Cell Structure 2.1

Learning Objectives

- List the main categories of cellular function
- Describe the general structure, location, and function of the plasma membrane
- Describe the composition, location, and function of the cytosol
- Compare the general structure and function of microtubules, microfilaments, and intermediate filaments
- Describe the general structure, location, and function of the nucleus and its envelope
- Describe the general structure, location, and function of the mitochondria
- Describe the general structure, location, and function of the endoplasmic reticulum
- Describe the general structure, location, and function of the Golgi apparatus
- Describe the general structure, location, and function of lysosomes and peroxisomes
- Describe the general structure, location, and function of proteasomes
- Explain the general purpose of ubiquitinylation of proteins
- List the different types of cell-to-cell junctions

FOR CELLS, FORM FOLLOWS FUNCTION

In Chapter 1.1, we discussed the following points:

- Cells are the organization unit of life.
- Cells come in a multitude of forms, specialized for their function.
- The vast majority of cells are somatic cells, and all of these contain the same set of genetic information, present in DNA and organized into genes.
- The multitude of forms comes from using only specific sets of the genes to make proteins.

Multicellular organisms such as ourselves evolved because multicellular structures can provide an internal environment that is more stable than the natural environment, and thereby enhance the survival of the component cells and of the organism. Free-floating, single-celled organisms live at the mercy of environmental conditions, whereas protected, multicellular organisms can better withstand changes in the environment. Single-celled organisms and cells in multicellular organisms must solve a number of problems in order to survive. These include:

- **Catalysis**: Cells must be able to change one metabolite into another in order to synthesize cellular constituents, degrade them, or provide energy.
- **Transport**: Cells must be able to move things from outside the cell to inside or from one compartment to another within the cell. This includes bulk secretion or uptake of materials from the extracellular fluid.
- **Signal transduction**: Cells must have mechanisms for responding to signals from other cells or from within the cell. These may be chemical signals or electrical signals.
- **Recognition**: Cells attach to other cells and to extracellular structures. They must be able to recognize where they should form attachments.
- **Movement**: At some stage of their development, all cells must be able to move so as to position themselves properly within the cellular matrix that makes us up.
- **Control**: All of the activities of the cell must be coordinated. Control here also means that cells must select the parts of the genome that they will use. "Control" thus implies **differentiation**—the formation of specialized cells uniquely suited to their task.
- **Proliferation**: At appropriate times of development, cells must make new cells. This involves cell division and its control.

ORGANELLES MAKE UP THE CELL LIKE THE ORGANS MAKE UP THE BODY

The cells of the body typically are composed of a relatively small number of **organelles** that carry out specific functions of the cell, much like our organs do for us. These are called organelles because their relation to the cell is like the organs' relation to the body. Table 2.1.1 lists these organelles along with their major function. The disposition of these organelles in a "typical" cell is shown in Figure 2.1.1.

THE *CELL MEMBRANE* MARKS THE LIMITS OF THE CELL

The cell membrane, also known as the plasma membrane, defines the inside and outside of the cell. Like all biological membranes, it consists of two lipid

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DOI: http://dx.doi.org/10.1016/B978-0-12-800883-6.00010-0

TABLE 2.1.1 Major Organelles of the Cell and Their Function

Organelles	Function
Plasma membrane	**Customs officer of the cell**: determines what gets into or out of the cell, also **signal transduction** and cell recognition
Cytosol	**Cell sap**: the fluid medium in which soluble biochemicals diffuse and in which the other organelles are suspended
Cytoskeleton	Support, movement, and cell attachment
Nucleus	**Command center**: contains the hereditary material and organizes and controls differentiation
Free ribosomes	**Factory of the cell** for soluble proteins
Rough ER	**Factory of the cell** for membrane proteins and secreted proteins
Smooth ER	Synthesis of lipids and steroids
Golgi apparatus	**Shipping department**: finishing and targeting of proteins to specific locations
Mitochondria	**Powerhouse of the cell**: site of oxidation and energy transfer
Lysosomes	**Garbage disposal**: destruction of worn-out organelles
Proteasomes	Destruction of tagged proteins
Peroxisomes	Oxidation of fatty acids and detoxification of xenobiotics

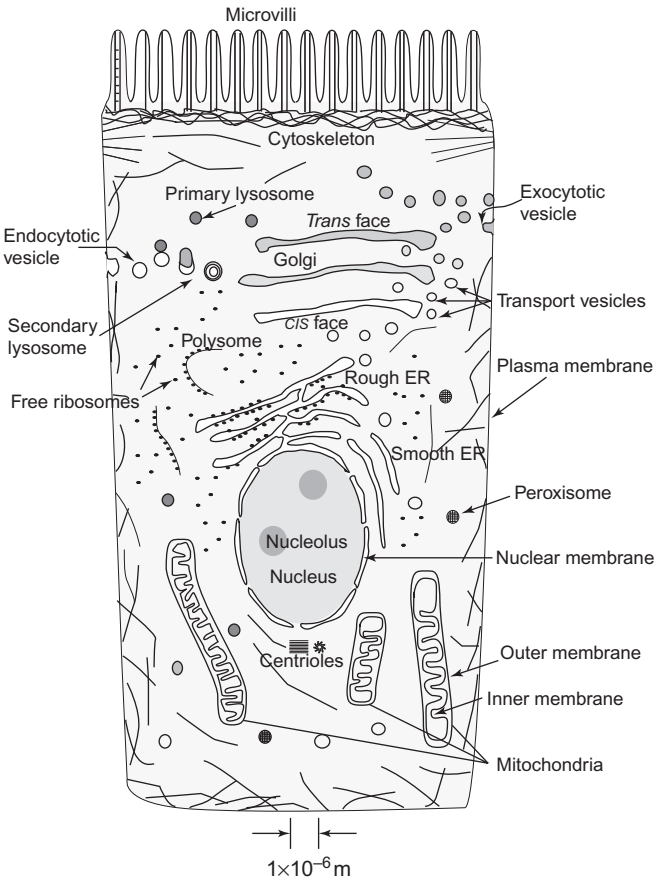

FIGURE 2.1.1 A "typical" human cell showing various subcellular organelles including the plasma or cell membrane, nucleus, nuclear membrane, rough ER, smooth ER, transport vesicles, Golgi apparatus, mitochondria, cytoskeletal elements, lysosomes, peroxisomes, and endocytotic and exocytotic vesicles. Although there is no "typical" cell, most cells contain this set of organelles. Exceptions to this rule include the adult erythrocytes.

layers, or leaflets, in which are embedded a variety of proteins that serve specific functions (see Chapter 2.4 for a discussion of the structure of lipids in biological membranes). Both the inside and outside layers may be coated with carbohydrate units that partly define the function of the lipids. An important function of this membrane is to determine what goes into and what comes out of the cell. The "customs officer" of the cell has several mechanisms that can transport materials into or out of the cell. These include:

- Passive transport
- Active transport
- Exocytosis
- Endocytosis.

We will discuss all of these in more detail in Chapters 2.5 and 2.6. **Passive transport** requires no metabolic energy and may involve diffusion through the lipid layer of the membrane or through water-like channels that are established by proteins that span the membrane. **Active transport** requires the input of cellular metabolic energy and may be primary or secondary. Primary active transport directly couples transport to metabolic energy. Secondary active transport indirectly links transport of a material to metabolic energy. **Exocytosis** and **endocytosis** refer to the movement of materials that are enclosed in **vesicles**. These vesicles are tiny hollow spheres of membrane. Secretory vesicles are filled with some material by the cell. These vesicles can fuse with the plasma membrane, and, in so doing, they dump their contents into the extracellular space. This process is called exocytosis. Endocytosis is similar but occurs in the opposite direction. In this case, parts of the plasma membrane invaginate and pinch off to form endocytotic vesicles inside the cell. Endocytosis of fluid is called **pinocytosis** or "cell drinking"; endocytosis of particulate stuff is called **phagocytosis** or "cell eating."

In addition to these functions, the cell membrane forms the hub of **signal transduction** and **surface recognition**. It must transfer extracellular signals originating from other cells to an intracellular signal inside the cell. This is what is meant by "signal transduction." The cell membrane receives the first messenger in the form of a chemical or electrical signal, and receipt of the first messenger causes the formation of a second messenger inside the cell. Surface recognition occurs through surface proteins that are members of the **major histocompatibility complex** or **MHC**. These proteins are responsible for beginning transplant rejections by recognizing the transplants as foreign matter.

THE *CYTOSOL* PROVIDES A MEDIUM FOR DISSOLUTION AND TRANSPORT OF MATERIALS

The cell membrane surrounds all the constituents of the cell, which themselves exist in a fluid medium that allows transfer of materials among them. This fluid medium is the **cytoplasm**, which literally means "cell fluid." It includes the **cytosol** and all of the organelles suspended in it. The cytosol itself is the fluid that contains dissolved ions and organic compounds of a bewildering variety. These include amino acids for building proteins, glucose for energy, a tremendous variety of metabolic intermediates, and cytoplasmic enzymes for glycolysis, the first stage in burning carbohydrates for energy. The ionic composition of the cytoplasm varies with different cell types, but Table 2.1.2 gives some reasonable approximate numbers for the "typical" cell. The cytoplasm also serves as a medium for the transmission of control signals from the outer surface of the cell to the interior, and from the nucleus to the rest of the cell. Although we describe it here as a fluid, the cytosol is not like water: ions diffuse through the cytosol slower than they do through water (see Chapters 1.6 and 1.PS2, Problem #20). Cutting a muscle fiber, for example, does not cause its fluid to leak out like water. The cytoplasm is more akin to a gel. Most of this behavior is due to the small volume of fluid and the abundance of cell surfaces. Although bulk water flows, a thin film adheres to any wettable surface. This thin film generally exceeds the thickness of a cell. Thus, at the cell level, surface forces govern much of bulk fluid flow.

TABLE 2.1.2 Selected Components of the Cytosol

Component	Concentration
Na^+	14×10^{-3} M
K^+	120×10^{-3} M
Cl^-	10×10^{-3} M
HCO_3^-	10×10^{-3} M
ATP	5×10^{-3} M
ADP	50×10^{-6} M
Mg^{2+}	0.5×10^{-3} M
Ca^{2+}	0.1×10^{-6} M
pH	7.1–7.2
Osmolarity	295 mOsm L^{-1}

THE *CYTOSKELETON* SUPPORTS THE CELL AND FORMS A NETWORK FOR VESICULAR TRANSPORT

Arrays of protein filaments form a network within the cytoplasm. These filaments determine the shape of the cell and provide for the movement of the cell as a whole or for the movement of organelles from one part of the cell to another. There are four major types of filaments comprising the cytoskeleton:

1. Microtubules
2. Intermediate filaments
3. Actin filaments or microfilaments
4. Myosin filaments.

MICROTUBULES ARE THE LARGEST CYTOSKELETAL FILAMENTS

The microtubules are about 25 nm in diameter and are constructed of heterodimers of **tubulin**, a globular polypeptide of 50,000 Da. These dimers are assembled into protofilaments of tubulin dimers with the β-tubulin of one joined to the α-tubulin of the next. The microtubules are assembled from 13 such protofilaments arranged in a cylinder with a hollow core (see Figure 2.1.2).

