NY: McGraw-Hill; . https://accessmedicine.mhmedical.com/content.aspx?bookid=2386&sectionid=187835479. Accessed November 21, 2017.

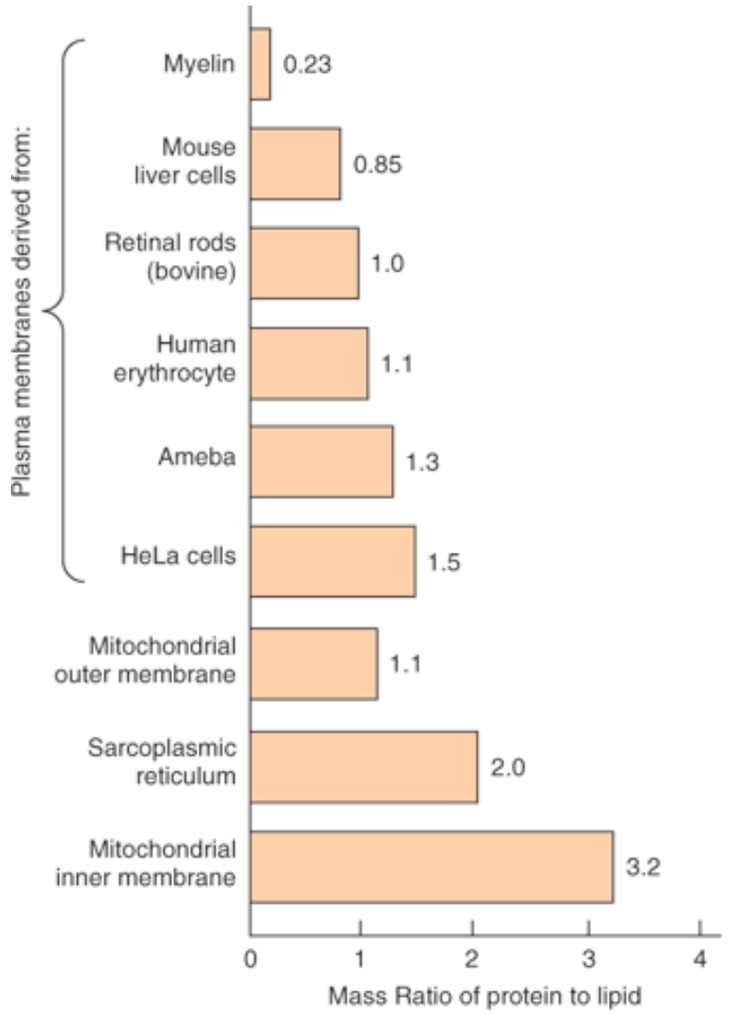


Interactive 2.1

Osmosis video: https://www.osmosis.org/home/learn?id=cell_membrane

MEMBRANES ARE COMPLEX STRUCTURES COMPOSED OF LIPIDS, PROTEINS, & CARBOHYDRATE-CONTAINING MOLECULES

We shall mainly discuss the membranes present in eukaryotic cells, although many of the principles described also apply to the membranes of prokaryotes. The various cellular membranes have different lipid (see below) and protein compositions. The ratio of protein to lipid in different membranes is presented in Figure 2.1, and is responsible for the many divergent functions of cellular organelles. Membranes are sheet-like enclosed structures consisting of an asymmetric lipid bilayer with distinct inner and outer surfaces or leaflets. These structures and surfaces are protein-studded, sheet-like, noncovalent assemblies that form spontaneously in



Source: V. W. Rodwell, D. A. Bender, K. M. Botham, P. J. Kennelly, P. A. Weil: Harper's Illustrated Biochemistry, 13th Edition
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Figure 2.1 Membrane protein content is highly variable. The amount of proteins equals or exceeds the quantity of lipid in nearly all membranes. The outstanding exception is myelin, an electrical insulator found on many nerve fibers.

water due to the amphipathic nature of lipids and the proteins contained within the membrane.

The Major Lipids in Mammalian Membranes Are Phospholipids, Glycosphingolipids & Cholesterol

Phospholipids

Of the two major phospholipid classes present in membranes, **phosphoglycerides** are the more common and consist of a glycerol-phosphate backbone to which are attached two fatty acids in ester linkages and an alcohol (Figure 2.2). The **fatty acid** constituents are usually even-numbered carbon molecules, most commonly containing 16 or 18 carbons. They are unbranched and can be saturated or unsaturated with one or more double bonds. The simplest phosphoglyceride is **phosphatidic acid**, a

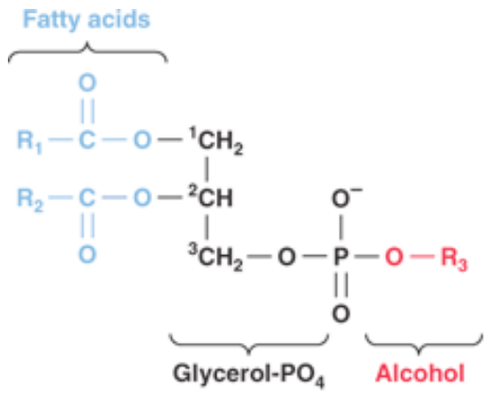


Figure 2.2 A phosphoglyceride showing the fatty acids (R_1 and R_2), glycerol, and a phosphorylated alcohol component. Saturated fatty acids are usually attached to carbon 1 of glycerol, and unsaturated fatty acids to carbon 2. In phosphatidic acid, R_3 is hydrogen.

1,2-diacylglycerol 3-phosphate, a key intermediate in the formation of other phosphoglycerides (see Chapter 24 ↻). In most phosphoglycerides present in membranes, the 3-phosphate is esterified to an **alcohol** such as choline, ethanolamine, glycerol, inositol or serine (see Chapter 21 ↻). Phosphatidylcholine is generally the major phosphoglyceride by mass in the membranes of human cells.

The second major class of phospholipids comprises **sphingomyelin** (see Figure 21–13 ↻), a phospholipid that contains a sphingosine rather than a glycerol backbone. A fatty acid is attached by an amide linkage to the amino group of sphingosine, forming **ceramide**. When the primary hydroxyl group of sphingosine is esterified to phosphorylcholine, sphingomyelin is formed. As the name suggests, sphingomyelin is prominent in myelin sheaths.

Glycosphingolipids

The glycosphingolipids (GSLs) are sugar-containing lipids built on a backbone of **ceramide**. GSLs include **galactosyl-** and **glucosyl-ceramides** (cerebrosides) and the **gangliosides** (see structures in Chapter 21 ↻), and are mainly located in the plasma membranes of cells, displaying their sugar components to the exterior of the cell.

Sterols

The most common sterol in the membranes of animal cells is **cholesterol** (see Chapter 21 ↻). The majority of cholesterol resides within **plasma membranes**, but smaller amounts are found within mitochondrial, Golgi complex, and nuclear membranes. Cholesterol intercalates among the phospholipids of the membrane, with its hydrophilic hydroxyl group at the aqueous interface and the remainder of the molecule buried within the lipid bilayer leaflet. From a nutritional viewpoint, it is important to know that cholesterol is not present in plants.

Lipids can be separated from one another and quantified by techniques such as column, thin-layer, and gas-liquid chromatography and their structures established by mass spectrometry and other techniques (see Chapter 4 ↻).

Membrane Lipids Are Amphipathic

All major lipids in membranes contain both hydrophobic and hydrophilic regions and are therefore termed **amphipathic**. If

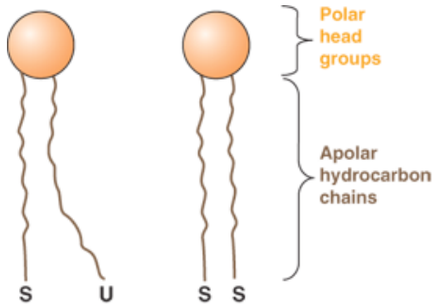


Figure 2.3 Diagrammatic representation of a phospholipid or other membrane lipid. The polar head group is hydrophilic, and the hydrocarbon tails are hydrophobic or lipophilic. The fatty acids in the tails are saturated (S) or unsaturated (U); the former are usually attached to carbon 1 of glycerol and the latter to carbon 2 (see Figure 40–2 ↻). Note the kink in the tail of the unsaturated fatty acid (U), which is important in conferring increased membrane fluidity.

the hydrophobic region were separated from the rest of the molecule, it would be insoluble in water but soluble in organic solvents. Conversely, if the hydrophilic region were separated from the rest of the molecule, it would be insoluble in organic solvents but soluble in water. The amphipathic nature of a phospholipid is represented in Figure 2.3 and also Figure 21–24 ↻. Thus, the **polar head groups** of the phospholipids and the hydroxyl group of cholesterol interface with the aqueous environment; a similar situation applies to the **sugar moieties** of the GSLs (see below).

Saturated fatty acids form relatively straight tails, whereas unsaturated fatty acids, which generally exist in the cis form in membranes, form "kinked" tails (Figure 2.3; see also Figures 21–1 ↻, 21–6 ↻). As the number of double bonds within the lipid side chains increase, the number of kinks in the tails increases. As a consequence, the membrane lipids become less tightly packed and the membrane more fluid. The problem caused by the presence of **trans fatty acids** in membrane lipids is described in Chapter 21 ↻.

Detergents are amphipathic molecules that are important in biochemistry as well as in the household. The molecular structure of a detergent is not unlike that of a phospholipid. Certain detergents are widely used to **solubilize** and purify membrane proteins. The hydrophobic end of the detergent binds to hydrophobic regions of the proteins, displacing most of their bound lipids. The polar end of the detergent is free,

bringing the proteins into solution as detergent-protein complexes, usually also containing some residual lipids.

Membrane Lipids Form Bilayers

The amphipathic character of phospholipids suggests that the two regions of the molecule have incompatible solubilities. However, in a solvent such as water, phospholipids spontaneously organize themselves into **micelles** (Figure 2.4 and Figure 21–24 ↻), an assembly that thermodynamically satisfies the solubility requirements of the two chemically distinct regions of these molecules. Within the micelle the hydrophobic regions of the amphipathic phospholipids are shielded from water, while the hydrophilic polar groups are immersed in the aqueous environment. Micelles are usually relatively small in size (eg, ~200 nm) and consequently are limited in their potential to form membranes. Detergents commonly form micelles.

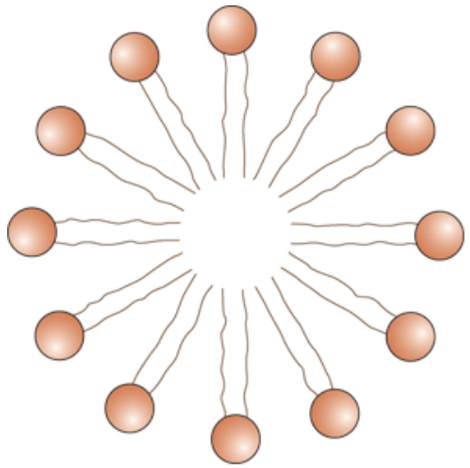


Figure 2.4 Diagrammatic cross-section of a micelle.
The polar head groups are bathed in water, whereas the hydrophobic hydrocarbon tails are surrounded by other hydrocarbons and thereby protected from water. Micelles are relatively small (compared with lipid bilayers) spherical structures.

