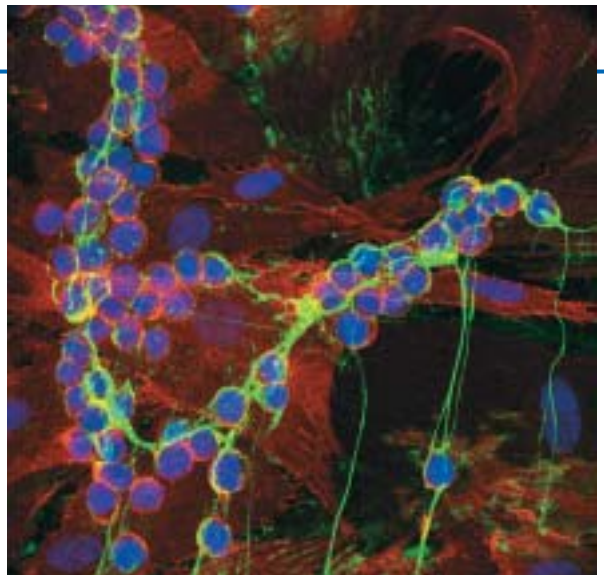
6

INTEGRATING CELLS INTO TISSUES



Model of inflammatory bowel disease in which cultured flat colonic smooth muscle cells were induced to secrete cables of hyaluronan (green) that bind to spheroidal mononuclear leukocytes via their CD44 receptors (red). Nuclei are stained blue. [Courtesy of C. de la Motte et al., Lerner Research Institute.]

In the development of complex multicellular organisms such as plants and animals, progenitor cells differentiate into distinct "types" that have characteristic compositions, structures, and functions. Cells of a given type often aggregate into a *tissue* to cooperatively perform a common function: muscle contracts; nervous tissues conduct electrical impulses; xylem tissue in plants transports water. Different tissues can be organized into an *organ,* again to perform one or more specific functions. For instance, the muscles, valves, and blood vessels of a heart work together to pump blood through the body. The coordinated functioning of many types of cells within tissues, as well as of multiple specialized tissues, permits the organism as a whole to move, metabolize, reproduce, and carry out other essential activities.