A number of **microtubule-associated proteins** (MAPs) bind to microtubules. Some of these MAPs are "motor

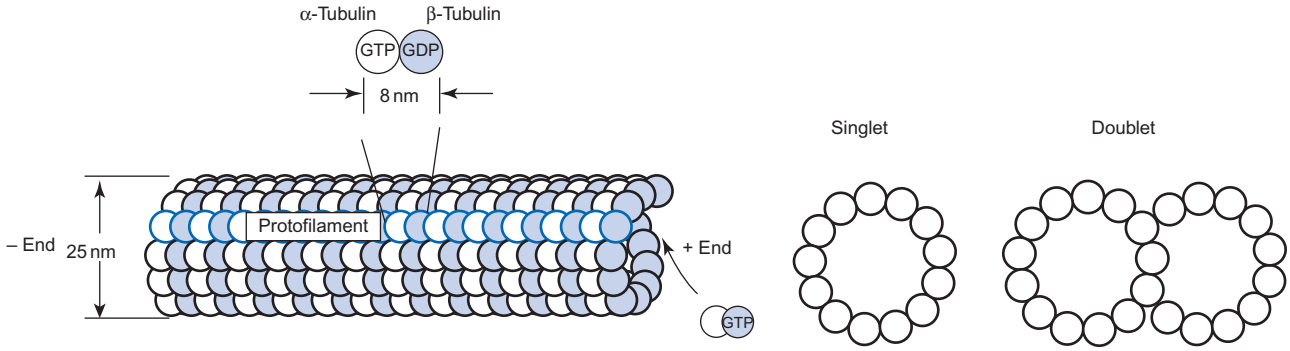

FIGURE 2.1.2 Schematic diagram of the structure of a microtubule. Microtubules consist of protofilaments composed of tubulin dimers, one α-tubulin and one β-tubulin. Both bind GTP, guanosine triphosphate. GTP, like ATP, stores chemical energy in its phosphate bonds. Hydrolysis of GTP to GDP can be used to stabilize protein shapes. The α-tubulin does not hydrolyze its GTP, whereas the β-tubulin hydrolyzes its GTP to GDP; 13 of the protofilaments assemble to form a singlet microtubule. Because of the asymmetrical arrangement of the tubulin monomers, the microtubule has an asymmetry, with a **plus** (+) end and a **minus** (–) end. The plus (+) end is the end pointing away from the origin of the microtubule and is the end to which monomers add to the growing microtubule. These ends differ in the rates of tubulin association and dissociation. Because these rates differ, the microtubule can treadmill—dissolve at one end while lengthening at the other. Tubulin can also form doublet and triplet structures. Cross-sections of a singlet microtubule and a doublet microtubule are shown.

proteins" that can "walk" along the microtubule. Two of these motor proteins are named **kinesin** and **dynein**. Kinesin forms a family of motor proteins with about 40 members. Most of these are "+ directed" motors, moving along the microtubules toward the + end. Dyneins comprise a family of − directed motors. These motor proteins can attach vesicles and then move along the microtubules, carrying their vesicles along. In this way, the microtubules can provide a track along which intracellular transport occurs. This is especially important in neurons in which transport must occur down a long narrow process of the cell. Figure 2.1.3 shows a highly schematic cartoon illustrating kinesin and dynein movement along a microtubule.

Microtubules can also form the interior of larger structures called **cilia** that extend out from the cell into the extracellular fluid. These cilia have a special arrangement of nine doublets arrayed circumferentially around two central microtubules. In this case, the microtubules are cross-linked such that the action of dynein causes the cilia to bend. The waving cilia move the extracellular fluid past the fixed cell. In this way, the movement of the cilia causes the movement of extracellular fluid. In the lungs, the cilia move mucus, trapped dust, and foreign material toward the pharynx where they can be expelled. In the Fallopian tubes, the cilia help move the ovum toward the uterus. Figure 2.1.4 shows a schematic diagram of the structure of a cilium. Activation of the dynein arms linking two isolated microtubules will ordinarily cause the two microtubules to slide past each other. In the cilia, linking proteins turn this motion into bending of the cilia.

Microtubules also form the **mitotic spindle** during cell division. Nearly all cells possess two **centrioles** oriented at right angles. These form a microtubule organizing center (MTOC) that provides a scaffold for the assembly of microtubules. Another kind of tubulin, γ-tubulin, binds to accessory proteins to form a γ-tubulin ring complex (γ-TuRC) that acts as a nucleation site for microtubules. The centrosome consists

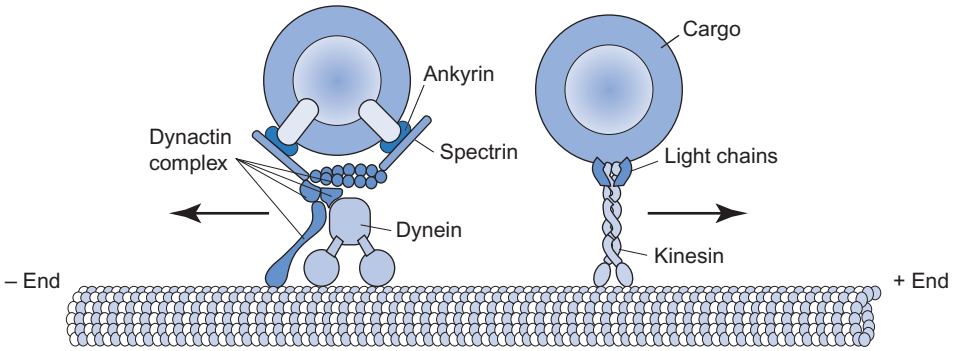

FIGURE 2.1.3 Schematic model of microtubule motor proteins. Kinesins typically have two globular heads and an elongated coiled tail. The tail regions of most kinesins bind cargo, either membrane-enclosed vesicles or microtubules. Dyneins may contain two or three globular heads and a large number of accessory proteins that bind vesicle cargo. Dynein itself is a complex assembly that requires a second complex assembly, dynactin, to transport cargo. A possible arrangement of some of these structures is shown.

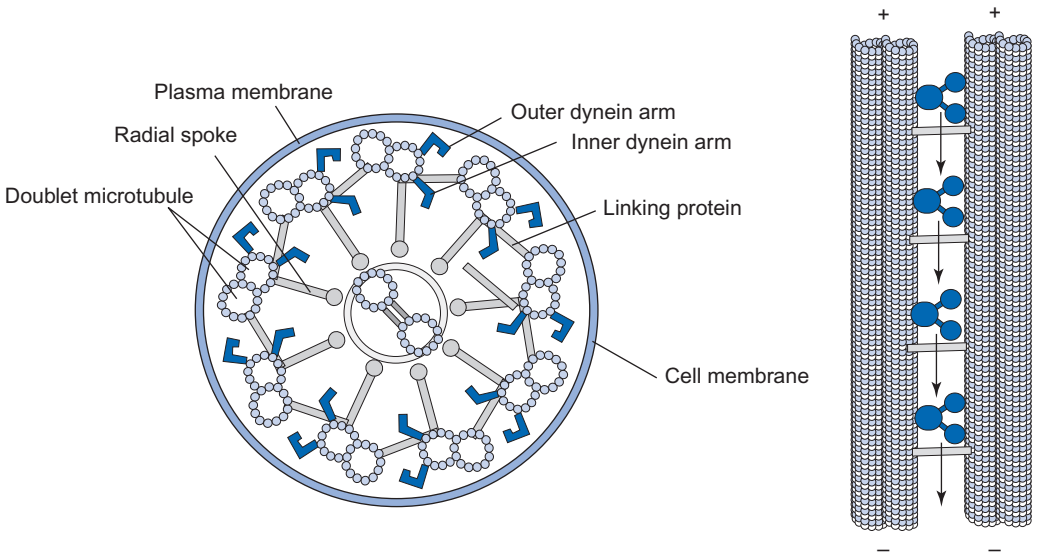

FIGURE 2.1.4 Structure of a cilium. A cilium contains a "9 + 2" arrangement of microtubules. Nine doublet microtubules containing A and B microtubules with 13 and 11 protomers, respectively, surround a central pair of microtubules. Multiple structures link these microtubules. The dynein arms move toward the minus end of the microtubule. In isolated tubules, this would cause sliding of one microtubule past another. The linking proteins turn this motion into a bending of the cilium.

of the two centrioles surrounded by a centrosome matrix containing many copies of the γ-TuRC. The microtubules that grow out of this centrosome complex provide tracks for chromosome movement during cell division.

ACTIN FILAMENTS ARISE FROM NUCLEATION SITES USUALLY IN THE CELL CORTEX

Actin filaments are present in most cells but are especially abundant in muscle cells. The monomer is a globular protein called **G-actin**, with a molecular weight of 41,800 Da. G-actin polymerizes noncovalently into actin filaments, called **F-actin**. Actin filaments consist of two strands of globular molecules twisted into a helix with a repeat distance of about 36 nm. The filament is asymmetric having distinguishable ends that are detectable by the way in which it interacts with **myosin**, another protein that is present in many cell types but is especially abundant in muscle. Thus the actin filament also has a **plus end** (the growing end) and a **minus end** (the nucleation or beginning end). Each individual actin filament is about 3.5 nm across, so that F-actin has a diameter of about 7 nm. Assembly and stabilization of filamentous, or F-actin, is described in Figure 2.1.5.

Actin filaments determine the shape and movement of the cell's surface, including structures such as **microvilli**, which are fingerlike extensions of epithelial cells that line internal structures like the intestinal villi and kidney tubules. The membrane of these cells anchors the actin filaments and extends them into a web of cytoskeletal elements in the main body of the cell. Other proteins can cross-link actin microfilaments together to form bundles of filaments or gel-like networks. These cross-linking proteins include **α-actinin**, **fimbrin**, and **villin**, which bundle actin filaments together. **Spectrin** and **filamin** both have two actin-binding sites. They join two actin filaments together to form a web of supporting filaments.

INTERMEDIATE FILAMENTS ARE DIVERSE

Intermediate filaments were originally named because with diameters between 8 and 10 nm, they are intermediate in size between the microtubules (at 25 nm) and the microfilaments at 7 nm. These intermediate filaments are composed of a number of different proteins. They play some structural or tension-bearing role. They differ from microtubules and microfilaments in that:

- Both microtubules and microfilaments are made by the polymerization of globular monomeric

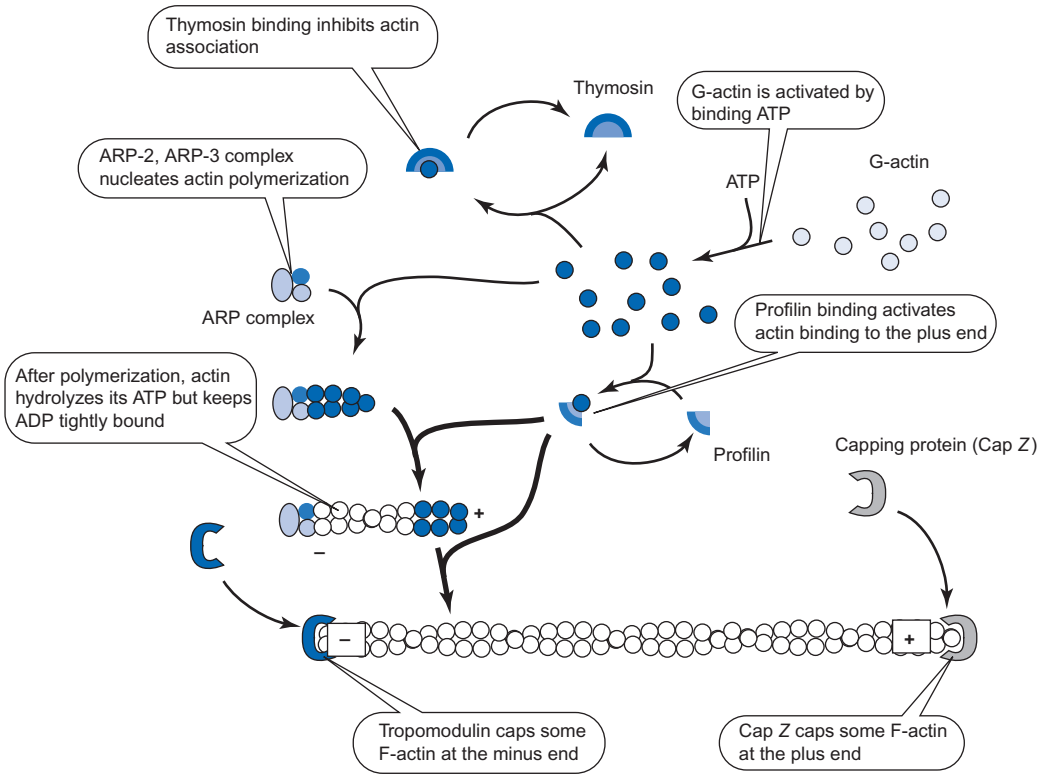

FIGURE 2.1.5 Assembly and stabilization of microfilaments (actin filaments). Actin binds ATP and begins assembly by binding to actin-related proteins (ARPs) that serve as a nucleation site, usually just under the cell membrane in the cortex of the cell. The ARP complex can also bind F-actin on the side of the filament, so it can build a tree-like web from individual actin filaments. After assembly, actin hydrolyzes its bound ATP, but the ADP remains tightly bound. Formation and stabilization of F-actin is regulated by proteins that bind the free monomer. Thymosin binds to the free monomer and inhibits its association with either the minus or plus end of the F-actin. Profilin binds to the free monomer and inhibits its association with the minus end but markedly enhances its association with the plus end. Cap *Z* binds to the plus end of the F-actin and stabilizes it. The minus end can be stabilized by remaining bound to the ARP complex. In muscle cells, tropomodulin binds to the minus end and stabilizes it.