Phospholipids and similar amphipathic molecules can form another structure, the **bimolecular lipid bilayer**, which also satisfies the thermodynamic requirements of amphipathic molecules in an aqueous environment. Bilayers are the key structures in biological membranes. Bilayers exist as sheets wherein the hydrophobic regions of the phospholipids are sequestered from the aqueous environment, while the hydrophilic, charged portions are exposed to water (Figure 2.5 and Figure 21–24 ↻). The ends or edges of the bilayer sheet can be eliminated by folding the sheet back upon itself to form an enclosed vesicle with no edges. The closed bilayer provides one of the most essential properties of membranes. The lipid

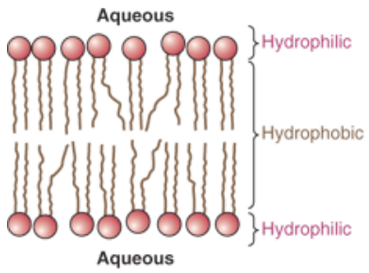


Figure 2.5 Diagram of a section of a bilayer membrane formed from phospholipid molecules. The unsaturated fatty acid tails are kinked and lead to more spacing between the polar head groups, hence to more room for movement. This in turn results in increased membrane fluidity. (Slightly modified and reproduced, with permission, from Stryer L: *Biochemistry*, 2nd ed. Freeman, 1981. Copyright ©1981 by W. H. Freeman and Company.)

bilayer is **impermeable to most water-soluble molecules** since such charged molecules would be insoluble in the hydrophobic core of the bilayer. The **self-assembly of lipid bilayers** is driven by the **hydrophobic effect** (see Chapter 2 ↻). When lipid molecules come together in a bilayer, the entropy of the surrounding solvent molecules increases due to the release of immobilized water.

Two questions arise from consideration of the information described above. First, how many biologically important molecules are **lipid-soluble** and can therefore readily enter the cell? Gases such as oxygen, CO_2, and nitrogen—small molecules with little interaction with solvents—readily diffuse through the hydrophobic regions of the membrane. The **permeability coefficients** of several ions and a number of other molecules in a lipid bilayer are shown in Figure 2.6. The electrolytes Na^+, K^+, and Cl^- cross the bilayer much more slowly than water. In general, the permeability coefficients of small molecules in a lipid bilayer **correlate with their solubilities in nonpolar solvents**. For instance, **steroids** more readily traverse the lipid bilayer compared with electrolytes. The high permeability coefficient of **water** itself is surprising, but is partly explained by its small size and relative lack of charge. Many **drugs** are hydrophobic and can readily cross membranes and enter cells.

The second question concerns **non-lipid-soluble molecules**. How are the transmembrane concentration gradients for these molecules maintained? The answer is that **membranes contain proteins**, many of which span the lipid bilayer. These

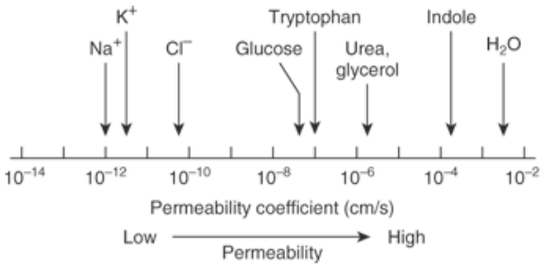


Figure 2.6 Permeability coefficients of water, some ions, and other small molecules in lipid bilayer membranes. The permeability coefficient is a measure of the ability of a molecule to diffuse across a permeability barrier. Molecules that move rapidly through a given membrane are said to have a high permeability coefficient. (Slightly modified and reproduced, with permission, from Stryer L: *Biochemistry,* 2nd ed. Freeman, 1981. Copyright © 1981.)

proteins either form **channels** for the movement of ions and small molecules or serve as **transporters** for molecules that otherwise could not readily traverse the lipid bilayer (membrane). The nature, properties, and structures of membrane channels and transporters are described below.

Membrane Proteins Are Associated with the Lipid Bilayer

Membrane **phospholipids** act as a solvent for membrane proteins, creating an environment in which the latter can function. As described in Chapter 5 ↻, the **α-helical structure of proteins** minimizes the hydrophilic character of the peptide bonds themselves. Thus, proteins can be amphipathic and form an integral part of the membrane by having hydrophilic regions protruding at the inside and outside faces of the membrane but connected by a hydrophobic region traversing the hydrophobic core of the bilayer. In fact, those portions of membrane proteins that traverse membranes do contain substantial numbers of hydrophobic amino acids and almost invariably have a high α-helical content. For most membranes, a stretch of ~20 amino acids in an α-helical configuration will span the lipid bilayer.

It is possible to calculate whether a particular sequence of amino acids present in a protein is consistent with a **transmembrane location**. This can be done by consulting a table that lists the hydrophobicities of each of the 20 common amino acids and the free energy values for their transfer from

the interior of a membrane to water. Hydrophobic amino acids have positive values; polar amino acids have negative values. The total free energy values for transferring successive sequences of 20 amino acids in the protein are plotted, yielding a so-called **hydropathy plot**. Values of over 20 kcal mol^{-1} are consistent with—but do not prove—the interpretation that the hydrophobic sequence is a transmembrane segment.

Another aspect of the interaction of lipids and proteins is that some proteins are anchored to one leaflet of the bilayer by covalent linkages to certain lipids; this process is termed **protein lipidation**. Lipidation can occur at protein termini (N- or C-) or internally. Common protein lipidation events are: C-terminal protein **isoprenylation**, **cholesterylation** and **glycophosphatidylinositol** (**GPI**; see Chapter 46 ↻); N-terminal protein **myristoylation** and internal cysteine ↻ **S-prenylation** and **S-acylation**. Such lipidation only occurs on a specific subset of proteins.

Different Membranes Have Different Protein Compositions

The **number of different proteins** in a membrane varies from less than a dozen very abundant proteins in the sarcoplasmic reticulum of muscle cells to hundreds in plasma membranes. Proteins are the **major functional molecules** of membranes and consist of **enzymes**, **pumps** and **transporters**, **channels**, **structural components**, **antigens** (eg, for histocompatibility), and **receptors** for various molecules. Because every type of membrane possesses a different complement of proteins, there is no such thing as a typical membrane structure.

Membranes Are Dynamic Structures

Membranes and their components are dynamic structures. Membrane lipids and proteins undergo turnover, just as they do in other compartments of the cell. Different lipids have different turnover rates, and the turnover rates of individual species of membrane proteins may vary widely. In some instances the membrane itself can turn over even more rapidly than any of its constituents. This is discussed in more detail in the section on endocytosis.

Another indicator of the dynamic nature of membranes is that a variety of studies have shown that lipids and certain proteins exhibit **lateral diffusion** in the plane of their membranes. Many nonmobile proteins do not exhibit lateral diffusion because they are anchored to the underlying actin cytoskeleton. By contrast, the **transverse** movement of lipids across the membrane (**flip-flop**) is extremely slow (see below) and does not appear to occur at an appreciable rate in the case of membrane proteins.

Membranes Are Asymmetric Structures

Proteins have unique orientations in membranes, making the **outside surfaces different from the inside surfaces**. An **inside-outside asymmetry** is also provided by the external location of the carbohydrates attached to membrane proteins. In addition, specific proteins are located exclusively on the outsides or insides of membranes.

There are also **regional heterogeneities** in membranes. Some, such as occur at the villous borders of mucosal cells, are almost macroscopically visible. Others, such as those at gap junctions, tight junctions, and synapses, occupy much smaller regions of the membrane and generate correspondingly smaller local asymmetries.

There is also inside-outside **asymmetry of the phospholipids**. The **choline-containing phospholipids** (phosphatidylcholine and sphingomyelin) are located mainly in the **outer leaflet**; the **aminophospholipids** (phosphatidylserine and phosphatidylethanolamine) are preferentially located in the **inner leaflet**. Obviously, if this asymmetry is to exist at all, there must be limited transverse mobility (flip-flop) of the membrane phospholipids. In fact, phospholipids in synthetic bilayers exhibit an **extraordinarily slow rate of flip-flop**; the half-life of the asymmetry in these synthetic bilayers is on the order of several weeks.

The mechanisms involved in the **establishment of lipid asymmetry** are not well understood. The enzymes involved in the synthesis of phospholipids are located on the cytoplasmic side of microsomal membrane vesicles. Translocases **(flippases)** exist that transfer certain phospholipids (eg, phosphatidylcholine) from the inner to the outer leaflet. Specific **proteins that preferentially bind** individual

phospholipids also appear to be present in the two leaflets; thus lipid binding also contributes to the asymmetric distribution of specific lipid molecules. In addition, **phospholipid exchange proteins** recognize certain phospholipids and transfer them from one membrane (eg, the endoplasmic reticulum [ER]) to others (eg, mitochondrial and peroxisomal). A related issue is **how lipids enter membranes**. This has not been studied as intensively as the topic of how proteins enter membranes (see Chapter 49 ↻) and knowledge is still relatively meager. Many membrane lipids are synthesized in the ER. At least three pathways have been recognized: (1) transport from the ER in vesicles, which then transfer the contained lipids to the recipient membrane; (2) entry via direct contact of one membrane (eg, the ER) with another, facilitated by specific proteins; and (3) transport via the phospholipid exchange proteins (also known as lipid transfer proteins) mentioned above, which only exchanges lipids, but does not cause net transfer.

There is **further asymmetry** with regard to glycosphingolipids and **glycoproteins**; the **sugar moieties** of these molecules all **protrude outward** from the plasma membrane and are absent from its inner face.

Membranes Contain Integral & Peripheral Proteins

It is useful to classify membrane proteins into two types: **integral** and **peripheral** (Figure 2.7). Most membrane proteins fall into the **integral class**, meaning that they interact extensively with the phospholipids and **require the use of detergents** for their solubilization. Also, they generally span the bilayer as a bundle of α-helical transmembrane segments. Integral proteins are usually globular and are themselves amphipathic. They consist of two hydrophilic ends separated by an intervening hydrophobic region that traverses the hydrophobic core of the bilayer. As the structures of integral membrane proteins were being elucidated, it became apparent that certain ones (eg, transporter molecules, ion channels, various receptors, and G proteins) span the bilayer many times, whereas other simple membrane proteins (eg, glycophorin A) span the membrane only once (see Figures 42–4 ↻ and 52–5 ↻). Integral proteins are asymmetrically distributed across the membrane bilayer. This asymmetric orientation is conferred at the time of their insertion in the lipid bilayer during biosynthesis in the ER. The molecular mechanisms involved in insertion of proteins into membranes and the topic of membrane assembly are discussed in Chapter 49 ↻.