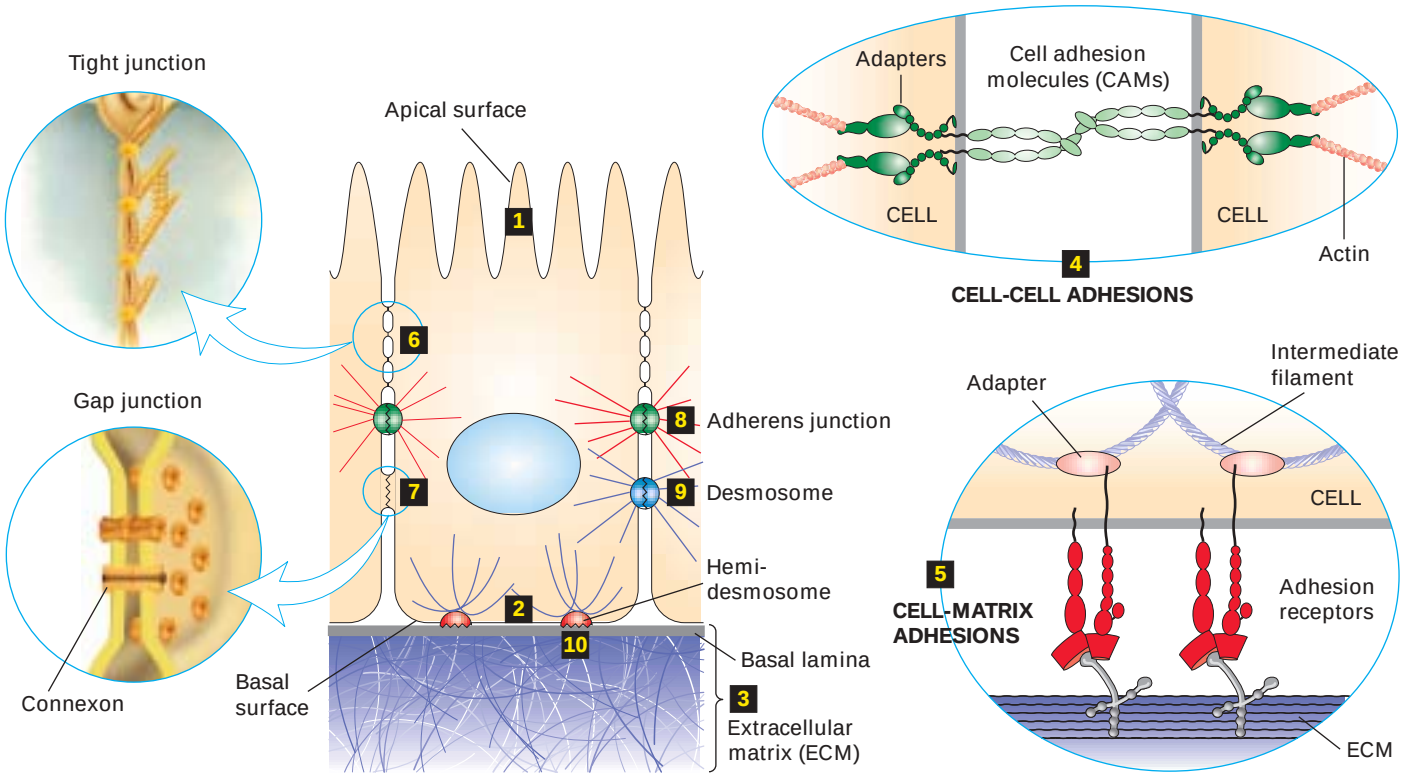
The adult form of the roundworm *Caenorhabditis elegans* contains a mere 959 cells, yet these cells fall into 12 different general cell types and many distinct subtypes. Vertebrates have hundreds of different cell types, including leukocytes (white blood cells), erythrocytes, and macrophages in the blood; photoreceptors in the retina; adipocytes that store fat; secretory α and β cells in the pancreas; fibroblasts in connective tissue; and hundreds of different subtypes of neurons in the human brain. Despite their diverse forms and functions, all animal cells can be classified as being components of just five main classes of tissue: *epithelial tissue, connective tissue, muscular tissue, nervous tissue,* and *blood.* Various cell types are arranged in precise patterns of staggering complexity to generate the different tissues and organs. The costs of such complexity include increased requirements for information, material, energy, and time during the development of an individual organism. Although the physiological costs of complex tissues and organs are high, they provide organisms with the ability to thrive in varied and variable environments, a major evolutionary advantage.

The complex and diverse morphologies of plants and animals are examples of the whole being greater than the sum of the individual parts, more technically described as the emergent properties of a complex system. For example, the root-stem-leaf organization of plants permits them to simultaneously obtain energy (sunlight) and carbon (CO_2) from

OUTLINE

the atmosphere and water and nutrients (e.g., minerals) from the soil. The distinct mechanical properties of rigid bones, flexible joints, and contracting muscles permit vertebrates to move efficiently and achieve substantial size. Sheets of tightly attached epithelial cells can act as regulatable, selective permeability barriers, which permit the generation of chemically and functionally distinct compartments in an organism (e.g., stomach, bloodstream). As a result, distinct and sometimes opposite functions (e.g., digestion and synthesis) can efficiently proceed simultaneously within an organism. Such compartmentalization also permits more sophisticated regulation of diverse biological functions. In many ways, the roles of complex tissues and organs in an organism are analogous to those of organelles and membranes in individual cells.

The assembly of distinct tissues and their organization into organs are determined by molecular interactions at the cellular level and would not be possible without the temporally and spatially regulated expression of a wide array of adhesive molecules. Cells in tissues can adhere directly to one another (*cell–cell adhesion*) through specialized integral membrane proteins called **cell-adhesion molecules (CAMs)** that often cluster into specialized cell junctions (Figure 6-1). Cells in animal tissues also adhere indirectly (*cell–matrix*