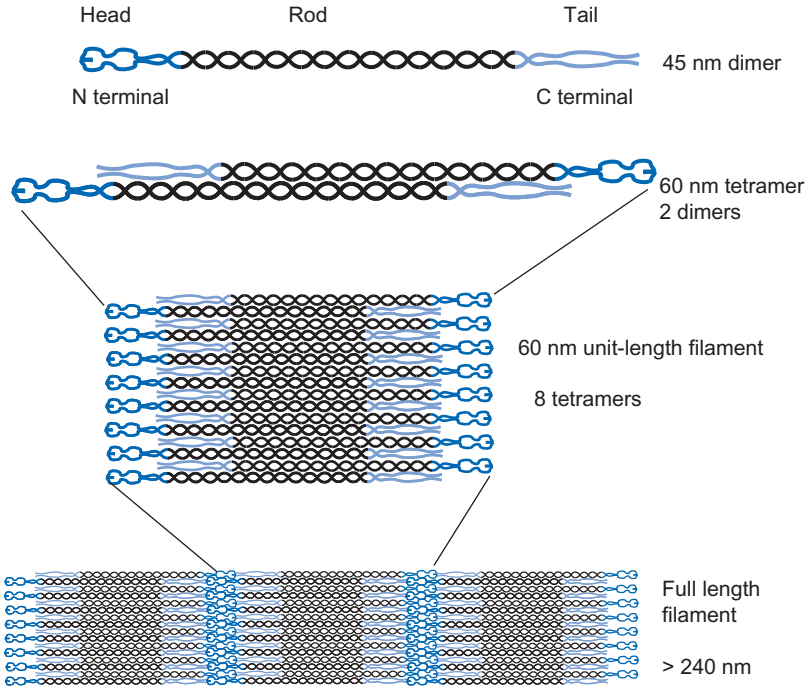

FIGURE 2.1.6 Highly schematic representation of the structure of intermediate filaments. The elementary subunit of the intermediate filaments consists of an elongated rod with an N-terminal "head" and a C-terminal "tail." A variety of these elementary subunits are made by the body. These associated laterally to form a homo- or heterodimer, approximately 45 nm long. These dimers further associate laterally, with an offset, to form a 60-nm-long tetramer. Eight of these tetramers further associate laterally to form a unit length filament. These unit length filaments can associate end to end to form longer filaments. The width of the mature filaments is not eight times the width of the tetramer, as these associate in three dimensions to form a mature filament about 11 nm in diameter. After the initial association into full length filaments, the filaments are radially compacted to form the mature filaments.

proteins, but the intermediate filaments are made of elongated (45 nm) and thin (2–3 nm) rod-like dimers. The intermediate filament units align with their long axis parallel to the filament axis, and filament width is determined by lateral association of the dimers (see Figure 2.1.6)

- Both microtubules and microfilaments are polar, which allows the active movement of motor proteins with their associated cargo along the filaments. Assembled intermediate filaments have no polarity because individual monomers are oriented in both directions along the axis of the filament.
- Intermediate filaments differ from both microtubules and microfilaments in that reversible association and dissociation of intermediate filament dimers can occur all along the length of the filament, whereas association and dissociation of microtubules and microfilaments occur only at their ends. This process is called **dynamic subunit exchange**. However, the exchange occurs much slower than the exchange of subunits in microtubules and microfilaments.
- Unlike tubulin and actin, the subunits of the intermediate filaments do not bind a nucleotide.

The intermediate filaments are diverse; some 73 separate proteins in humans have been identified encoded by over 70 genes. They all consist of three parts: a "head," a long rod-like central part, and a "tail." The members of the IF family have been subdivided into five distinct groups based on their structure, mode of assembly, and developmental expression in different tissues. These groups and their types are summarized in Table 2.1.3. There is considerable variation within types. For example, there are over 50 different varieties of keratin.

CYTOSKELETAL UNITS FORM FREE-FLOATING STRUCTURES BASED ON *TENSEGRITY*

Buckminster Fuller in the 1960s invented the word "tensegrity" as a blend of tension and integrity. He used it to describe architectural structures of remarkable rigidity that were composed of compressive rods and elastic cables. These two elements can be combined to form stable structures. Cells cannot rely on their membranes for structural stability because the membranes themselves are weak. But if you drape the membranes over cytoskeletal elements, structural strength can be achieved. In this view, the microtubules are the rigid rods and intermediate filaments are the tension-bearing elements. The actin and myosin elements allow for the movement of the cytoskeleton and the consequent movement of the attached membrane. In this way, the cell can extend processes or move from one place to another.

TABLE 2.1.3 Classification of the Intermediate Filaments

Types of IFs	Protein	Tissue Distribution	Proposed Function	Associated Diseases
Type I Type II	Acidic keratins Basic keratins	Epithelial tissues Epithelial tissues	Tissues strength and integrity	Epidermolytic hyperkeratosis
Type III	Desmin GFAP Peripherin Vimentin Syncoilin	Muscle Glial cells	Sarcomere organization	Dilated cardiomyopathy Alexander disease
Type IV	Neurofilaments NF-L, NF-M, and NF-H Nestin Synemin α, β	Neurons	Axon organization	Amyotrophic lateral sclerosis; Parkinson disease
Type V	Nuclear lamins type A, B1, B2, C1,C2	Nucleus	Nuclear organization and signaling	Hutchinson–Gilford progeria; limb–girdle muscle dystrophy; Emery–Dreifuss muscular dystrophy
Type VI	Filensin Phakinin	Lens		Cataracts

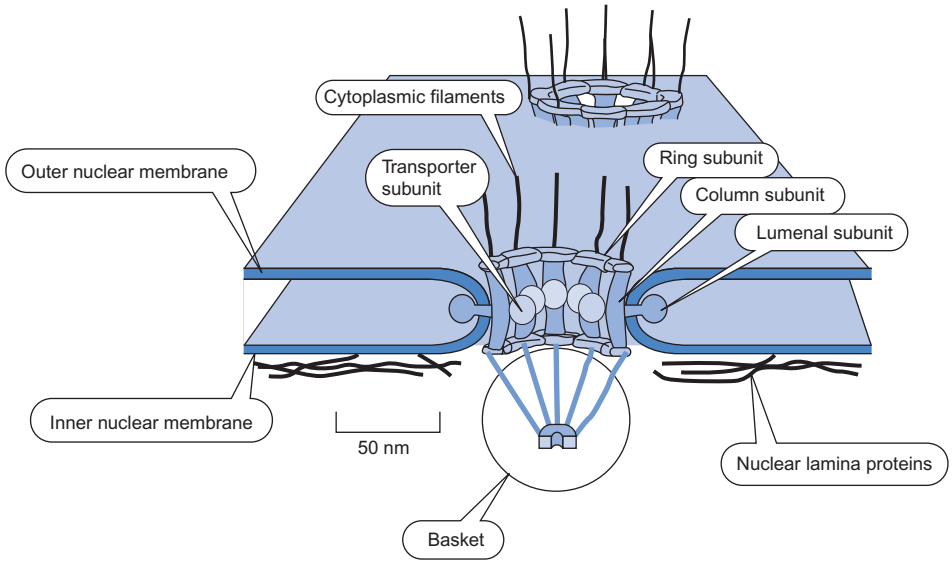

FIGURE 2.1.7 Cartoon of the structure of the nuclear pore. The nuclear pores consist of a complex of more than 50 different proteins that form a complicated structure with octagonal symmetry. Small molecular weight materials (< 20 kD) can pass through these pores in both directions, but the movement of larger materials, such as RNA and ribosomes, is regulated. The outer membrane faces the cytosolic compartment and the inner membrane faces the nuclear compartment. Two rings made of eight subunits each are connected by columnar scaffold subunits. Lumenal subunits anchor the scaffold to the membrane. The ring subunits connect to fibrils that form a basket structure on the nuclear side. Transport subunits in the interior of the pore actively transport materials into or out of the nucleus.

MYOSIN INTERACTS WITH ACTIN TO PRODUCE FORCE OR SHORTENING

Myosin also exists in multiple isoforms. A major form is a protein of about 200 kDa that forms a homodimer with two long tails forming a coil, a hinge region, and a head region that binds actin filaments, hydrolyzes ATP, and "walks" along the actin filament. The interaction of the actin filament relative to the myosin filament causes either shortening of the acto-myosin thread or production of force. This mechanism is responsible for muscle force and also produces movement in nonmuscle cells.

THE *NUCLEUS* IS THE COMMAND CENTER OF THE CELL

Most cells have linear dimensions on the order of 20–50 μm. The nucleus is the largest organelle, with a diameter of about 3–5 μm. The nucleus is bounded by a double membrane, the **nuclear envelope**, that has pores for materials to move between nucleus and cytoplasm, as shown in Figure 2.1.7. The nucleus contains nearly all of the DNA of the cell. As described in Chapter 2.2, this DNA carries the information that allows the synthesis of specific proteins. The nucleus

also contains a specialized region called the **nucleolus**. This is a diffuse region that is not delimited by a membrane. The nucleolus is involved in the synthesis of **ribosomes**.

RIBOSOMES ARE THE SITE OF PROTEIN SYNTHESIS

Ribosomes exist either free in the cytoplasm or bound to membranes of the rough endoplasmic reticulum (ER). Both types consist of two main subunits, designated 60S and 40S, where the S refers to Svedbergs and describes how fast the particles sediment during centrifugation (see Appendix 2.1.A1). The larger subunit consists of three different strands of ribonucleic acid (RNA) and about 49 different proteins. The smaller subunit has a single RNA strand and about 33 other proteins. Both are assembled in the nucleolus, a specialized region of the nucleus that is not membrane bound. There, large loops of DNA containing ribosomal RNA genes are transcribed by the enzyme **RNA polymerase I** to ribosomal RNA or **rRNA**. Ribosomal proteins made in the cytoplasm are imported back into the nucleus and assembled, along with the rRNA, into the two subunits. The two subunits join to form a functional ribosome in the cytoplasm that makes a platform on which proteins are synthesized, as described more fully in Chapter 2.2.