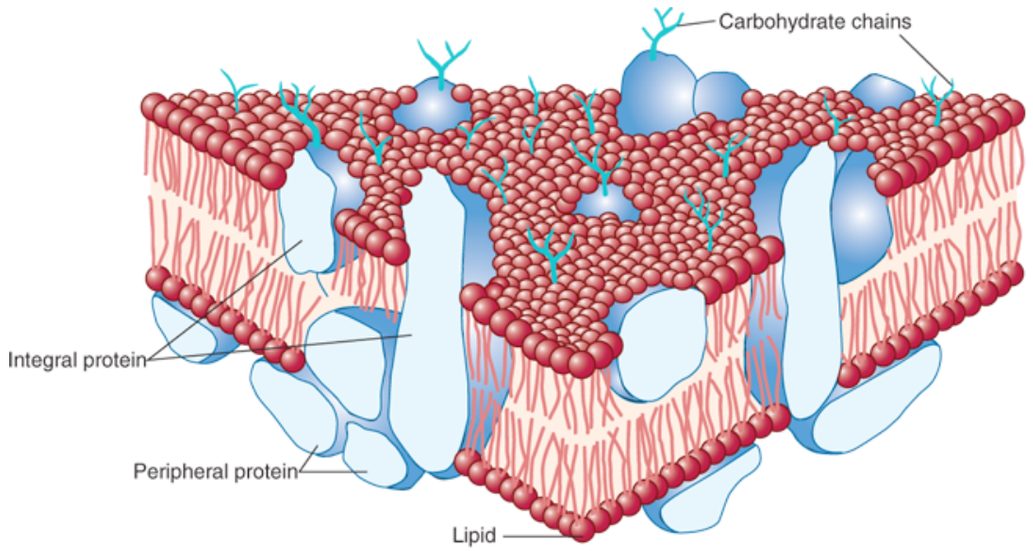


Figure 2.7 The fluid mosaic model of membrane structure. The membrane consists of a bimolecular lipid layer with proteins inserted in it or bound to either surface. Integral membrane proteins are firmly embedded in the lipid layers. Some of these proteins completely span the bilayer and are called transmembrane proteins, while others are embedded in either the outer or inner leaflet of the lipid bilayer. Loosely bound to the outer or inner surface of the membrane are the peripheral proteins. Many of the proteins and all the glycolipids have externally exposed oligosaccharide carbohydrate chains. (Reproduced, with permission, from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas,* 10th ed., McGraw-Hill, 2003.)

Peripheral proteins do not interact directly with the hydrophobic cores of the phospholipids in the bilayer and thus **do not require use of detergents** for their release. They are bound to the hydrophilic regions of specific integral proteins and head groups of phospholipids and can be released from them by treatment with salt solutions of high ionic strength.

For example, ankyrin, a peripheral protein, is bound to the inner aspect of the integral protein "band 3" of the erythrocyte membrane. Spectrin, a cytoskeletal structure within the erythrocyte, is in turn bound to ankyrin and thereby plays an important role in maintenance of the biconcave shape of the erythrocyte.

3

Diagram and describe the cell, including organelles, cellular membranes, and the cytoskeleton

THE FOLLOWING is taken from:

Kennelly PJ, Murray RK. Muscle & the Cytoskeleton. In: Rodwell VW, Bender DA, Botham KM, Kennelly PJ, Weil P. eds. Harper's Illustrated Biochemistry, 30e New York, NY: McGraw-Hill; . https://accessmedicine.mhmedical.com/content.aspx?bookid=2386&sectionid=187838753. Accessed November 27, 2017.

Interactive 3.1

Osmosis video: https://www.osmosis.org/home/learn?id=cytoskeleton

THE CYTOSKELETON PERFORMS MULTIPLE CELLULAR FUNCTIONS

Nonmuscle cells also perform mechanical work, including self-propulsion, morphogenesis, cleavage, endocytosis, exocytosis, intracellular transport, and changing cell shape. These cellular functions are carried out by an extensive intracellular network of filamentous structures constituting the **cytoskeleton**. The cell cytoplasm is not a sac of fluid, as once thought. Essentially all eukaryotic cells contain three types of filamentous structures: **actin filaments** (also known as microfilaments), **microtubules**, and **intermediate filaments**. Each type of filament can be distinguished biochemically and by the electron microscope.

Nonmuscle Cells Contain Actin That Forms Microfilaments

G-actin is present in most if not all cells of the body. With appropriate concentrations of magnesium and potassium chloride, it spontaneously polymerizes to form double helical **F-actin** filaments like those seen in muscle. There are at least two types of actin in nonmuscle cells: β-actin and γ-actin. Both types can coexist in the same cell and probably even copolymerize in the same filament. In the cytoplasm, **F-actin** forms **microfilaments** of 7 to 9.5 nm that frequently exist as bundles of a tangled-appearing meshwork. These prominent bundles, which just underly the plasma membranes of many cells, are referred to as **stress fibers**. Stress fibers disappear as cell motility increases or upon malignant transformation of cells by chemicals or oncogenic viruses.

Although not organized as in muscle, actin filaments in nonmuscle cells interact with **myosin** to cause cellular movements.

Microtubules Contain α- & β-Tubulins

Microtubules, an integral component of the cellular cytoskeleton, consist of cytoplasmic tubes 25 nm in diameter and often of extreme length (Figure 3.1). Microtubules are necessary for the formation and function of the **mitotic spindle** and thus are present in all eukaryotic cells. They are also involved in the intracellular movement of endocytic and exocytic **vesicles** and form the major structural components of **cilia** and **flagella**. Microtubules are a major component of **axons** and **dendrites**, in which they maintain structure and participate in the axoplasmic flow of material along these neuronal processes.

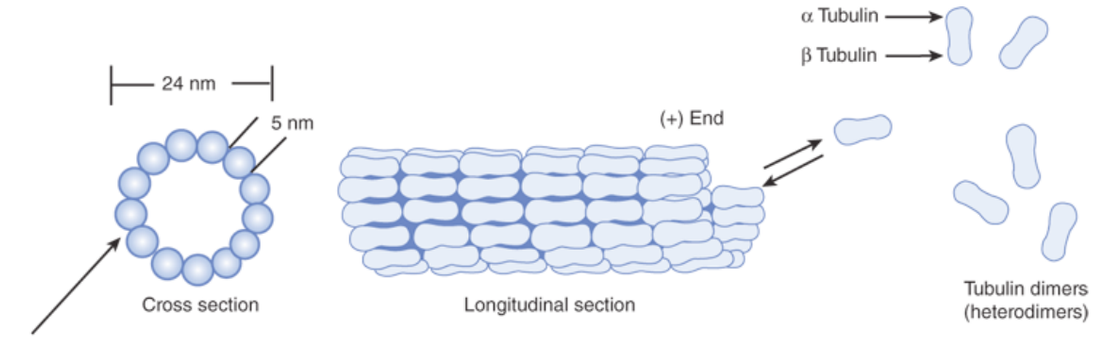


Figure 3.1 Schematic representation of microtubules. The upper left-hand corner shows a drawing of microtubules as seen in the electron microscope following fixation with tannic acid in glutaraldehyde. The unstained tubulin subunits are delineated by the dense tannic acid. Cross sections of tubules reveal a ring of 13 subunits of dimers arranged in a spiral. Changes in microtubule length are due to the addition or loss of individual tubulin subunits. Characteristic arrangements of microtubules (not shown here) are found in centrioles, basal bodies, cilia, and flagellae. (Reproduced, with permission, from Junqueirai LC, Carneiro J, Kelley RO: *Basic Histology,* 7th ed. Appleton & Lange, 1992.)

Microtubules are cylinders of 13 longitudinally arranged protofilaments, each consisting of dimers of **α-tubulin** and **β-tubulin**, closely related proteins of approximately 50 kDa molecular mass. The tubulin dimers assemble into protofilaments and subsequently into sheets and then cylinders. A microtubule-organizing center, located around a pair of centrioles, nucleates the growth of new microtubules. A third species of tubulin, **γ-tubulin**, appears to play an important role in this assembly. **GTP** is required for assembly. A variety of proteins are associated with microtubules (**microtubule-associated proteins** [**MAPs**], one of which is **tau**) and play important roles in microtubule assembly and stabilization. Microtubules are in a state of dynamic instability, constantly assembling and disassembling. They exhibit **polarity** (plus and minus ends); this is important in their growth from centrioles and in their ability to direct intracellular movement. For instance, in axonal transport, the protein **kinesin**, with a myosin-like ATPase activity, uses hydrolysis of ATP to move vesicles down the axon toward the positive end of the microtubular formation. Flow of materials in the opposite direction, toward the negative end, is powered by **cytosolic dynein**, another protein with ATPase activity.

Similarly, **axonemal dyneins** power ciliary and flagellar movement. Another protein, **dynamin**, uses GTP and is involved in endocytosis. Kinesins, dyneins, dynamin, and myosins are referred to as **molecular motors**.

An absence of dynein in cilia and flagella results in immotile cilia and flagella, leading to male sterility, situs inversus and chronic respiratory infection, a condition known as **Kartagener syndrome** (OMIM 244400). Mutations in genes affecting the synthesis of dynein have been detected in individuals with this syndrome.

Certain **drugs** bind to microtubules and thus interfere with their assembly or disassembly. These include **colchicine**(used for treatment of acute gouty arthritis), **vinblastine** (a vinca alkaloid used for treating certain types of cancer), **paclitaxel** (Taxol) (effective against ovarian cancer), and **griseofulvin** (an antifungal agent).

Intermediate Filaments Differ from Microfilaments & Microtubules

An intracellular fibrous system exists of filaments with an axial periodicity of 21 nm and a diameter of 8 to 10 nm that is intermediate between that of microfilaments (6 nm) and microtubules (23 nm). At least four classes of **intermediate filaments** are found, as indicated in Table 3.1.

They are all elongated, fibrous molecules, with a central rod domain, an amino terminal head, and a carboxyl terminal tail. They form a structure like a rope, and the mature filaments are composed of tetramers packed together in a helical manner. They are important structural components of cells, and most are **relatively stable** components of the cytoskeleton, not undergoing rapid assembly and disassembly and not disappearing during mitosis, as do actin and many microtubular filaments.

An important exception to this is provided by the **lamins**, which, subsequent to phosphorylation, disassemble at mitosis and reappear when it terminates. **Lamins** form a meshwork in positioned in apposition to the inner nuclear membrane.

Mutations in the gene encoding **lamin A** and **lamin C** cause Hutchinson-Gilford progeria syndrome **(progeria)** [OMIM

176670], characterized by the appearance of **accelerated aging** and other features. A farnesylated form (see Figure 26–2 ↻ for the structure of farnesyl) of prelamin A accumulates in this condition, because the site at which the farnesylated portion of lamin A is normally cleaved by proteases has been altered by mutation. Lamin A is an important component of the structural scaffolding that maintains the integrity of the nucleus of a cell. It appears that the accumulation of farnesylated prelamin A destabilizes nuclei, altering their shape, somehow predisposing victims to manifest signs of premature aging. Experiments in mice have indicated that administration of a farnesyltransferase inhibitor may ameliorate the development of misshapen nuclei. Children affected by this condition often die in their teens of atherosclerosis. A brief scheme of the causation of progeria is shown in Figure 51–18 ↻.