▲ FIGURE 6-1 **Schematic overview of major adhesive interactions that bind cells to each other and to the extracellular matrix.** Schematic cutaway drawing of a typical epithelial tissue, such as the intestines. The apical (upper) surface of these cells is packed with fingerlike microvilli 1 that project into the intestinal lumen, and the basal (bottom) surface 2 rests on extracellular matrix (ECM). The ECM associated with epithelial cells is usually organized into various interconnected layers (e.g., the basal lamina, connecting fibers, connective tissue), in which large, interdigitating ECM macromolecules bind to one another and to the cells 3. Cell-adhesion molecules (CAMs) bind to CAMs on other cells, mediating cell–cell adhesions 4, and adhesion receptors bind to various components of the ECM, mediating cell–matrix adhesions 5. Both types of cell-surface adhesion molecules are usually integral membrane proteins whose cytosolic domains often bind to multiple intracellular adapter proteins. These adapters, directly or indirectly, link the CAM to the cytoskeleton (actin or intermediate filaments) and to intracellular signaling pathways. As a consequence, information can be transferred by CAMs and the macromolecules to which they bind from the cell exterior into the intracellular environment, and vice versa. In some cases, a complex aggregate of CAMs, adapters, and associated proteins is assembled. Specific localized aggregates of CAMs or adhesion receptors form various types of cell junctions that play important roles in holding tissues together and facilitating communication between cells and their environment. Tight junctions 6, lying just under the microvilli, prevent the diffusion of many substances through the extracellular spaces between the cells. Gap junctions 7 allow the movement through connexon channels of small molecules and ions between the cytosols of adjacent cells. The remaining three types of junctions, adherens junctions 8, spot desmosomes 9, and hemidesmosomes 10, link the cytoskeleton of a cell to other cells or the ECM. [See V. Vasioukhin and E. Fuchs, 2001, *Curr. Opin. Cell Biol.* **13**:76.]

adhesion) through the binding of **adhesion receptors** in the plasma membrane to components of the surrounding **extracellular matrix (ECM),** a complex interdigitating meshwork of proteins and polysaccharides secreted by cells into the spaces between them. These two basic types of interactions not only allow cells to aggregate into distinct tissues but also provide a means for the bidirectional transfer of information between the exterior and the interior of cells.

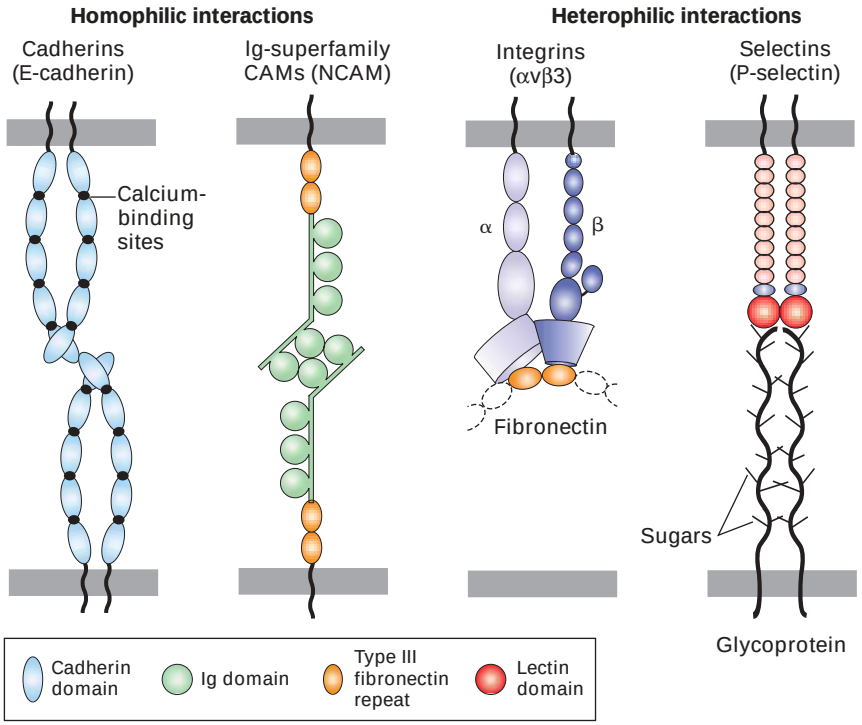
In this chapter, we examine the various types of adhesive molecules and how they interact. The evolution of plants and animals is thought to have diverged before multicellular organisms arose. Thus multicellularity and the molecular means for assembling tissues and organs must have arisen independently in animal and plant lineages. Not surprisingly, then, animals and plants exhibit many differences in the organization and development of tissues. For this reason, we first consider the organization of epithelial and nonepithelial tissues in animals and then deal separately with plant tissues. Although most cells in living organisms exist within tissues, our understanding about cells depends greatly on the study of isolated cells. Hence, we present some general features of working with populations of cells removed from tissues and organisms in the last section of this chapter.