THE *ER* IS THE SITE OF PROTEIN AND LIPID SYNTHESIS AND PROCESSING

The ER is a membranous network within the cell fluid, or cytoplasm, that is continuous with both the outer nuclear membrane and the plasma membrane. The membrane forms flattened disks with an enclosed space called **cisternae**. Electron micrographs and density gradient centrifugation reveal two functionally distinct regions of the ER; these are **rough ER** and **smooth ER**. The "rough" ER was given that name because the many ribosomes attached to the membrane give it a granular appearance in electron micrographs. The smooth ER lacks these attached ribosomes.

Special proteins, called **translocons**, span the ER membrane and bind ribosomes to the cytoplasmic face of the rough ER. Protein synthesis occurs on the ribosomes. Some of these are free within the cytoplasm, and the proteins made there are generally soluble proteins that remain in the cytoplasm. The ribosomes on the ER synthesize proteins that pass through the ER membrane as they are being synthesized. These proteins may become embedded in membranes or they may be destined for secretion from the cell. After synthesis, many proteins are further processed within the ER cisternae, preparing them for secretion or targeting them for some location within the cell.

THE *GOLGI APPARATUS* PACKAGES SECRETORY MATERIALS

The Golgi apparatus consists of sets of membrane-delimited smooth-surfaced cisternae. Each set of flattened, disk-shaped cisternae resembles a stack of pancakes. This structure is called a **Golgi stack** or **dictyosome**. It is about 1 μm in diameter and is usually located near the nucleus and near the centrioles that define the cell center. The number of cisternae in a stack varies from 6 to 30, and the number of Golgi stacks in the cell varies enormously with the biochemical activity of the cell.

Golgi stacks are polarized with two distinct faces. The *cis* or **forming face** is nearest a smooth transitional portion of the rough ER. The *trans* or **maturing face** typically faces the plasma membrane. Swarms of small vesicles (about 50 nm in diameter) cluster on the *cis* face of the Golgi stack. A large number of vesicles associate with the sides of the stack near the dilated rims of each cisternae. In electron micrographs, these vesicles sometimes appear to bud off the Golgi cisternae. In secretory cells, larger vesicles containing high concentrations of secreted proteins appear to originate from the *trans* face of a Golgi stack. The Golgi stacks are a processing station for proteins manufactured in the rough ER. Proteins made in the rough ER travel to the Golgi through the small transport vesicles. In the Golgi, the proteins are processed and packaged for delivery to various locations throughout the cell, including packaging for eventual fusion with the plasma membrane and secretion into the extracellular space (see Figure 2.1.8).

THE *MITOCHONDRION* IS THE POWERHOUSE OF THE CELL

Mitochondria produce much of the cell's ATP by coupling the chemical energy of oxidation of metabolites to the synthesis of ATP. Their main structural features are shown schematically in Figure 2.1.9. The matrix contains many different enzymes required for the oxidation of pyruvic acid and fatty acids, including those involved in the **tricarboxylic acid cycle**. The mitochondrial matrix also includes mitochondrial DNA, special mitochondrial ribosomes, tRNAs, and enzymes that are required for the expression of mitochondrial genes. The inner mitochondrial membrane contains a number of important proteins that collectively comprise the **electron transport chain** and another enzyme complex called the **ATP synthetase** that makes ATP from ADP and Pi, the reverse of the ATP hydrolysis reaction discussed in Chapter 1.7. These complexes are discussed further in Chapter 2.10. Briefly, these complexes couple the chemical energy derived from the oxidation of fuels obtained from food to the synthesis of ATP. This is the site of oxygen consumption by aerobic cells.

Lynn Margulis originally postulated that the mitochondria in aerobic cells that contain a nucleus (eukaryotic cells)

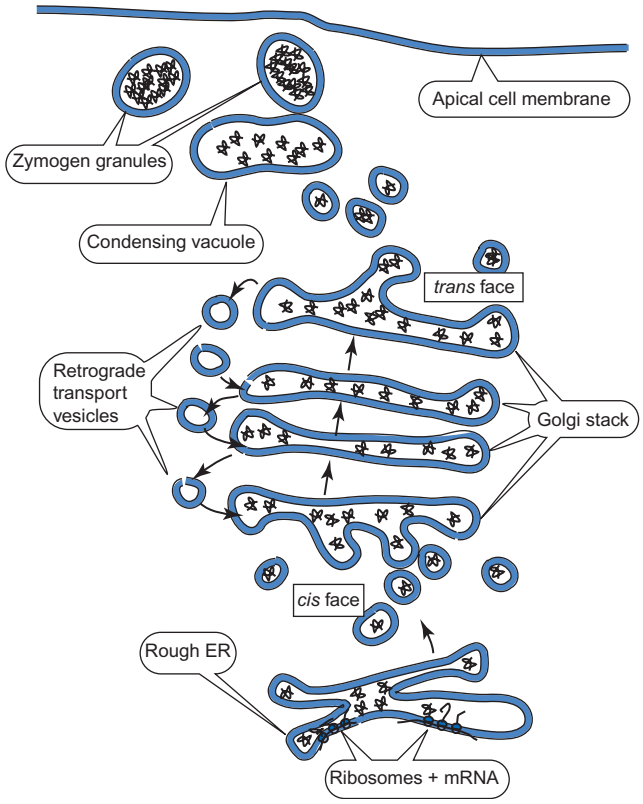

FIGURE 2.1.8 Packaging of secreted proteins in secretory cells of the pancreas. Proteins are synthesized on the membrane of the rough ER and translocated into the lumen of the ER as they are being made. The ER forms transport vesicles that fuse to form the *cis* face of the Golgi stack. These membranes progress through the stack as new layers are added on the *cis* face and taken away on the *trans* face. On the *trans*, or maturing, face of the Golgi stack, the enclosed proteins are collected in secretory vesicles. These then fuse into condensing vacuoles that concentrate the proteins to form zymogen granules. These granules lie in the apical aspect of the secretory cells, adjacent to the plasma membrane. Upon stimulation, these granules fuse with the apical membrane and release their contents of secretory enzymes into the lumen of a duct, or channel, that takes the enzymes into the intestine.

originated from the engulfment of aerobic bacteria by anaerobic single-celled organisms. This hypothesis, called the **endosymbiotic hypothesis**, derives from the similarity of mitochondria to bacteria. Both have circular DNA; both are approximately the same size; both reproduce by dividing into two, asexually; mitochondrial ribosomes resemble bacterial ribosomes rather than eukaryotic ribosomes, and both bacteria and mitochondria share a slightly different genetic code from that in the nucleus.

LYSOSOMES AND *PEROXISOMES* ARE BAGS OF ENZYMES

Lysosomes are membranous bags of **hydrolytic enzymes** including **proteases**, **nucleases**, **glycosidases**, **lipases**, **phospholipases**, and **phosphatases**. These hydrolytic enzymes are acid hydrolases, being optimally active in an acid environment. Lysosomes are typically 0.2–0.3 μm in diameter. They originate from the *trans* face of the Golgi stack and are formed first as **primary lysosomes**. The primary lysosome fuses repeatedly with a variety of membrane-bound substrates including endocytotic vesicles, phagocytotic vesicles, and worn-out intracellular organelles. After fusion, the combined vesicle forms a **secondary lysosome**. Because of its diverse substrate contents, the secondary lysosomes have a diverse morphology. Lysosomes degrade phagocytosed material and worn-out parts of the cell.

The peroxisome is another membrane-bounded vesicle, with a diameter of about 0.5 μm. It contains oxidative enzymes such as catalase, d-amino acid oxidase, and urate oxidase. Like the mitochondria, the peroxisomes are a major site of O_2 utilization. The peroxisome detoxifies foreign chemicals and metabolizes fatty acids. Beta-oxidation, a process in which fatty acids are shortened by two carbons to form acetyl-coenzyme A, occurs in both mitochondria and peroxisomes (see Chapter 2.11).

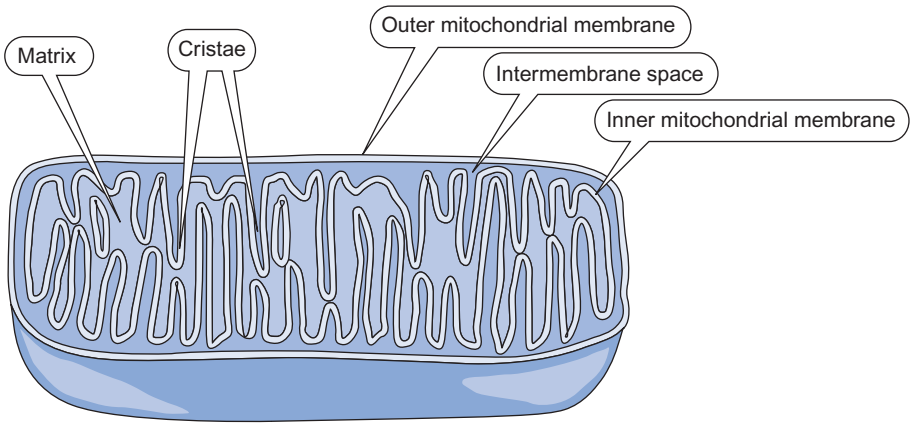

FIGURE 2.1.9 Typical features of a mitochondrion. The mitochondria, shown cut longitudinally, are membrane-delimited structures about 0.5–1.0 μm wide and 1–4 μm long. The **outer mitochondrial membrane** is permeable to many materials with molecular weights below 10 kDa. The **inner mitochondrial membrane** is impermeable to most materials but can transport specific materials. It is folded to form the shelf-like **cristae** and encloses the **matrix**. The intermembrane space lies between the inner and outer membranes.

PROTEASOMES DEGRADE MARKED PROTEINS

Proteasomes are large, multisubunit protein complexes that are scattered throughout the cytoplasm and that degrade cell proteins. Proteins are tagged for degradation by the attachment of a **ubiquitin** molecule to the proteins. Ubiquitin is a protein consisting of 76 amino acids. Proteins can be marked by more than one ubiquitin chain through a complex series of reactions involving several different enzymes. This process is called **ubiquitinylation**, in which a series of enzymes recognizes the proteins to be degraded and adds activated ubiquitin onto them. Different sets of enzymes in this pathway recognize different degradation signals on proteins. In this way, each set of enzymes targets distinct subsets of proteins that bear particular degradation signals. The process of ubiquitinylation is illustrated in Figure 2.1.10.

The proteasome consists of a central hollow cylinder capped at both ends. The central cylinder consists of a stack of four 7-membered rings. The caps recognize the ubiquitin coats of proteins that have been tagged for degradation. Each cap contains ATPase activity, releasing chemical energy in the process. These caps use the energy to unfold the ubiquitinylated proteins and move them into the central cylinder where proteases cleave the protein into its constituent amino acids. The structure of the proteasome is shown in Figure 2.1.11.