Keratins form a large family, with about 30 members being distinguished. As indicated in Table 3.1, two major types of keratins are found; all individual keratins are **heterodimers** made up of one member of each class.

Vimentins are widely distributed in mesodermal cells, and desmin, glial fibrillary acidic protein, and peripherin are related to them. All members of the vimentin-like family can copolymerize with each other.

Intermediate filaments are very prominent in nerve cells; neurofilaments are classified as low, medium, and high on the basis of their molecular masses. The **distribution of intermediate filaments** in normal and abnormal (eg, cancer) cells can be studied by the use of immunofluorescent techniques, using antibodies of appropriate specificities. These antibodies to specific intermediate filaments can also be of use to pathologists in helping to decide the origin of certain dedifferentiated malignant tumors. These tumors may still retain the type of intermediate filaments found in their cell of origin.

A number of **skin diseases**, mainly characterized by blistering, have been found to be due to mutations in genes encoding **various keratins**. Two of these disorders are epidermolysis bullosa simplex (OMIM 131800) and epidermolytic palmoplantar keratoderma (OMIM 144200). The **blistering**

found in these disorders probably reflects a diminished capacity of various layers of the skin to resist mechanical stresses due to abnormalities in the keratin structure.

Table 3.1

Proteins	*Molecular Mass (kDa)*	*Distributions*
Lamins		
A, B, and C	65–75	Nuclear lamina
Keratins		
Type I (acidic)	40–60	Epithelial cells, hair, nails
Type II (basic)	50–70	As for type I (acidic)
Vimentin-like		
Vimentin	54	Various mesenchymal cells
Desmin	53	Muscle
Glial fibrillary acid protein	50	Glial cells
Peripherin	66	Neurons
Neurofilaments		
Low (L), medium (M), and high (H)	60–130	Neurons

NOTE

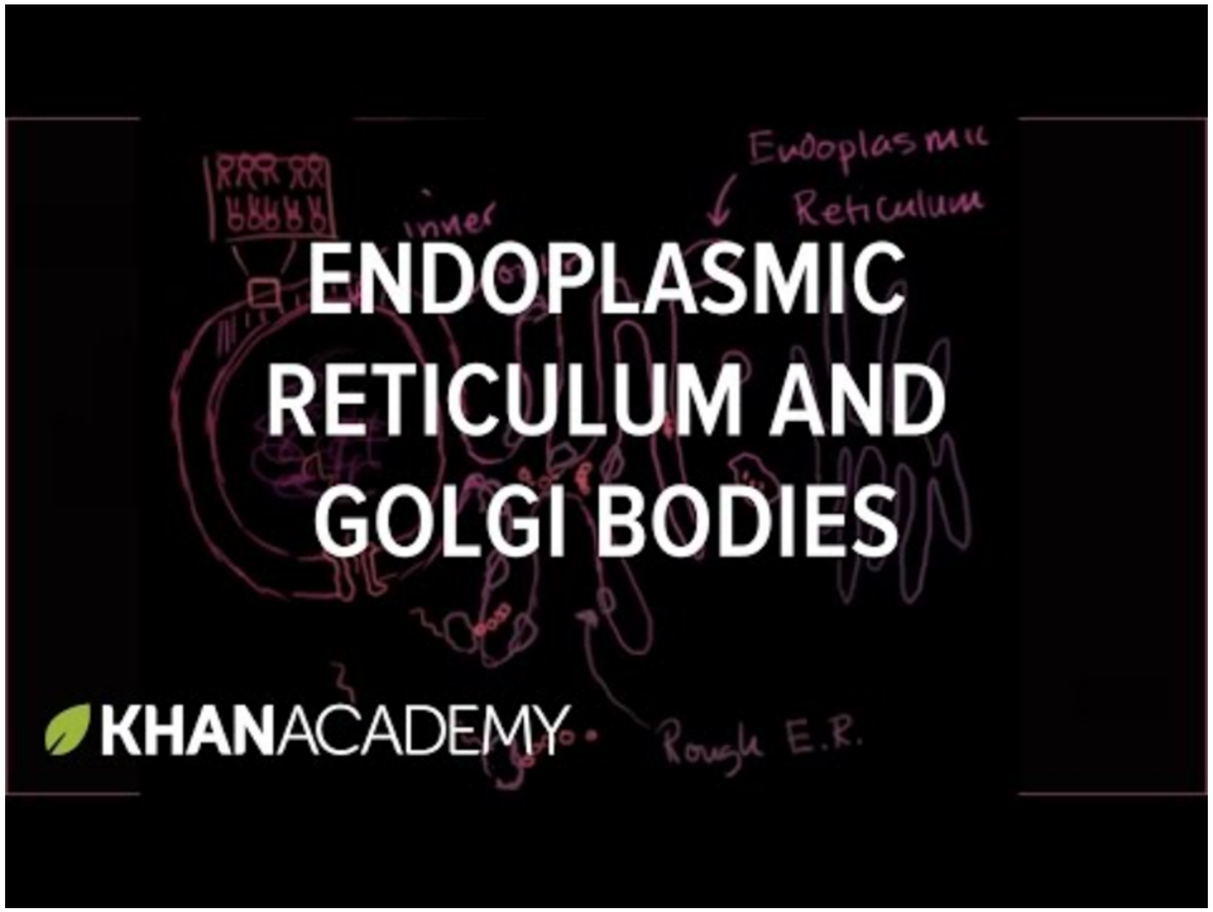
Note: Intermediate filaments have an approximate diameter of 10 nm and have various functions. For example, keratins are distributed widely in epithelial cells and adhere via adapter proteins to desmosomes and hemidesmosomes. Lamins provide support for the nuclear membrane.

4

Diagram and describe how lipids and proteins are distributed in a cell

THE FOLLOWING is taken from:

The Cytoplasm. In: Mescher AL. eds. Junqueira's Basic Histology, 14e New York, NY: McGraw-Hill; . http://accessmedicine.mhmedical.com/content.aspx?bookid=1687&sectionid=109632115. Accessed November 27, 2017.



Interactive 4.1

Osmosis video: https://www.osmosis.org/home/learn?id=Endoplasmic_reticulum_and_Golgi_bodies&focus=golgi_apparatus

GOLGI APPARATUS

The dynamic organelle called the **Golgi apparatus**, or Golgi complex, completes posttranslational modifications of proteins produced in the RER and then packages and addresses these proteins to their proper destinations. The organelle was named after histologist Camillo Golgi who discovered it in 1898. The Golgi apparatus consists of many smooth membranous saccules, some vesicular, others flattened, but all containing enzymes and proteins being processed (Figure 4.1). In most cells the small Golgi complexes are located near the nucleus.

As shown in Figure 4.1, the Golgi apparatus has two distinct functional sides or faces, formed by the complex traffic of vesicles within cells. Material moves from the RER cisternae to the Golgi apparatus in small, membrane-enclosed carriers called **transport vesicles** that are transported along cytoskeletal polymers by motor proteins. The transport vesicles merge with the Golgi-receiving region, or *cis face*. On the opposite side of the Golgi network, at its shipping or *trans face*, larger saccules or vacuoles accumulate, condense, and generate other vesicles that carry completed protein products to organelles away from the Golgi (Figure 4.1).

Formation of transport vesicles and secretory vesicles is driven by assembly of various coat proteins (including clathrin), which also regulate vesicular traffic to, through, and beyond the Golgi apparatus. Forward movement of vesicles in the *cis* Golgi network of saccules is promoted by the **coat protein COP-II**, while retrograde movements in that region involve **COP-I**. Other membrane proteins important for directed vesicle fusion include various Rab proteins and other enzymes, receptors and specific binding proteins, and fusion-promoting proteins that organize and shape membranes. Depending on the activity of these proteins, vesicles are directed toward different Golgi regions and give rise to lysosomes or secretory vesicles for exocytosis.

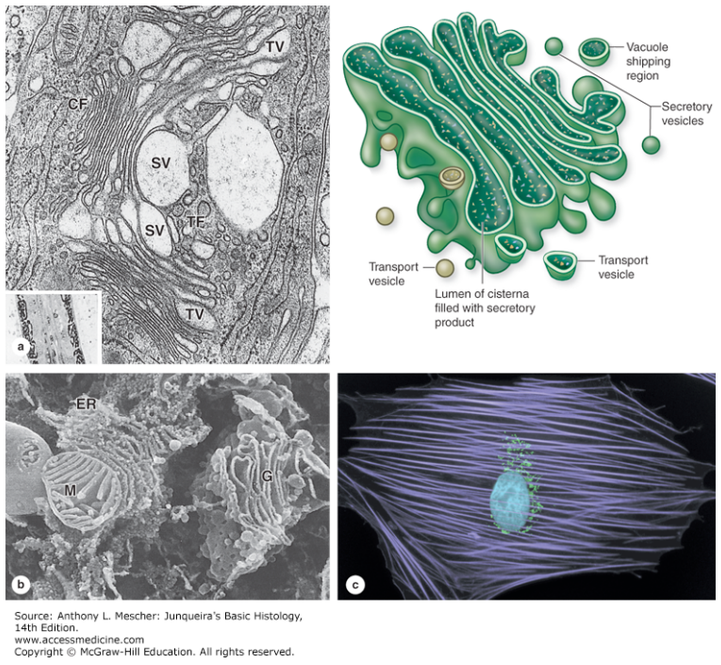


Figure 4.1 Golgi apparatus.
The **Golgi apparatus** is a highly plastic, morphologically complex system of membrane vesicles and cisternae in which proteins and other molecules made in the RER undergo further modification and sorting into specific vesicles destined for different roles in the cell.
(Figure b reproduced, with permission from Naguro T, Iino A. Prog Clin Biol Res. 1989;295:250; Figure c, © 2015 Thermo Fisher Scientific, Inc. Used under permission.)

(a) TEM of the Golgi apparatus provided early evidence about how this organelle functions. To the left is a cisterna of RER and close to it are many small vesicles at the *cis* face **(CF)**, or receiving face, of the Golgi apparatus, merging with the first of several flattened Golgi cisternae. In the center are the characteristic flattened, curved, and stacked medial cisternae of the complex. Cytological and molecular data suggest that other transport vesicles **(TV)** move proteins serially through the cisternae until at the *trans* face **(TF)**, or shipping region, larger condensing secretory vesicles **(SV)** and other vacuoles emerge to carry the modified proteins. elsewhere in the cell. Formation and fusion of the vesicles through the Golgi apparatus is controlled by specific membrane proteins. (X30,000) **Inset:** A small region of a Golgi apparatus in a 1-µm section from a silver-stained cell, demonstrating abundant glycoproteins within cisternae.
(b) Morphological aspects of the Golgi apparatus are revealed more clearly by SEM, which shows a three-dimensional snapshot of the region between RER and the Golgi membrane compartments. Cells may have multiple Golgi apparatuses, each with the general organization shown here and typically situated near the cell nucleus. (X30,000)
(c) The Golgi apparatus location can be clearly seen in intact cultured cells processed by immunocytochemistry using an antibody against golgin-97 to show the many complexes of Golgi vesicles (green), all near the nucleus, against a background of microfilaments organized as stress fibers and stained with fluorescent phalloidin (violet). Because of the abundance of lipids in its many membranes, the Golgi apparatus is difficult to visualize in typical paraffin-embedded, H&E-stained sections. In developing white blood cells with active Golgi complexes, the organelle can sometimes be seen as a faint unstained juxtanuclear region (sometimes called a "Golgi ghost") surrounded by basophilic cytoplasm.