6.1 Cell–Cell and Cell–Matrix Adhesion: An Overview

We begin with a brief orientation to the various types of adhesive molecules, their major functions in organisms, and their evolutionary origin. In subsequent sections, we examine in detail the unique structures and properties of the various participants in cell–cell and cell–matrix interactions in animals.

Cell-Adhesion Molecules Bind to One Another and to Intracellular Proteins

A large number of CAMs fall into four major families: the **cadherins, immunoglobulin (Ig) superfamily, integrins,** and **selectins.** As the schematic structures in Figure 6-2 illustrate, many CAMs are mosaics of multiple distinct domains, many



▲ FIGURE 6-2 **Major families of cell-adhesion molecules (CAMs) and adhesion receptors.** Dimeric E-cadherins most commonly form homophilic (self) cross-bridges with E-cadherins on adjacent cells. Members of the immunoglobulin (Ig) superfamily of CAMs can form both homophilic linkages (shown here) and heterophilic (nonself) linkages. Selectins, shown as dimers, contain a carbohydrate-binding lectin domain that recognizes specialized sugar structures on glycoproteins (shown here) and glycolipids on adjacent cells. Heterodimeric integrins (for example, αv and β3 chains) function as CAMs or as adhesion receptors (shown here) that bind to very large, multiadhesive matrix proteins such as fibronectin, only a small part of which is shown here (see also Figure 6-25). Note that CAMs often form higher-order oligomers within the plane of the plasma membrane. Many adhesive molecules contain multiple distinct domains, some of which are found in more than one kind of CAM. The cytoplasmic domains of these proteins are often associated with adapter proteins that link them to the cytoskeleton or to signaling pathways. [See R. O. Hynes, 1999, *Trends Cell Biol.* **9**(12):M33, and R. O. Hynes, 2002, *Cell* **110**:673–687.]

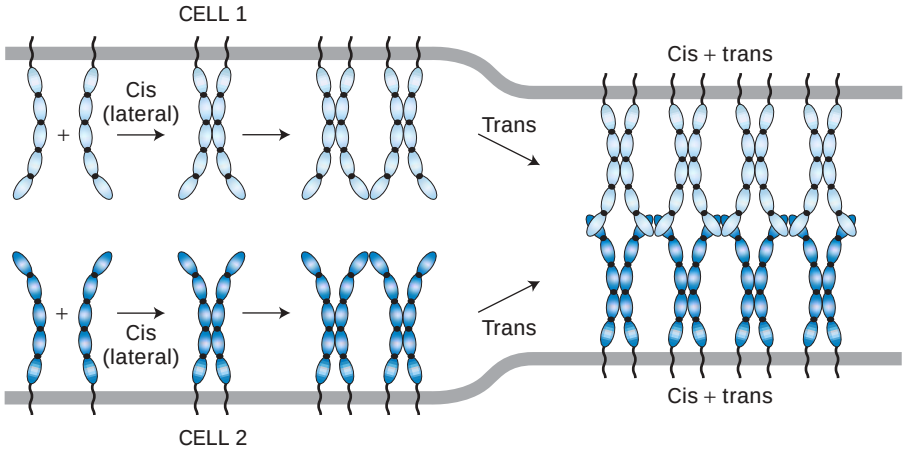
of which can be found in more than one kind of CAM. They are called "repeats" when they exist multiple times in the same molecule. Some of these domains confer the binding specificity that characterizes a particular protein. Some other membrane proteins, whose structures do not belong to any of the major classes of CAMs, also participate in cell–cell adhesion in various tissues.