CELLS ATTACH TO EACH OTHER THROUGH A VARIETY OF JUNCTIONS

Cells form a variety of attachments to each other or to the extracellular matrix. These include:

- **Zonula occludens**: This "tight" junction joins epithelial cells in an occluding zone at one pole of the epithelium. It has three functions:
  1. a ***barrier function*** that disallows free movement of materials between cells in an epithelial sheet. This is the so-called "tight" part of tight junctions, but in reality the junctions vary considerably in their permeabilities.
  2. a ***fence function*** that prevents the free migration of membrane components from the apical surface

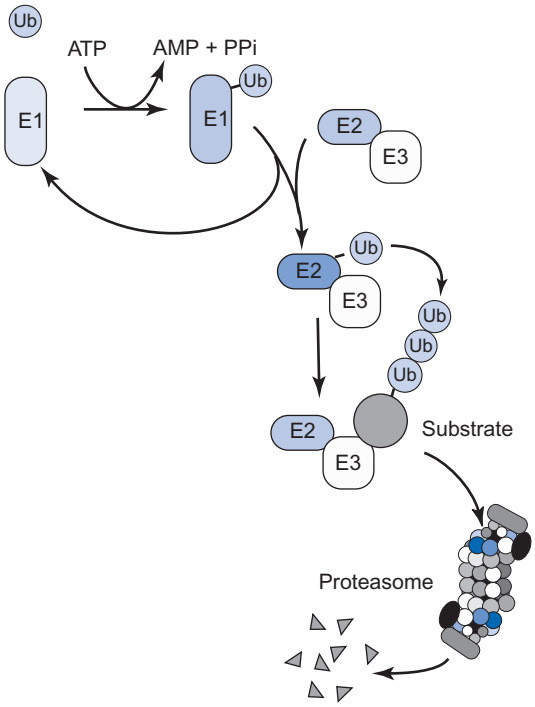

FIGURE 2.1.10 The ubiquitinylation reaction. Ubiquitin, noted as Ub in the figure, is a 76 amino acid protein that is used to "tag" proteins as being ready for degradation. First, Ub is attached to E1 through an ATP-requiring reaction. It is then passed from E1 to E2, and from there to the protein substrate being tagged for demolition. The ubiquitinylated protein is then degraded by a specialized cell structure called the proteasome.

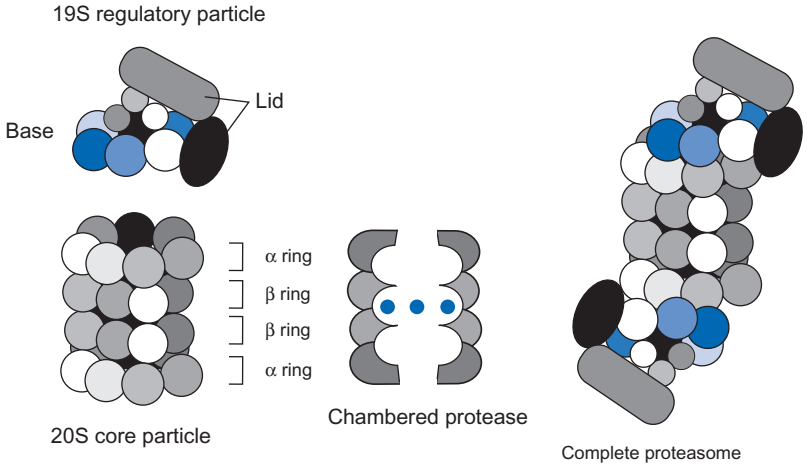

FIGURE 2.1.11 Structure of the proteasome. The overall structure consists of a 20S core particle of 28 subunits capped on one or both ends by a 19S regulatory particle containing at least 19 subunits. The core particle consists of four 7-membered rings that is symmetrical about a plane perpendicular to the long axis of the particle. Proteolytic activity resides in the two middle layers of the core particle.

of the cell to the lateral surface. This effectively partitions the cell membrane into components.

3. a ***signal function*** that help regulate cell proliferation, differentiation, and polarity.

- **Zonula adherens**: This is a belt of attachment that typically surrounds epithelial cells just below the zonula occludens—meaning toward the basolateral pole of the cell.
- **Desmosomes**: These are "spot welds" between cells and are constructed of different proteins than those that make up zonula adherens.
- **Gap junctions**: These junctions serve to electrically connect cells because they allow small ions to pass from one cell to another, and these ions carry electrical current.

Each of these junctions is made up of complexes of many proteins and a variety of proteins can associate with these. Figure 2.1.12 illustrates the structure of the desmosome, zonula adherens, zonula occludens, and gap junction, and Figure 2.1.13 shows their use in an epithelial sheet in the small intestine, consisting of a lining of cells that separate the ingested food and gastrointestinal secretions from the blood. Many of the proteins that make up these structures are present in the body in various **isoforms**—variants of the protein that serve the same basic function but in different tissues. For example, there are some 20 different types of connexins that associate as hexamers to form the connexons in gap junctions.

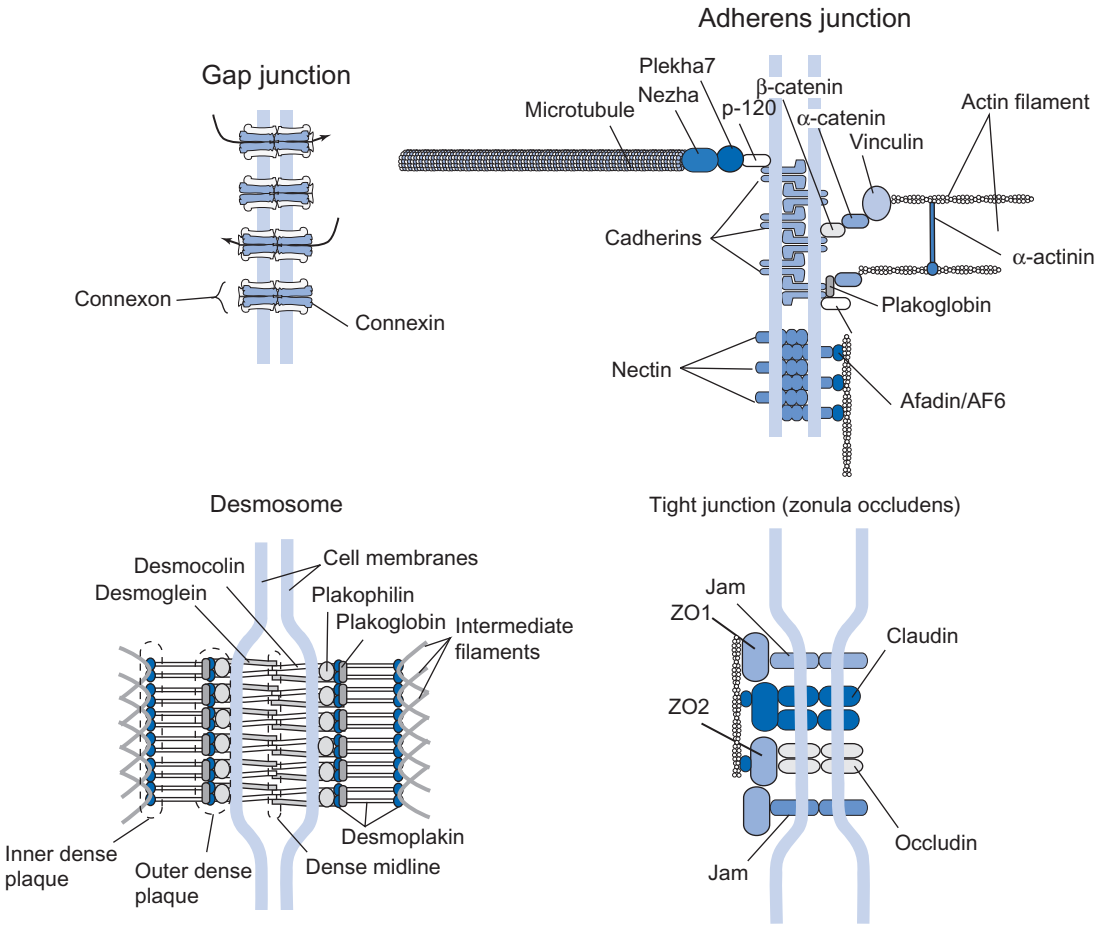

FIGURE 2.1.12 Schematic diagram of the proposed structures of gap junctions, adherens junctions, and desmosomes. Gap junctions form by linking of connexons on opposing membranes. Each connexon consists of a hexamer of connexin units that form a central pore. When two connexons link, the pore of one lines up with the pore of another, forming a watery path between the cells. This allows diffusion of small, soluble materials from one cell to another without crossing the cells' membranes. Adherens junctions form by interaction of extracellular parts of cadherin molecules. These proteins are embedded in the cell membrane and have a short cytoplasmic tail. This cytoplasmic tail binds to a variety of proteins including p-120, plakoglobin, and β-catenin. These in turn bind other proteins that eventually form initiation sites for actin polymerization. Cadherin also directly binds microtubules, although other proteins may stabilize this interaction. Desmosomes adhere two cells together because of the interaction of extracellular domains of desmoglein and desmocolin. These penetrate the membrane and their cytoplasmic domains bind other proteins, plakophilin, plakoglobin, and desmoplakin, that eventually connect to intermediate filaments. Tight junctions consist of binding of claudins and occludins, along with junctional adhesion molecule (JAM). There are multiple isoforms of each of these that produce junctions of varying permeability. These are stabilized by multiple cytoplasmic accessory proteins, including zonula occludens proteins (ZO-1, ZO-2, and ZO-3). These can connect to the cytoskeleton through other proteins such as afadin. In the junction between three cells, a special protein called tricellulin is required to seal the gap between the cells.

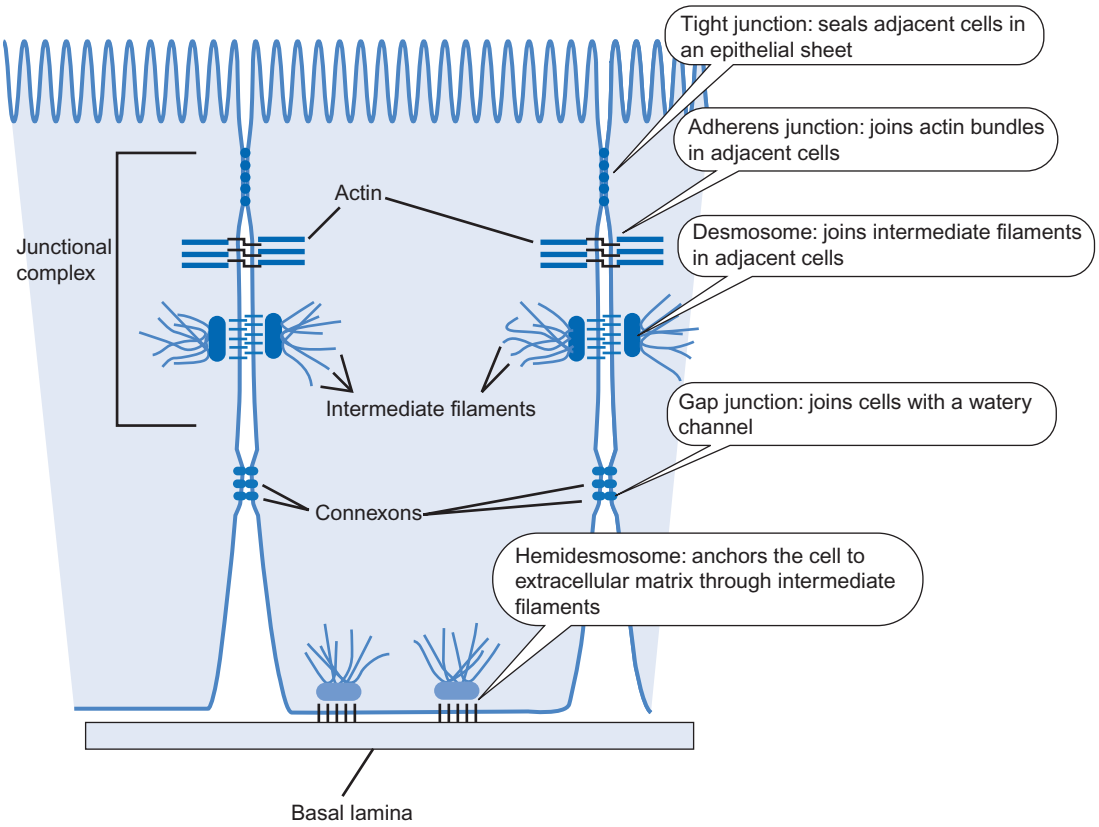

FIGURE 2.1.13 Cell attachments found in an epithelial sheet. The intestinal cells form a layer, referred to as an epithelium, that lines the intestine. The intestinal cells are bound at their apical pole, the side facing the gastrointestinal lumen, by a junctional complex consisting of tight junction, adherens junction, and desmosomes. The tight junction and adherens junction form a belt completely surrounding the cell. The desmosomes are located in spots. The cells are also joined by gap junctions that allow free passage of small molecular weight materials between the cells. The cells attach to the extracellular basal lamina through hemidesmosomes that connect the fibers in the extracellular matrix with the cytoskeletal intermediate filaments.