As indicated in Figure 4.2, Golgi saccules at sequential locations contain different enzymes at different *cis*, *medial*, and *trans* levels. Enzymes of the Golgi apparatus are important for glycosylation, sulfation, phosphorylation, and limited proteolysis of proteins. Along with these activities, the Golgi apparatus initiates packing, concentration, and storage of secretory products. Protein movements through the Golgi and the control of protein processing are subjects of active research.

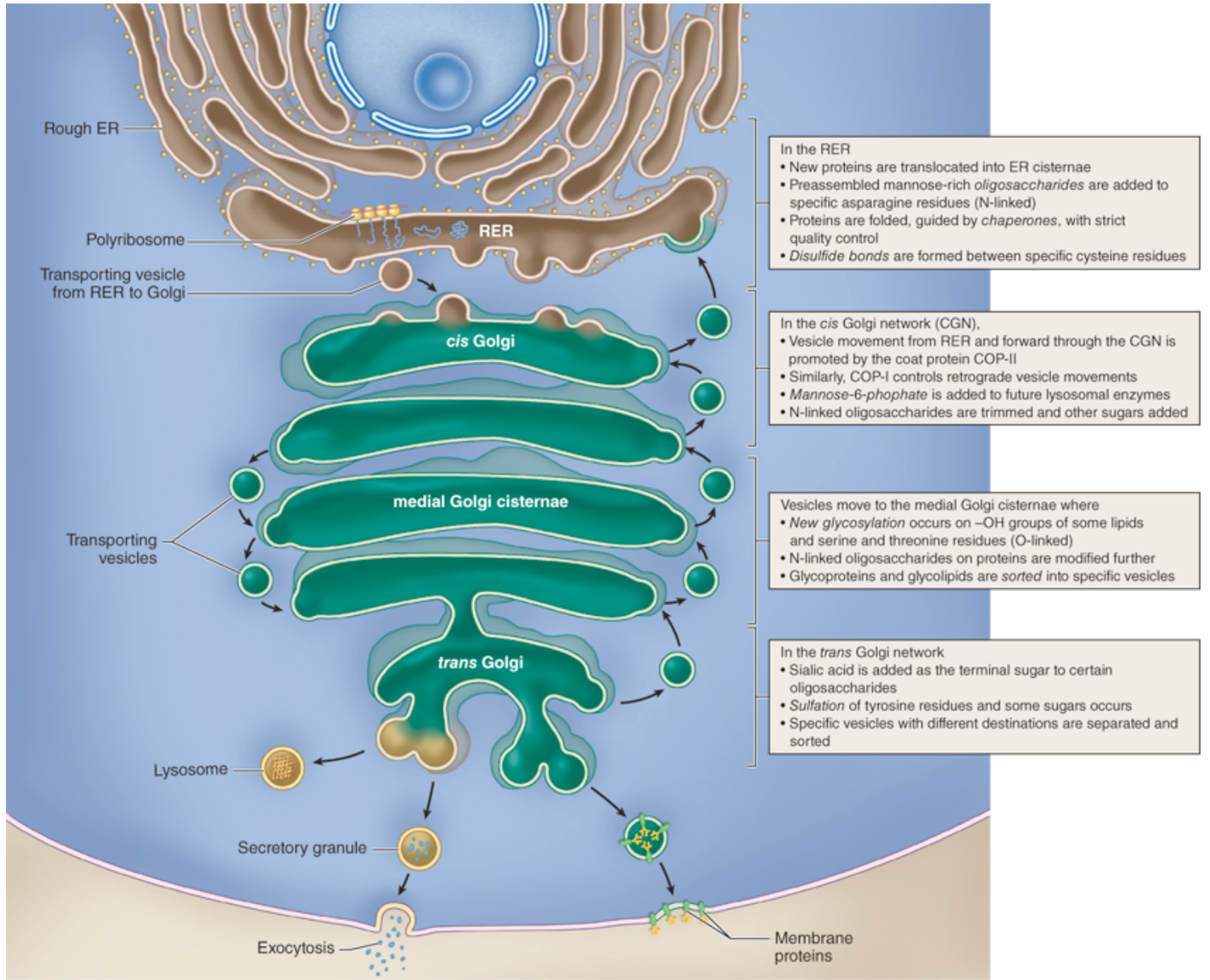


Figure 4.2 Summary of functions within the Golgi apparatus. The main molecular processes are listed at the right, with the major compartments where they occur. In the *trans* Golgi network, the proteins and glycoproteins combine with specific receptors that guide them to the next stages toward their destinations.

5

Diagram and describe the cell cycle including the function of cyclins and cyclin-dependent kinases (CDKs)

THE FOLLOWING is taken from:

Regulation of the Cell Cycle. In: Nalini Chandar, Susan Viselli. Lippincott's Illustrated Reviews: Cell and Molecular Biology, 2e. Philidelphia: Wolters Kluwer, 2019. Accessed December 2, 2019.

OVERVIEW

Numerous checks and balances ensure that the cell cycle is highly regulated, establishing a state of balance or **homeostasis** between cell proliferation, cell differentiation, and cell death. Certain cell types retain the ability to divide throughout their life spans. Others permanently leave the active phases of the cell cycle ($G_1 \rightarrow S \rightarrow G_2$) after their differentiation. Yet, other cells exit and then reenter the cell cycle. According to their type and function, cells receive developmental and environmental cues and respond in accord.

Cells that temporarily or reversibly stop dividing are viewed as being in a state of **quiescence** in the **G_0 phase** (Chapter 20 ↻) (Figure 5.1).

Distinct from temporarily resting quiescent cells, senescent cells have permanently stopped dividing, either **due to age or due to accumulated DNA damage**. For example, neurons are considered **senescent** and will not reenter the active phases of the cell cycle. (See Chapter 24 ↻ for further discussion of senescence.) Intestinal epithelial cells and bone marrow hematopoietic cells, on the other hand, undergo continuous, rapid cell turnover in the course of their normal function and must be continuously replaced. Liver hepatocytes do not continuously traverse the cell cycle but retain the ability to do so if needed. This ability of hepatocytes to reenter the active cell cycle accounts for liver regrowth following injury or disease; a property that has been successfully exploited in live-donor liver transplantation where

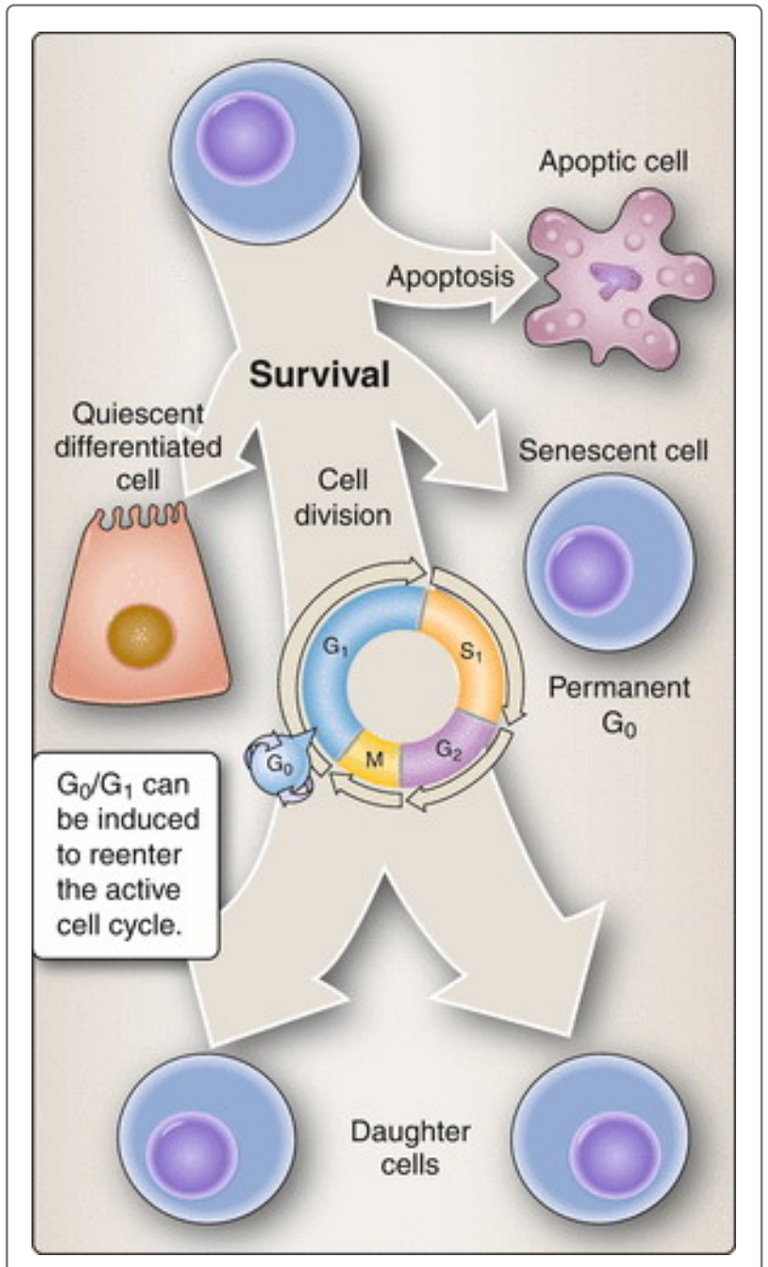


Figure 5.1 Tissue homeostasis requires a balance between differentiation, cell growth, and cell death.

portions of the liver from a donor are given to a patient in need of a liver transplant. Within several weeks after surgery, the liver tissue doubles in size in both the donor and the recipient.

CELL CYCLE REGULATORS

Cell cycle regulators control progression through the various stages of the cell cycle. Cell cycle mediators are categorized as **cyclins** or as **cyclin-dependent kinases (CDKs)**. The patterns of expression of these proteins and enzymes depend upon the cell cycle phase. Complexes of certain cyclins with specific CDKs (**cyclin-CDKs**) possess enzymatic (kinase) activity. Whenever necessary, **cyclin-dependent kinase inhibitors** (CKI) can be recruited to inhibit cyclin-CDK complexes (Figure 5.2).

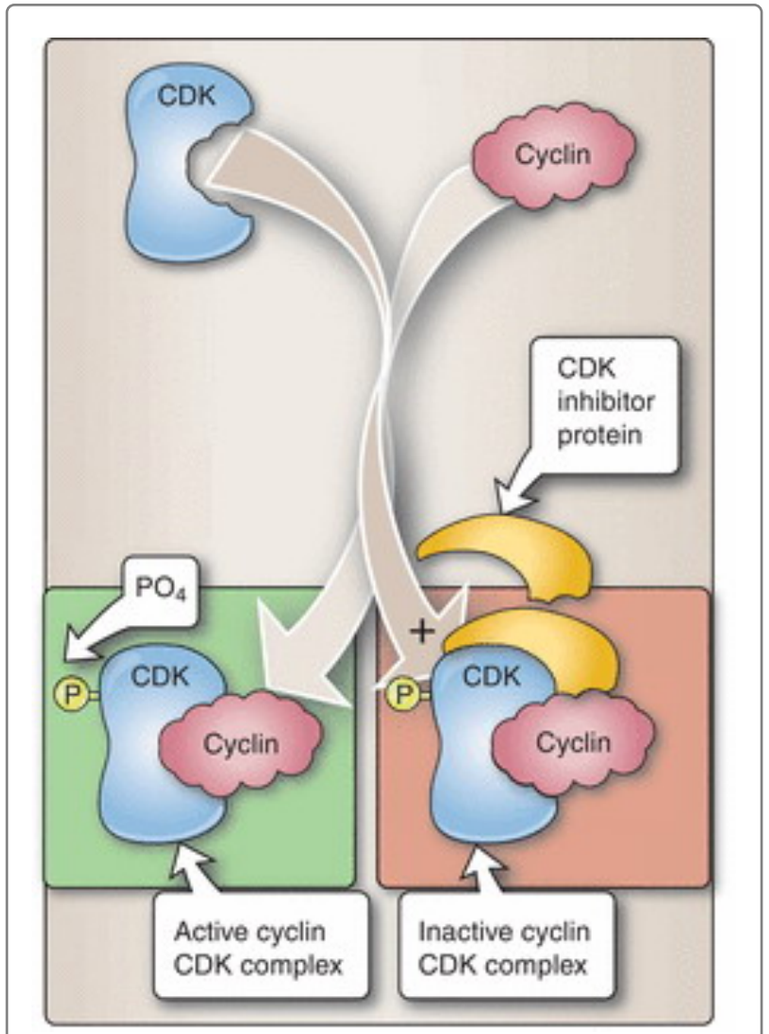


Figure 5.2 Cell cycle mediators and their formation of complexes.

Cyclins

The cyclins are a family of cell cycle regulatory proteins that are categorized as D, E, A, or B cyclins, which are expressed to regulate specific phases of the cell cycle. Cyclin concentrations rise and fall throughout the cell cycle due to synthesis and degradation (via the proteosomal pathway, Chapter 12 ↻) (Figure 5.3).

D-type cyclins (cyclins D1, D2, and D3) are G1 regulators that are critical for progression through the **restriction point**, the point beyond which a cell will irrevocably proceed through the remainder of the cell cycle. S phase cyclins include type E cyclins and cyclin A (Table 5.1). Mitotic cyclins include cyclins B and A.

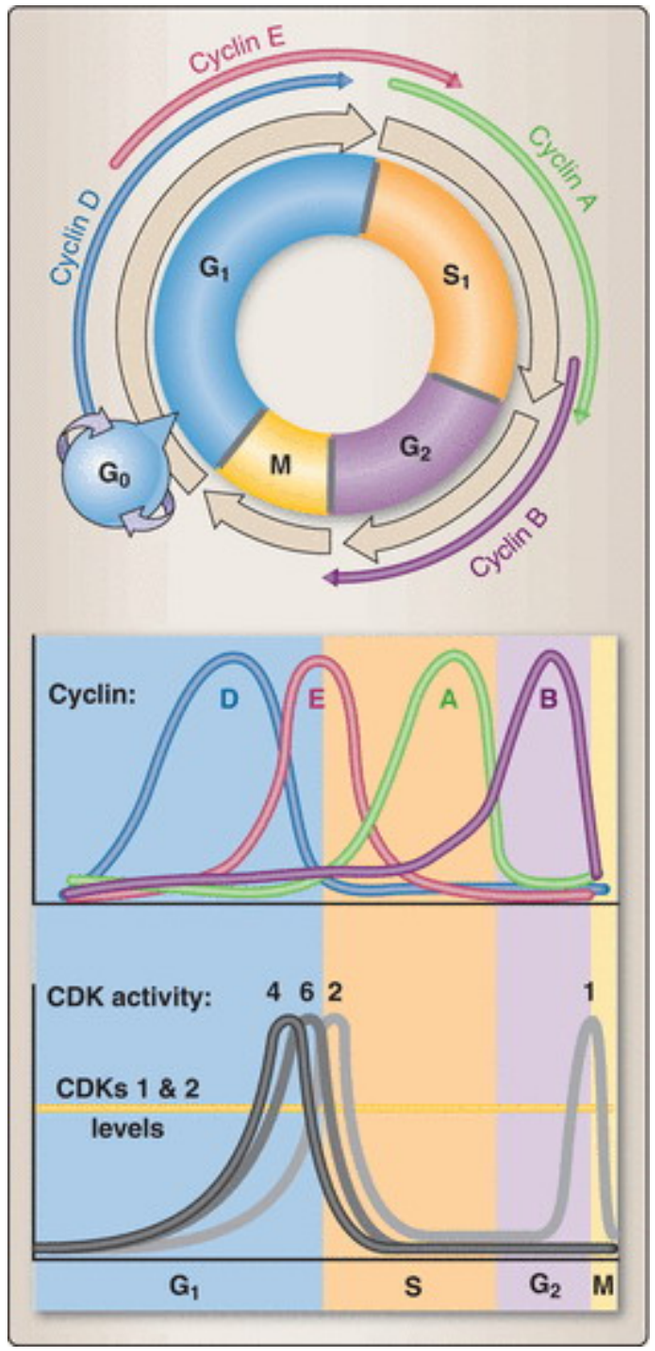


Figure 5.3 Cell cycle–specific expression of cyclins and activation of CDKs.

Cyclin-dependent kinases

CDKs are serine/threonine kinases that are present in constant amounts during the cell cycle. However, their enzyme activities fluctuate depending upon available concentrations of cyclins required for CDK activation (Figure 5.3). The specific cyclin binds first to the CDK and then **CDK-activating kinase** (CAK) phosphorylates the CDK on a threonine residue, completing its activation. Next, the active **cyclin-CDK complex** catalyzes the phosphorylation of substrate proteins on serine and threonine amino acid residues. Phosphorylation changes the activation status of substrate proteins. Such alteration of regulatory proteins allows for initiation of the next phase of the cell cycle.

Active CDK2 is responsible for activating target proteins involved in movement from G_1 to S (S phase transition) and for initiation of DNA synthesis. CDK1 targets activated proteins critical for the initiation of mitosis.

Table 5.1 Cell Cycle Function of Cyclins and CDKs

Cyclin	*Kinase*	*Function*
D	CDK4 CDK6	Progression past the restriction point at the G_1/S boundary
E, A	CDK2	Initiation of DNA synthesis in early S phase
B	CDK1	Transition from G2 to M

CHECKPOINT REGULATION

Checkpoints placed at critical points in the cell cycle monitor the completion of critical events and, if necessary, delay the progression to the next stage of the cell cycle (Figure 5.4). One such checkpoint is the restriction point in G1. Before it reaches the **restriction point**, a cell requires external, growth factor stimulation to progress through G_1. After that, the cell will continue through the cell cycle without the need for further stimulation. Another is the **G_2 checkpoint**, as described below. The **S phase checkpoint** includes monitoring of cell cycle progression and if DNA damage occurs to an S-phase cell, the rate of DNA synthesis will be slowed to attempt to allow for repair.

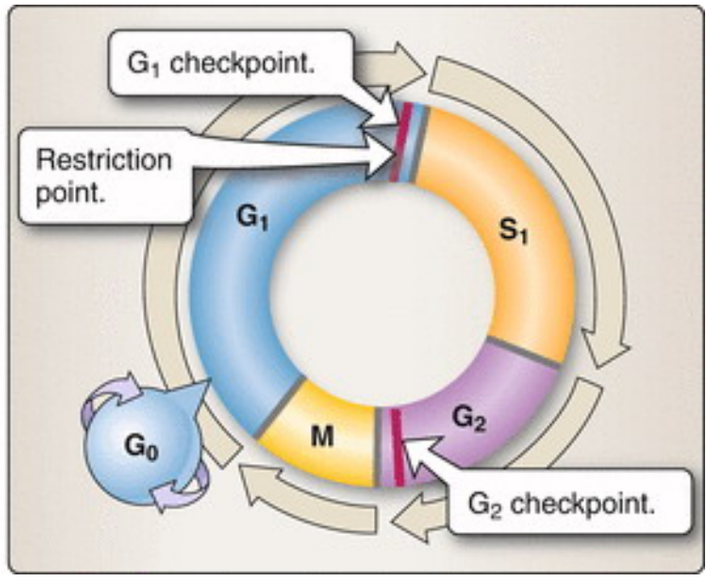


Figure 5.4 Progression through the cell cycle requires cyclin activation of specific CDKs.

G_1 checkpoint

It is important that nuclear synthesis of DNA not begin until all the appropriate cellular growth has occurred during G_1. Therefore, there are key regulators that ensure that G_1 is completed prior to the start of S phase, including tumor suppressors and CDK inhibitors. Tumor suppressor proteins normally function to halt cell cycle progression within G_1 when continued growth is not needed or is undesirable or when DNA is damaged. Mutated versions of tumor suppressor genes encode proteins that permit cell cycle progression at inappropriate times. Cancer cells often show mutations of tumor suppressor genes.

Retinoblastoma (RB) protein

The tumor suppressor **RB** normally halts cells in the G_1 phase of the cell cycle. When RB is mutated, such as in an inherited eye malignancy known as hereditary retinoblastoma, the cell is not stopped in G_1 and continues unregulated progression through the remainder of the cell cycle.

In normal, **resting cells**, the RB protein contains few phosphorylated amino acid residues. In this state, RB prevents a cell's entry into S phase by binding to transcription factor E2F and its binding partner DP1/2 which are critical for the G_1/S transition (Figure 5.5). Therefore, RB normally prevents progression out of early G_1 and into S phase in a resting cell.