SUMMARY

The cell is the fundamental organizational unit of the body. Although cells come in many different forms, they share many features. Each cell consists of a number of organelles, so named because they contribute to overall cell function in much the same way as our organs contribute to bodily function.

The cell membrane determines the inside and outside of a cell. This is the "customs officer" of the cell, determining what enters or exits the cell. Materials move through the cytoplasm, the watery cell fluid that transports materials from one place to another, largely by diffusion. A cytoskeleton maintains cell shape and provides movement. Cytoskeletal elements include microtubules, which are hollow rods formed from 13 protomer filaments of a heterodimer of tubulin, actin filaments, composed of a double helix of actin monomers strung together, intermediate filaments of various descriptions, and myosin filaments. These cytoskeletal elements are dynamic and allow the cell to change shape and to transport materials along cytoskeletal tracks. The nucleus is the single largest organelle, and it is the "command post" of the cell. It is enclosed by a double membrane that is pierced by numerous nuclear pores. In response to signals, the nucleus "expresses" select regions of the genome. This means that the nucleus specifically converts some DNA into mRNA, but not all. The mRNA then makes proteins in the "factory" of the cell located on ribosomes either free in the cytoplasm or bound to the surface of the rough ER, another membranous network in the cytoplasm. The smooth ER makes lipids; the rough ER makes membrane-bound proteins and proteins destined for export from the cell. The rough ER transfers its protein content to the *cis* side of the Golgi apparatus, which is the "shipping and packaging" department of the cell. The materials move from one part of the Golgi apparatus to another in tiny membrane spheres called vesicles. At each stage, the proteins are processed further. The final vesicles leaving the *trans* face of the Golgi stack are ready for export from the cell by exocytosis.

All of these activities of the cell require chemical energy supplied as ATP. The mitochondria make ATP by coupling the chemical energy liberated by oxidation of foodstuffs to the synthesis of ATP. Thus the mitochondrion is the "powerhouse" of the cell. It consists of a double membrane structure. The electron transport chain is on the highly folded inner membrane. This membrane synthesizes ATP from chemical precursors and is the main site of oxygen consumption by the cell.

The cell also contains a variety of other organelles including the lysosome, peroxisome, and proteasome. These structures degrade material engulfed by the cell

by endocytosis and also degrade worn-out organelles and cell proteins.

Cells form a variety of attachments to other cells and to the extracellular matrix. These include zona occludens and adherens junctions, gap junctions, and desmosomes. These form by the complex association of a variety of different proteins: connexons on opposite membranes link up to form the gap junction; cadherins link up to form adherens junctions; desmoglein and desmocolin form the desmosomes; claudin and occludin link up in the tight junction. These junctions form by the association of extracellular parts of these transmembrane proteins. The intracellular parts join up with still other proteins that eventually connect the junctions with the cell's cytoskeleton.

REVIEW QUESTIONS

1. What is the plasma membrane? What compartments does it separate? What is endocytosis? Exocytosis? Pinocytosis? Phagocytosis?
2. What is the major cation (positively charged ion that migrates toward the cathode, the negative electrode) of the cytosol? Is the cytosol comparable to water?
3. What makes up the cytoskeleton? What is a microtubule? What is a microfilament? What is an intermediate filament? How do intermediate filaments differ from microtubules and microfilaments?
4. What does the plus (+) end of a microtubule or microfilament mean? What does the minus (−) end mean? What are "motor proteins"? What does it mean to be "+directed"? Name the family of +directed motor proteins. Name the family of −directed motor proteins. How do motor proteins carry cargo?
5. Describe the structure of a nuclear membrane. Why does the nuclear membrane have an elaborate pore structure? How big is the nucleus? What is the nucleolus?
6. What do ribosomes do? What are they made of? Where are they made? What do "40S" and "60S" descriptions of the major ribosomal subunits mean?
7. What distinguishes "rough ER" from "smooth ER"? What does each make?
8. What is the Golgi apparatus? What is the *cis* face? *Trans* face? What are all those vesicles doing hanging around the Golgi rims and *cis* and *trans* faces? What goes on inside the Golgi cisternae?
9. How big is a mitochondrion? Why do you suppose it has two membranes? Where is the electron transport chain? What do the mitochondria do?
10. What do lysosomes contain? What is the difference between a primary and a secondary lysosome? What goes on inside peroxisomes?
11. What do proteasomes do? How do they know which proteins to degrade?
12. Name four junctions between cells. Which ones involve connexons? Which ones join actin filaments from one cell to another? Which ones join intermediate filaments from one cell to another?

APPENDIX 2.1.A1 SOME METHODS FOR STUDYING CELL STRUCTURE AND FUNCTION

THE MICROSCOPE HAS REVOLUTIONIZED OUR UNDERSTANDING OF BIOLOGY

The invention of the microscope literally opened up a new world view in biology. For the first time, we could look upon the microscopic world and we discovered that it was full of animated objects. Robert Hooke (1635−1703) first used the microscope to study biological material in 1665 and coined the term "cell" from the likeness of the empty cells in cork to the monks' cells within a monastery. Antoine van Leeuwenhoek (1632−1723) was the first to observe live cells with hand-made microscopes that could magnify up to 500 times. He observed an astonishing array of cells from single-celled protists to red blood cells, bacteria, sperm, and muscle fibers. These observations, along with many others, prompted Theodor Schwann to enunciate the Cell Theory in 1839, based in part on conversations with Matthias Schleiden, whom Schwann did not credit. It contained three important elements:

1. The cell is the unit of structure, physiology, and organization in living things.
2. The cell is a distinct entity but is the building block of complicated organisms.
3. Cells form from cell-free material, as in crystallization.

We now know that this last point is incorrect. Its first correction was famously uttered by the German pathologist, Rudolf Virchow, when he said "Omnis cellula e cellula"—all cells come from cells. The entire development of the cell theory was supported by a single advance—the light microscope.

MICROSCOPIC RESOLUTION IS THE ABILITY TO DISTINGUISH BETWEEN TWO SEPARATED OBJECTS

What do we mean when we say that red blood cells, erythrocytes, are invisible to the naked eye? Surely we can see blood. What we mean here is that we cannot make out the individual cells. The ability to distinguish two objects separated by a distance is called the **resolution**.

Light produces a diffraction pattern around all objects. This pattern consists of areas of maximum and minimum intensity of light. The resolution of optical devices is determined by considering the diffraction pattern produced by a circular aperture of radius a. This circular aperture is called an **Airy disk**. The convention is that two apertures can be distinguished if the first maximum

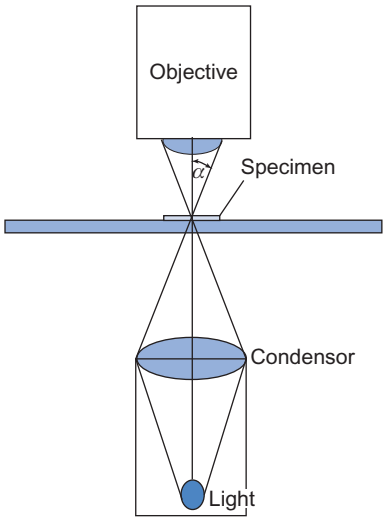

FIGURE 2.1.A1.1 Angle of the cone of light passing through the specimen and incident to the objective.

of the diffraction pattern from one pattern falls on the first minimum of the second. This is called the Rayleigh criterion. The diffraction pattern can be described analytically using Bessel functions, which we will not do here. The result is given by

$$\text{[2.1.A1.1]} \qquad \text{Resolution} = \frac{0.61\ \lambda}{\eta \sin \alpha}$$

where λ is the wavelength of light, η is the **refractive index** of the medium between the specimen and the lens, and α is the angle of the cone of light that passes between the specimen and the lens (see Figure 2.1.A1.1). The expression $\eta \sin \alpha$ is called the **numerical aperture** of the lens being used. This description of resolution is the inverse of what is ordinarily meant. Eqn [2.1.A1.1] defines resolution in terms of a distance. However, when we can resolve two objects that are close together, we ordinarily speak of a high resolution. According to Eqn [2.1.A1.1], the resolved distance is smallest (and the resolution is highest) when α is 90° and the refractive index is increased by placing oil between the specimen and lens. The refractive index of air is close to 1.0, whereas that of oil is 1.4–1.5.

THE ELECTRON MICROSCOPE HAS ADVANCED OUR UNDERSTANDING OF CELL STRUCTURE

Until the 1950s, the workhorse of cellular structure was the optical microscope. Our ideas of the structure and composition of cells were further enhanced by the electron microscope. This device allows tremendous magnification of cell components but suffers from the disadvantage that the cells must be fixed and stained before viewing their structures because the electron microscope views specimens in a vacuum. Otherwise, the incident electron beam would be scattered by air molecules. The electron microscope illuminates the structure of cells only after artificial preparation. Without functional studies, the activity going on in the structures remains elusive.

The electron microscope can achieve much higher resolution (it can resolve objects that are separated by a smaller distance) than the optical microscope because the wavelength of electrons is so much shorter than the wavelength of visible light. The fact that electrons have a wavelength at all was an astounding discovery. The first clue to the wavelength of electrons was the **Compton effect**, reported by Arthur H. Compton in 1922. Compton found that incident X-rays were scattered by a carbon target, subsequently shifting the X-rays to a lower wavelength and dislodging an electron from the crystal. The dislodged electron has a momentum. To describe this collision of light with electrons, it was necessary to postulate that the incident light possessed momentum, in order to preserve the conservation of momentum theorem of physics. Compton produced a quantum-mechanical analysis of the scattering that differed from classical explanations, but agreed with the experimental observations.

The photoelectric effect and Compton effects showed that light had distinctive particle-like aspects. It was already known, from interference and diffraction experiments, that light also had distinctive wave-like aspects. Louis de Broglie in 1924 postulated that the wave–particle duality of photons might also apply to particles; if photons have momentum, particles such as the electrons ought to have a wavelength. From the Compton experiments, the wavelength would be given by

$$\text{[2.1.A1.2]} \qquad \lambda = \frac{h}{mv}$$

where h is Planck's constant $= 6.625 \times 10^{-34}$ J s. The wave nature of electrons was confirmed in 1927 by Davisson and Germer, who exposed a single crystal of nickel to electrons having 54 electron volts of kinetic energy. They observed an electron diffraction pattern that confirmed de Broglie's relation.

The short wavelength of electrons opened the possibility of a microscope with astounding resolution. The electron microscope, invented in the 1930s, was first applied to living tissues by Albert Claude, Keith Porter, and George Palade in the late 1940s and 1950s.

The resolution of the electron microscope is determined not only by the wavelength of the incident radiation, which in this case is a beam of electrons, but also by the numerical aperture. Although theoretically the resolution of the electron microscope should be close to 0.002 nm, in principle it is much larger than this because the inherent properties of magnetic lenses limit the aperture angle to about 0.5°.