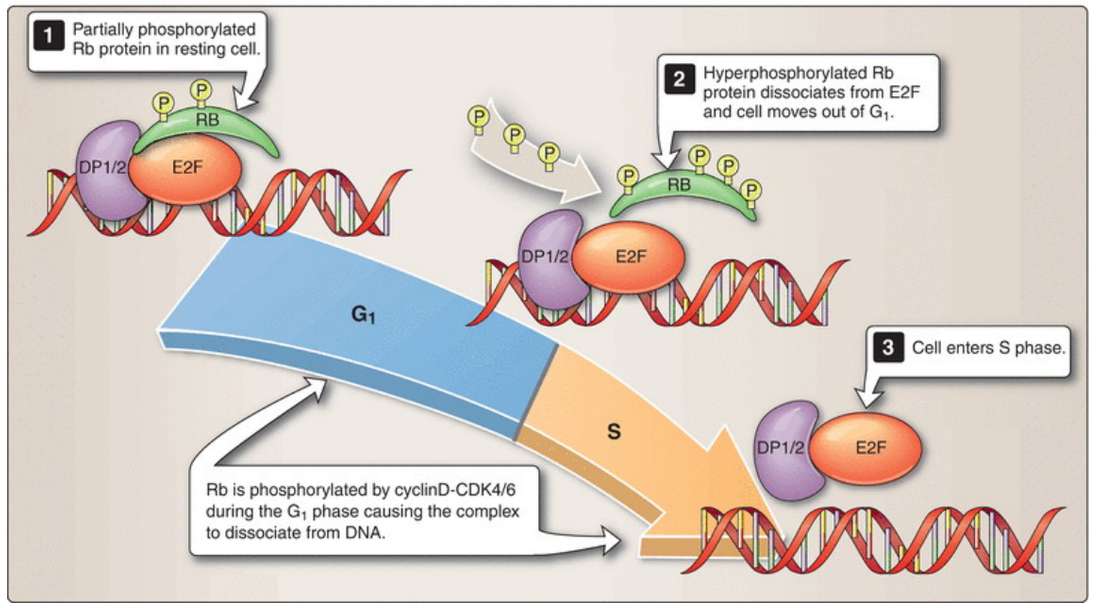


Figure 5.5 RB activation of transcription of S-phase genes.

In **actively cycling cells**, RB is progressively hyperphosphorylated as a consequence of growth factor stimulation and signaling via the MAP kinase cascade (Chapter 17 ↻). Subsequently, cyclin D-CDK4/6 complexes are activated and they phosphorylate RB. Further phosphorylation of RB by cyclin E-CDK2 allows the cell to move out of G1. Hyperphosphorylated RB can no longer inhibit transcription factor E2F binding to DNA. Therefore, E2F is able to bind to DNA and activate genes whose products are important for S phase. Examples of E2F-regulated genes include thymidine kinase and DNA polymerase, both of which are involved in the synthesis of DNA.

p53

The **p53** tumor suppressor protein plays a major regulatory role in G_1. When DNA is damaged, p53 becomes phosphorylated, stabilized, and activated. Activated p53 stimulates transcription of **CKI** (see Figure 5.2) to produce a protein named **p21**, to halt cell cycle progression to allow for DNA repair. If the DNA damage is irreparable, p53 instead triggers apoptosis (Chapter 23 ↻).

If p53 is mutated and unable to arrest the cell cycle, then unregulated cell cycle progression can occur. Over 50% of all human cancers show p53 mutations (Figure 5.6).

Cyclin-dependent kinase inhibitors

Two classes of these cyclin-dependent kinases inhibitors are recognized. **INK4A** family members inhibit D-type cyclins from associating with and activating CDK4 and CDK6. **CIP/KIP** family members are potent inhibitors of CDK2 kinases. p21 (p21CIP1), described above, is a member of the CIP/KIP family.

HUMAN PAPILLOMAVIRUS, CERVICAL CANCER, AND TUMOR SUPPRESSORS

Human papillomavirus strains 16 and 18 are established etiological agents of cervical cancer. In cervical cells infected with these viral strains, viral protein E6 binds to p53 and viral protein E7 binds to RB. As a result of viral protein binding, both RB and p53 are inactivated and unregulated cell cycle progression and malignancy may result.

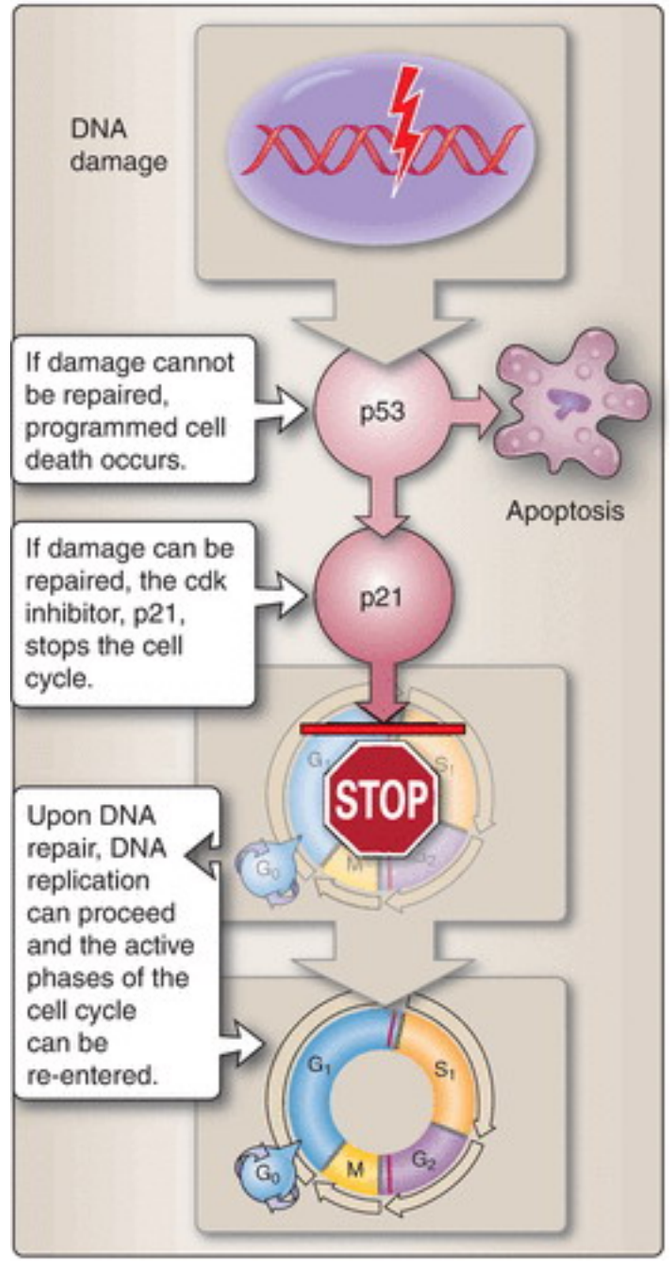


Figure 5.6 FIGURE 21.6 p53 control of cell fate following DNA damage.

G_2 checkpoint

It is important for the integrity of the genome that nuclear division (mitosis) does not begin before DNA is completely duplicated during S phase. Therefore, the G_2 checkpoint, which occurs after S and before the initiation of mitosis, is also a critical regulatory point within the cell cycle. CDK inhibitors and phosphatases function at the G_2 checkpoint.

Cyclin-dependent kinase inhibitor 1 (CDK1)

Cyclin-dependent kinase inhibitor 1 controls entry into mitosis. During G_1, S, and into G_2, CDK1 is phosphorylated on tyrosine residues, inhibiting its activity. In order for the cell to progress through G2 and into M, those inhibitory phosphorylations must be removed from CDK1.

cdc25C phosphatase

cdc25C phosphatase is the enzyme that catalyzes the removal of inhibitory phosphorylations from CDK1 (Figure 5.7). Following its dephosphorylation, CDK1 can bind to cyclin B and the activated CDK1-cyclin B complex moves into the nucleus where it activates mitosis by phosphorylating key components of subcellular structures (e.g., microtubules). If the cell cycle must be suspended prior to chromosome segregation in mitosis, then cdc25C can be inactivated through actions of tumor suppressors **ATM** and **ATR** (see below).

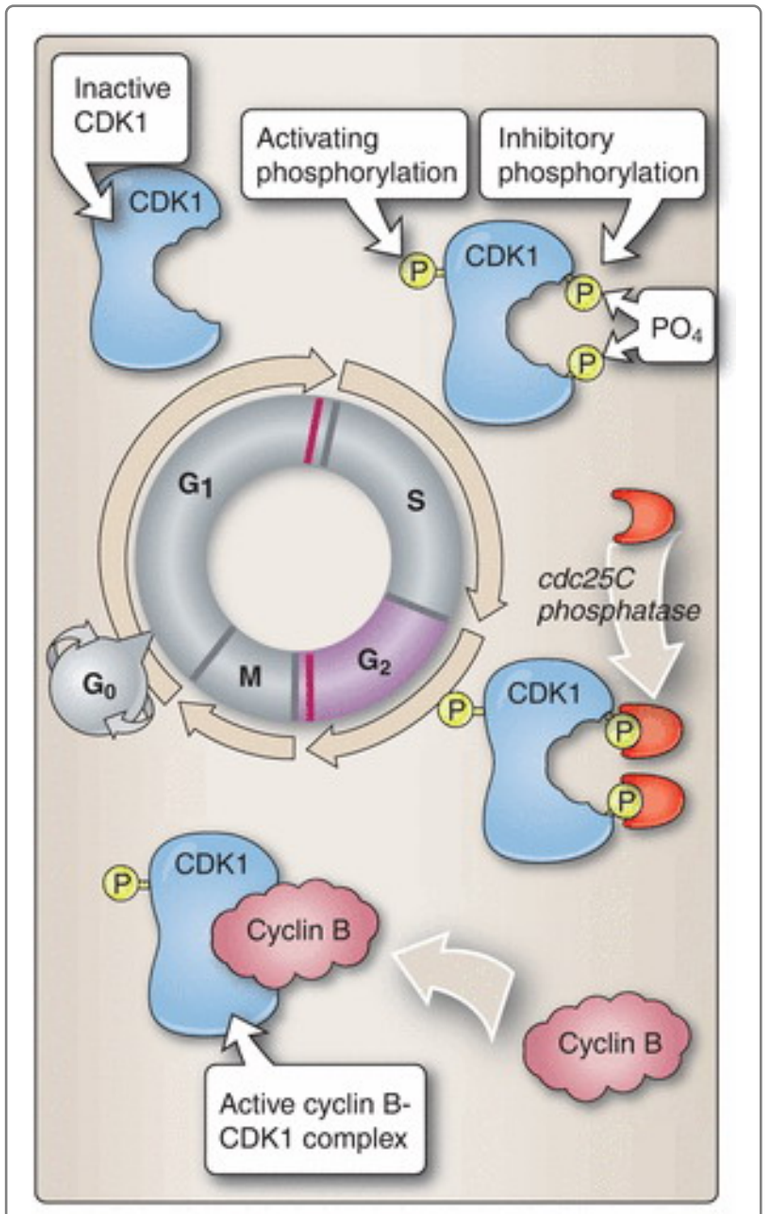


Figure 5.7 Progression from G2 to M is controlled by cdc25C phosphatase and CDK1.

DNA DAMAGE AND CELL CYCLE CHECKPOINTS

DNA damage within cells may be caused in a variety of ways including replication errors, chemical exposure, oxidative insults, and cellular metabolism. The usual response to DNA damage is to halt the cell cycle in G1 until DNA repair (Chapter 7 ↻) can be accomplished. As previously described, the tumor suppressor p53 responds to DNA damage by halting the cell in G_1. However, depending on the type of DNA damage, different cell cycle regulatory systems may be utilized, and other phases of the cell cycle may be halted. Additional tumor suppressor proteins can play a role in the control of checkpoints in cases of DNA damage.

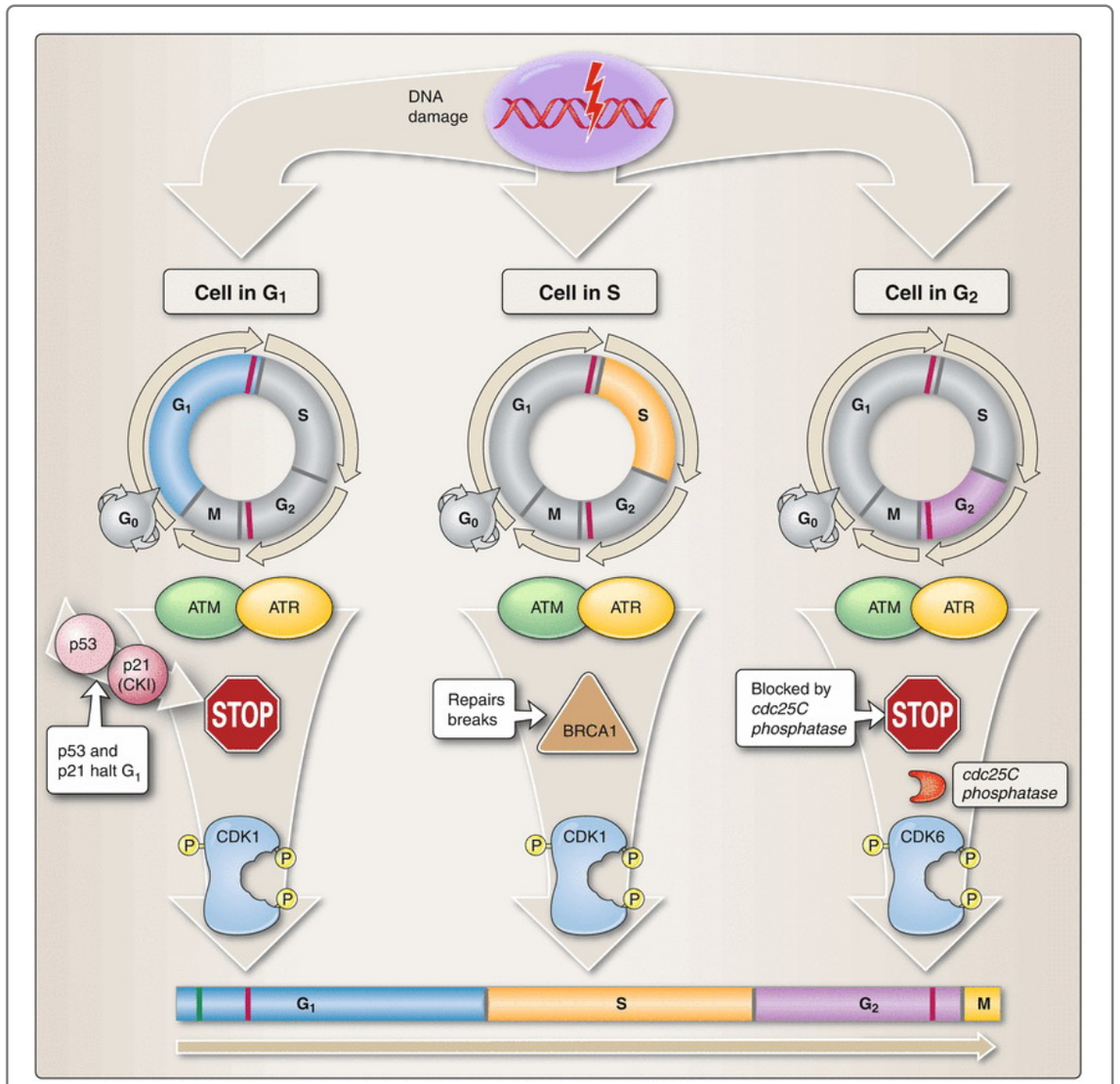


Figure 5.8 ATM/ATR in G_1 and G_2 checkpoint regulation.

ATM and ATR response to DNA damage

Tumor suppressors **ATM** (ataxia telangiectasia, mutated) and **ATR** (ATM and Rad3 related) are serine and threonine protein kinases that are important in the cellular response to DNA damage (Figure 5.8).

ATM is activated by ionizing radiation and is the primary mediator of the response to double-strand DNA breaks. It can induce cell cycle arrest at G_1/S, in S and at G_2/M transitions.

ATR plays a role in arresting the cell cycle in response to UV-induced DNA damage and has a secondary role in the response to double-strand DNA breaks.

BRCA1

BRCA1, the protein product of the breast cancer susceptibility gene 1, plays a role in the repair of double-strand DNA breaks. It is involved in all phases of the cell cycle. The mechanistic details and other proteins involved remain to be elucidated.

ANTICANCER DRUGS AND THE CELL CYCLE

Both normal and tumor cells utilize the same cell cycle. But, normal and **neoplastic** (cancerous) tissue may differ in the total number of cells in active phases of the cell cycle. Some chemotherapeutic agents are effective only in actively cycling cells (Figure 5.9). These therapies are considered to be cell cycle–specific agents and are generally used for tumors with a high percentage of dividing cells. Normal, actively cycling cells are also damaged by such therapies. When tumors have a low percentage of dividing cells, then cell cycle–nonspecific agents can be used therapeutically.

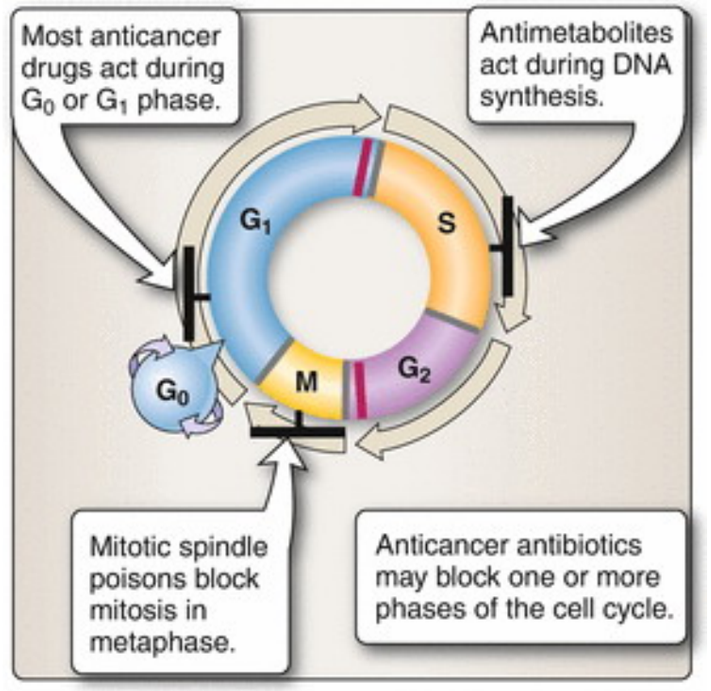


Figure 5.9 Anticancer drugs and the cell cycle.

Antimetabolites

Compounds that are structurally related to normal purine or pyrimidine nucleotide precursors are called antimetabolites. They exert their toxic effects on cells in the S phase of the cell cycle. They can also compete with nucleotides in DNA and RNA

synthesis (Chapters 7 and 8 and *LIR Biochemistry*). Examples of drugs in this category are methotrexate and 5-fluorouracil.

Anticancer antibiotics

While some anticancer antibiotics, such as bleomycin, cause cells to accumulate in G_2. Other agents in this category are not specific to any particular phase of the cell cycle, but impact actively cycling cells more than resting cells by binding DNA and disrupting its function. Some alkylating agents and nitrosoureas are cell cycle–nonspecific anticancer antibiotics. These agents are often used to treat solid tumors with low growth fractions.

Mitotic spindle poisons

Drugs that act as mitotic spindle poisons inhibit mitotic or M-phase cells, specifically during metaphase (Chapter 20). Their mechanism of action involves binding to tubulin (Chapter 4) and disrupting the spindle apparatus of the microtubules required for chromosome segregation. Such therapies are often used to treat high growth fraction cancers such as leukemias. Vincristine and vinblastine (the Vinca alkaloids), as well as Taxol, are examples of mitotic spindle poisons. Taxol is used in combination with other chemotherapy drugs to treat certain cancers, including metastatic breast cancer, ovarian cancer, and testicular cancer.

ORAL DRUGS AND CANCER TREATMENT

Newly available drugs target CDK4/6 and can be ingested orally, unlike traditional cytotoxic drugs that interfere with DNA replication or mitosis and are administered intravenously. In continuously cycling cancer cells, D-type cyclins are degraded in S phase but accumulate again in G_2 phase, in an attempt to recombine with CDK4/6 in subsequent G_1 phases to allow for uninterrupted cell cycles. The CDK4/6 inhibitors impair cyclin D recombination with CDK4/6. Since the stability and assembly of cyclins with CDK4/6 are dependent on mitogen-activated signaling pathways, using drugs that inhibit the MAP kinase pathway along with drug inhibitors of cyclin-CDK4/6 interactions appears to produce synergistic effects. This combination of drugs allows the cell to arrest in G_1 and exit from the cell cycle into a quiescent (G0) state. Monotherapy with CDK4/6 inhibitors has not yet proven to be effective in controlling cancers, but their use in combination with these MAP kinase inhibitors appears to hold promise.

CHAPTER SUMMARY

- Cyclins and CDKs control cell cycle progression.
- Specific cyclins are made and degraded during particular points in the cell cycle.
- CDKs are enzymatically active during a narrow window of the cell cycle and are important for driving the cell cycle forward.
- Cells are stimulated to enter the cell cycle by the action of growth factors, which directly activate a specific cyclin belonging to the cyclin D family of G_1 cyclins.
- Tumor suppressor proteins inhibit the cell cycle. Mutated tumor suppressors allow unregulated cell cycle progression that may result in malignancy.
- Checkpoint regulation is a safety measure to prevent accumulated DNA damage.
- RB protein controls movement into the S phase by inhibiting S phase–specific transcription factors. RB is active in its underphosphorylated form and inactive in its hyperphosphorylated form.
- p53 guards the genome from damage. In the event of DNA damage, p53 can induce the synthesis of a CKI.
- CDK1 controls the G2M transition and is activated by the phosphatase action of cdc25C.
- ATM and ATR are kinases that sense and respond to specific types of DNA damage.
- External and internal factors contribute to different types of DNA damage.
- Anticancer drugs may have cell cycle–specific or cell cycle–nonspecific actions.
- Antimetabolites inhibit S-phase cells, while anticancer antibiotics may cause accumulation of G_2-phase cells or act without regard to cell cycle phase. Mitotic spindle poisons disrupt spindle formation and affect cells in mitosis.

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COVER AND TABLE OF CONTENTS IMAGE

Human karyotype with color added to distinguish chromosome pairs. "Human genome," by Webridge (CC BY 2.0). https://commons.wikimedia.org/wiki/File:Human_genome.png