Because biological specimens lack inherent contrast, the practical resolution is further reduced to about 1–2 nm. Nevertheless, this is a marked improvement over the optical microscope. The optical microscope was useful to a magnification of about 1000×; the electron microscope could attain more than a 100-fold better magnification.

SUBCELLULAR FRACTIONATION ALLOWS STUDIES OF ISOLATED ORGANELLE BUT REQUIRES DISRUPTION OF CELL FUNCTION AND STRUCTURE

Although the optical microscope and electron microscope provided keen insights into the structure of living things, the function of the structures could not be directly investigated. In the late 1940s and 1950s, Albert Claude and Christian de Duve developed methods for **subcellular fractionation** for separating cells into their component parts and elucidating the function of the cellular constituents. This method disrupted the cells and separated the parts by **differential centrifugation**.

DIFFERENTIAL CENTRIFUGATION PRODUCES ENRICHED FRACTIONS OF SUBCELLULAR ORGANELLES

The first step in subcellular fractionation is the disruption of the cell into its component subcellular **organelles**. This process usually uses homogenization, and its aim is to break the plasma membrane that delimits the cell, thereby releasing the cellular contents, without damaging those contents. Cells can be homogenized by sonication (exposure to high-frequency sound waves), shearing the cells between two surfaces such as a Teflon mortar and glass pestle, or placing the cells in a high-speed blender. These treatments break the cell membrane and leave the remaining parts of the cells relatively intact. These relatively harsh treatments obviously disrupt some of the normal relationships between parts of the cell, and may scramble normal constituents of cells.

The resulting mixture, the homogenate, can be separated into its component parts on the basis of their size and density (see Figures 2.1.A1.2 and 2.1.A1.3). Following lysis of the cells, the homogenate is placed in a tube in a centrifuge and spun at relatively low speed (1000 × gravity) for a short time (10–20 minutes). The centrifugation causes materials to move away from the axis of centrifugation. When the particles reach the bottom of the tube, they form a pellet. This process is called **sedimentation**. Particles that sediment quickly reach a terminal velocity in the centrifuge tube. At this terminal velocity, the frictional drag on the particle provides the acceleration necessary to keep the particle in approximate uniform circular motion (see below for an explanation of the forces during sedimentation). The frictional drag depends on the density of the particle, viscosity of the medium, and speed of centrifugation. The first centrifugation, at low speed and short times, sediments unbroken cells, and the nuclei because these

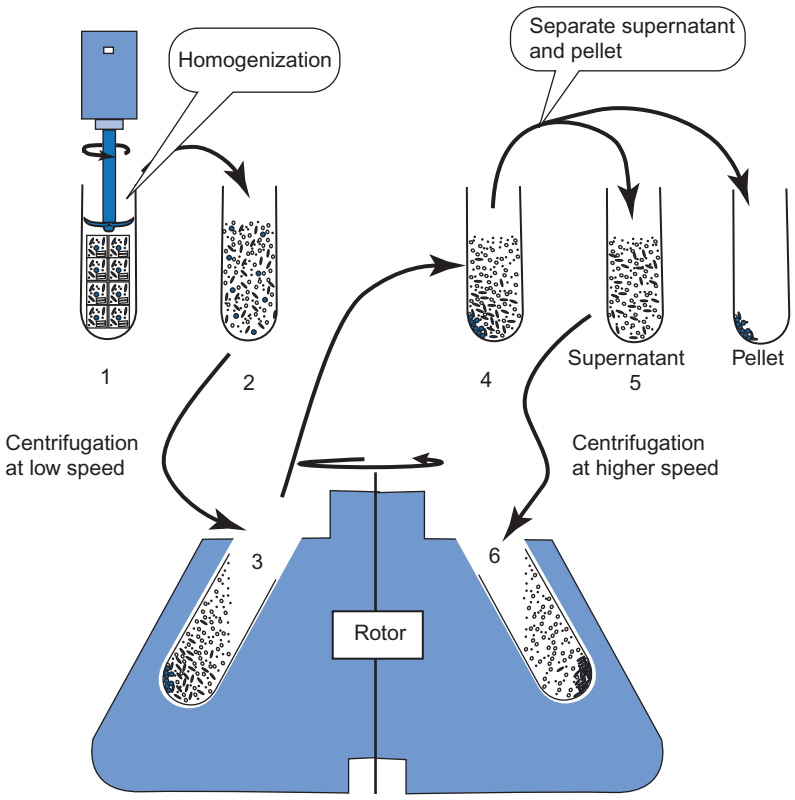

FIGURE 2.1.A1.2 Separation of subcellular organelles by differential centrifugation. Whole tissue is first homogenized, which disrupts cell membranes and releases subcellular organelles. The homogenate is then centrifuged to separate out particles on the basis of their sedimentation. In general, large particles sediment with small centrifugal forces and smaller particles require larger forces. Successive centrifugation at progressively faster revolutions per minute (RPM) separates organelles on the basis of their sedimentation characteristics.

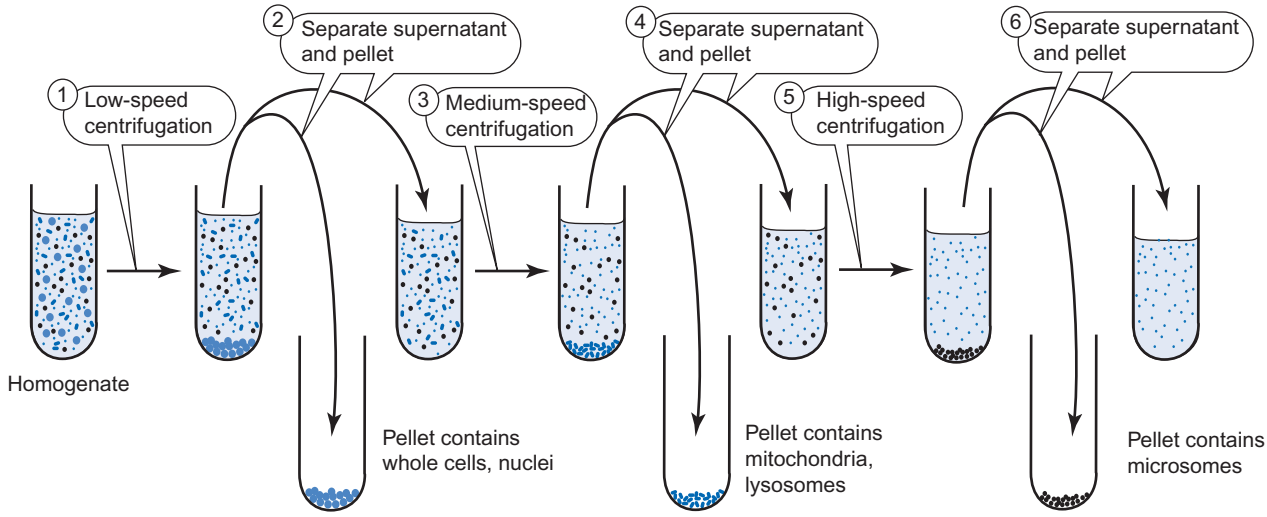

FIGURE 2.1.A1.3 Differential centrifugation. The first slow speed spin causes large and heavy particles to sediment. These are separated from the particles that do not sediment. Successively faster centrifugations cause progressively smaller and lighter particles to sediment.

are the largest and heaviest of subcellular structures. Therefore, a fraction of the homogenate is produced that is enriched in nuclei. The supernatant fraction, the suspension that lies above the first pellet, is cleared of nuclei but still contains other subcellular particles.

Successive centrifugations at higher speeds and longer times produce fractions enriched in the **mitochondria**, **lysosomes**, and **peroxisomes**. Further centrifugation at still higher speeds sediments the broken fragments of the **plasma membrane** and **endoplasmic reticulum**. De Duve identified these subcellular fractions through the use of **marker enzymes**. The basic idea here is that each of the subcellular structures has a unique biochemical composition that enables their unique biochemical function within the cell. Part of their biochemical composition is their component enzymes. By measuring the distribution of an enzyme, the scientist can track the distribution of the particles that contain it. Markers for the mitochondria, for example, include cytochrome C oxidase and succinate dehydrogenase, among others. By measuring cytochrome C oxidase in the various fractions, one can estimate the amount of mitochondria in those fractions. De Duve deduced the existence of the lysosome on the basis of an enzyme that distributed itself differently from all other known markers.

DENSITY GRADIENT CENTRIFUGATION ENHANCES PURITY OF THE FRACTIONS

The centrifuge is a crude instrument for the separation of subcellular fractions because of the way in which it separates the different subcellular organelles. Differential centrifugation can produce fractions enriched in one particle or another, but the fractions are not pure. This is due to the fact that sedimentation occurs over the considerable length of the centrifuge tube. When the tube is spun, particles throughout the tube are subject to centrifugal forces that cause them to sediment. Although heavier particles sediment more quickly, the heavier particles at the top of the tube have much further to travel than those near the bottom. By the time all of the heavy particles sediment, some of the lighter particles near the bottom of the tube, or at the middle, also sediment. Therefore, pure fractions of subcellular particles cannot be achieved easily by simple differential centrifugation. Further purification can be achieved by using **density gradient centrifugation**. In this method, subcellular organelles are separated by centrifugation through a gradient of a dense substance, such as sucrose. In **velocity centrifugation**, the material to be separated is layered on top of a sucrose gradient, and then centrifuged. Particles of different sizes and density sediment through the gradient at different rates moving as discrete bands. At the end of the centrifugation, the different layers consist of purified organelles, and they can be collected for further experiments. In **equilibrium centrifugation**, the density gradient is used to separate particles based on their buoyant density. Instead of being separated by their sedimentation velocity, particles will sediment until they reach a layer with the same density as the particles. At this point, sedimentation stops and the purified organelles can be collected at the equilibrium position. These methods of separating subcellular organelles are illustrated in Figure 2.1.A1.4.

ANALYSIS OF CENTRIFUGATION SEPARATION

CIRCULAR MOTION REQUIRES AN INWARD CENTRIPETAL FORCE

Centrifugation typically involves spinning tubes of material at a constant angular velocity, except for the angular acceleration to that velocity, and the deceleration when the spin stops. The position of any particle in the tube at any time may be represented by the vector **r**, as shown in Figure 2.1.A1.5.

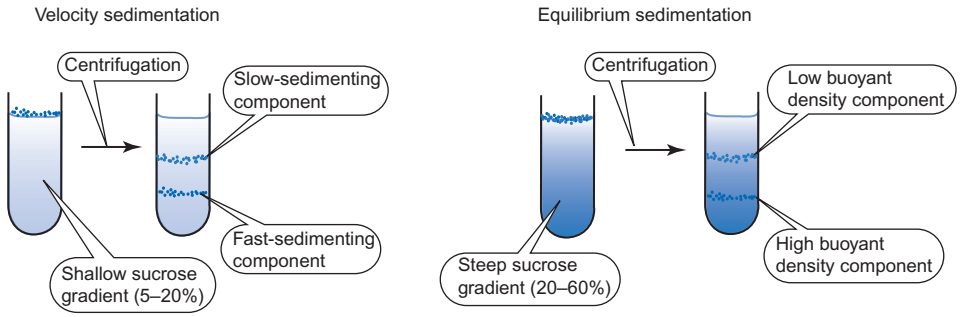

FIGURE 2.1.A1.4 Comparison of velocity sedimentation and equilibrium sedimentation for the separation of subcellular particles. In velocity sedimentation, separation relies on different velocity of sedimentation through a shallow sucrose (or other material) gradient. The sucrose is added to prevent mixing by convection. In equilibrium sedimentation, subcellular particles sediment until they reach a zone of solution that matches their density. At equilibrium, the organelles distribute themselves over a narrow region of the tube corresponding to the density of the organelle. The organelles can then be harvested from a narrow band.

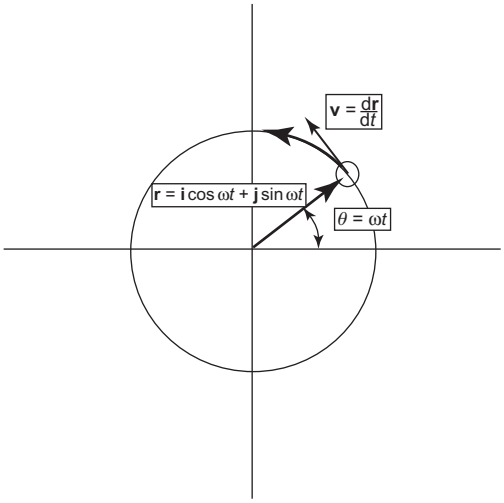

FIGURE 2.1.A1.5 A particle in uniform circular motion around a central pivot point. The angular displacement is a linear function of time.

This vector makes the angle θ from an arbitrary zero reference, the x-axis, and this angle increases with time. The angular velocity is defined as

$$\omega = \frac{d\theta}{dt} \quad [2.1.A1.3]$$

By integrating this we see easily that:

$$\theta = \omega t \quad [2.1.A1.4]$$

The position vector indicating the location of the point relative to the center of rotation is thus given as

$$\vec{r} = \vec{i}\ \cos \omega t + \vec{j}\ \sin \omega t \quad [2.1.A1.5]$$

where **r** is the position vector and **i** and **j** are unit vectors along the x-axis and y-axis, respectively. The velocity vector at any time is the derivative of this position vector:

$$\vec{V} = d\frac{\vec{r}}{dt} = -\vec{i}\ \omega \sin \omega t + \vec{j}\ \omega \cos \omega t \quad [2.1.A1.6]$$

This velocity vector is orthogonal to the position vector as seen by the dot product: it is given as

$$\vec{r} \cdot \vec{V} = -\omega \sin \omega t \cos \omega t + \omega \sin \omega t \cos \omega t = 0 \quad [2.1.A1.7]$$

This means that the velocity vector is oriented at 90° to the position vector, as shown in Figure 2.1.A1.5. The acceleration at any time is the derivative of the velocity:

$$\vec{a} = d\frac{\mathbf{V}}{dt} = -\vec{i}\ \omega^2 \cos \omega t - \vec{j}\ \omega^2 \sin \omega t$$
$$\vec{a} = -\omega^2 \vec{r} \quad [2.1.A1.8]$$

We see here that the magnitude of the acceleration is $\omega^2\mathbf{r}$ and its direction is directly opposite that of the position vector (the negative sign in Eqn [2.1.A1.8]). (Compare Eqn [2.1.A1.8] to [2.1.A1.5] for the identity of **r** in **a**.) This is the centripetal acceleration. In order for the particle to remain in uniform circular motion, the velocity must be continuously bent toward the center. The acceleration vector is orthogonal to the velocity vector, so that all of the acceleration is used to change the direction of the velocity, and not its magnitude.

CENTRIPETAL FORCE IN A SPINNING TUBE IS PROVIDED BY THE SOLVENT

As described above, particles that are spinning in a rotor at a constant angular velocity, ω, are subjected to a centripetal acceleration given by

$$\mathbf{a} = \omega^2 r \quad [2.1.A1.9]$$

where **a** is the acceleration, r is the radius, and ω is the angular velocity, in radians per second ($=2\pi \times$ revolutions per second). This **centripetal acceleration** is the acceleration necessary to keep a particle rotating about the axis, at a distance r. If the actual force is less than this, the particle will move away from the axis of rotation. In the centrifuge, the centripetal acceleration is provided by collisions with solvent particles. The net force of these collisions under ordinary conditions (i.e., not in the centrifuge) adds to the force of gravity on the particle. In the centrifuge, the centripetal force necessary to keep the particle rotating at angular velocity ω at distance r from the axis is

$$F_c = (m_{\text{particle}} - m_{\text{solution}})\omega^2 r \quad [2.1.A1.10]$$

Here the mass of solution is the mass of the volume of solution which is displaced by the particle and is

the origin of the buoyant force. Eqn [2.1.A1.10] can be rewritten as

$$[2.1.A1.11] \qquad \begin{aligned} F_c &= (m - V_p\rho)\omega^2 r \\ &= (m - m\bar{v}\rho)\omega^2 r \\ &= m(1 - \bar{v}\rho)\omega^2 r \end{aligned}$$

where m is the mass of the particle, $\overline{V}$ is the partial specific volume, equal to $1/\rho$ for the particle, and ρ is the density of the solution.

As pointed out above, this centripetal force is the force required to maintain an orbit at angular velocity ω at a distance r from the axis of revolution. The source of this force is the collisions with solvent molecules, which have a net direction toward the axis of revolution only when there is a net velocity of the particle in the opposite direction. That is, a particle more dense than the solution will move toward the bottom of the tube (away from the axis of revolution) with some velocity relative to the tube. Because it is moving through the solution, it experiences a drag force that is proportional to the velocity. This drag force on a particle moving outward from the axis of revolution is given by

$$[2.1.A1.12] \qquad F_D = -\beta V$$

where β is a frictional coefficient. This equation says that the drag force is proportional to the velocity but opposite in direction. A terminal velocity, v, is reached when there is a balance between F_c and F_D. From Eqns [2.1.A1.11] and [2.1.A1.12], this is

$$[2.1.A1.13] \qquad m(1 - \bar{v}\rho)\omega^2 r = \beta v$$

THE MAGNITUDE OF THE CENTRIPETAL FORCE CAN BE EXPRESSED AS RELATIVE CENTRIFUGAL FORCE

The frame of reference for the analysis presented so far is the nonrotating frame of the laboratory. One can also view the situation from the accelerated, rotating frame of reference of the solution within the rotor. In this case, there is an apparent force on every particle, the centrifugal force, which is equal but opposite to the centripetal force, which appears to drive particles heavier than the solution to the bottom of the tube. This motion within the tube is opposed by the frictional force, given above, which is opposite to the direction of motion. The terminal velocity is reached when the centrifugal force is equal to the frictional force, as given by Eqn [2.1.A1.13]. Note that the centrifugal force is a fictional force which must be invented in order to apply Newton's laws in a uniformly accelerated frame of reference. The centrifugal force is just that force given by Eqns [2.1.A1.10] and [2.1.A1.11]. Usually the centrifugal force is given in multiples of g, the acceleration due to gravity:

$$[2.1.A1.14] \qquad a = \frac{\omega^2 r}{g} g$$

The relative centrifugal field (RCF) is given as

$$[2.1.A1.15] \qquad \text{RCF} = \frac{\omega^2 r}{g}$$

where $g = 981$ cm s^{-2} or 9.81 m s^{-2}.

THE VELOCITY OF SEDIMENTATION IS MEASURED IN SVEDBERGS OR S UNITS

The terminal velocity within the tube can be written as

$$[2.1.A1.16] \qquad V = \frac{dr}{dt}$$

where r is the distance from a sedimenting particle and the center of rotation. Insertion of this definition into Eqn [2.1.A1.13] and rearranging, we obtain:

$$[2.1.A1.17] \qquad m(1 - \bar{v}\rho) = \beta \left[\frac{\frac{dr}{dt}}{\omega^2 r} \right]$$

The term in the brackets on the right-hand side of the equation defines the **sedimentation coefficient**, **s**. **The sedimentation coefficient is the rate of sedimentation per unit of centrifugal force**. Sedimentation coefficients are usually of the order of 10^{-13} s. They are reported in **Svedberg** units, where 1 **Svedberg** $= 10^{-13}$ s. This unit is named after T. Svedberg, an early pioneer in the design of centrifuges and their use in investigations of biological material.

The sedimentation coefficient is obtained experimentally by plotting the logarithm of the radius of the maximum of the concentration profile against the time. To see this, consider again the definition of the sedimentation coefficient:

$$[2.1.A1.18] \qquad s = \frac{\frac{dt}{dr}}{\omega^2 r}$$

multiplying through by ω^2.

$$[2.1.A1.19] \qquad \begin{aligned} \omega^2 s &= \frac{\frac{dr}{dt}}{r} \\ &= \frac{d \ln r}{dt} \end{aligned}$$

Thus the slope of a plot of $\ln r$ against t will give $\omega^2 s$.

The sedimentation coefficient varies with the concentration of solute, usually decreasing as the total protein concentration increases. The sedimentation coefficient at infinite dilution, s^0, is usually obtained by extrapolation of plots of $1/s$ against protein concentration to zero protein concentration.

Sedimentation information can sometimes inform us about molecular dimensions. Eqn [2.1.A1.17] can be rearranged to give

$$[2.1.A1.20] \qquad \frac{m(1 - \bar{v}\rho)}{s} = \beta$$

where β is the frictional coefficient described in Eqn [1.2.A1.12]. The **Stokes equation** gives the frictional coefficient as

[2.1.A1.21] $$\beta = 6\pi\eta a_s$$

where η is the viscosity of the solution and a_s is the radius of the molecule, assuming spherical geometry. Thus the measurement of s and the knowledge of the molecular mass, m, and its partial molar volume allow estimate of its size.

DENSITY GRADIENT CENTRIFUGATION

Macromolecules or subcellular organelles can be sedimented through gradients of increasing density on a preparative scale to purify them. There are a variety of materials which can be used to prepare such density gradients, including CsCl, D_2O, Ficoll, glycerol, sorbitol, sucrose, and percoll. The gradients usually are formed by mixing two limiting solutions in varying proportions in order to produce the desired gradient. Gradients can be discontinuous, in which solutions of varying densities are layered on top of each other manually, or continuous. Continuous gradients can have a variety of shapes, although linear gradients are most common. Materials will sediment until they reach a solution with the same density as the particles, and then they stop sedimenting.

OTHER OPTICAL METHODS

A number of other optical approaches have proven useful for the modern investigation of cell function. These include **phase-contrast microscopy** and **differential interference microscopy**. Both of these optical techniques use variation in the refractive index of cell structures, rather than variation in light absorption, to produce contrast between the structures. These images can be clarified by using video cameras and computerized image analysis and processing. Other techniques include **fluorescence microscopy** and **confocal microscopy**. The main advantage in these techniques is that intact and living cells can be investigated.

In fluorescence microscopy, specific components of the cells can be labeled by attaching a **fluorophore** to them. The fluorophore is a fluorescent molecule. It absorbs light at one wavelength and emits it at a second, lower wavelength. The location of the fluorescent molecule is achieved by illuminating the specimen at the excitation wavelength and using filters to collect the emitted light. For example, incubating cells with a fluorophore-tagged antibody directed against a specific protein allows the study of the distribution of the protein. This technique can be used with either living or fixed cells. In other experiments, native proteins expressed by cells can be "tagged" with a fluorescent protein, green fluorescent protein (GFP by incorporating the gene for GFP into the gene for the protein. Thus the location of the tagged protein can be followed by fluorescent microscopy.

Confocal microscopy allows for optical sectioning of live cells. Confocal microscopy refers to the idea that both incident and emitted light are in focus. In a bright field, light illuminates the specimen and the objective lens is moved to focus the light. However, out-of-focus light still reaches the detector. In confocal microscopy, an aperture is placed in front of the detector so that out-of-focus light is eliminated. The result is that the image is formed from a narrow plane of in-focus light. By moving the plane up and down, one can obtain a series of optical sections. By computer techniques, three-dimensional reconstructions of the object can be obtained. Because the image is obtained and stored digitally, the resulting image can be viewed from any perspective. Confocal microscopy can be combined with fluorescent probes to achieve outstanding detail of localization.