CAMs mediate, through their extracellular domains, adhesive interactions between cells of the same type (*homotypic* adhesion) or between cells of different types (*heterotypic* adhesion). A CAM on one cell can directly bind to the same kind of CAM on an adjacent cell (*homophilic* binding) or to a different class of CAM (*heterophilic* binding). CAMs can be broadly distributed along the regions of plasma membranes that contact other cells or clustered in discrete patches or spots called **cell junctions.** Cell–cell adhesions can be tight and long lasting or relatively weak and transient. The associations between nerve cells in the spinal cord or the metabolic cells in the liver exhibit tight adhesion. In contrast, immune-system cells in the blood can exhibit only weak, short-lasting interactions, allowing them to roll along and pass through a blood vessel wall on their way to fight an infection within a tissue.

The cytosol-facing domains of CAMs recruit sets of multifunctional adapter proteins (see Figure 6-1). These adapters act as linkers that directly or indirectly connect CAMs to elements of the cytoskeleton (Chapter 5); they can also recruit intracellular molecules that function in signaling pathways to control protein activity and gene expression (Chapters 13 and 14). In some cases, a complex aggregate of CAMs, adapter proteins, and other associated proteins is assembled at the inner surface of the plasma membrane. Because cell–cell adhesions are intrinsically associated with the cytoskeleton and signaling pathways, a cell's surroundings influence its shape and functional properties ("outside-in" effects); likewise, cellular shape and function influence a cell's surroundings ("inside-out" effects). Thus *connectivity* and *communication* are intimately related properties of cells in tissues.

The formation of many cell–cell adhesions entails two types of molecular interactions (Figure 6-3). First, CAMs on one cell associate laterally through their extracellular domains or cytosolic domains or both into homodimers or higher-order oligomers in the plane of the cell's plasma membrane; these interactions are called intracellular, lateral, or cis interactions. Second, CAM oligomers on one cell bind to the same or different CAMs on an adjacent cell; these interactions are called intercellular or trans interactions. Trans interactions sometimes induce additional cis interactions and, as a consequence, yet even more trans interactions.

Adhesive interactions between cells vary considerably, depending on the particular CAMs participating and the tissue. Just like Velcro, very tight adhesion can be generated when many weak interactions are combined together in a small, well-defined area. Furthermore, the association of intracellular molecules with the cytosolic domains of CAMs can dramatically influence the intermolecular interactions of CAMs by promoting their cis association (clustering) or by altering their conformation. Among the many variables that determine the nature of adhesion between two cells are the binding affinity of the interacting molecules (thermodynamic properties); the overall "on" and "off" rates of association and dissociation for each interacting molecule (kinetic properties); the spatial distribution (clustering, high or low density) of adhesion molecules (geometric properties); the active versus inactive states of CAMs with respect to adhesion (biochemical properties); and external forces such as the laminar and turbulent flow of cells in the circulatory system (mechanical properties).



▲ FIGURE 6-3 **Schematic model for the generation of cell–cell adhesions.** Lateral interactions between cell-adhesion molecules (CAMs) within the plasma membrane of a cell form dimers and larger oligomers. The parts of the molecules that participate in these cis interactions vary among the different CAMs. Subsequent trans interactions between distal domains of CAMs on adjacent cells generate a zipperlike strong adhesion between the cells. [Adapted from M. S. Steinberg and P. M. McNutt, 1999, *Curr. Opin. Cell Biol.* **11**:554.]

The Extracellular Matrix Participates in Adhesion and Other Functions

Certain cell-surface receptors, including some integrins, can bind components of the extracellular matrix (ECM), thereby indirectly adhering cells to each other through their interactions with the matrix. Three abundant ECM components are proteoglycans, a unique type of glycoprotein; collagens, proteins that often form fibers; and soluble multiadhesive matrix proteins (e.g., fibronectin). The relative volumes of cells versus matrix vary greatly among different animal tissues and organs. Some connective tissue, for instance, is mostly matrix, whereas many organs are composed of very densely packed cells with relatively little matrix.

Although the extracellular matrix generally provides mechanical support to tissues, it serves several other functions as well. Different combinations of ECM components tailor the extracellular matrix for specific purposes: strength in a tendon, tooth, or bone; cushioning in cartilage; and adhesion in most tissues. In addition, the composition of the matrix, which can vary, depending on the anatomical site and physiological status of a tissue, can let a cell know where it is and what it should do (environmental cues). Changes in ECM components, which are constantly being remodeled, degraded, and resynthesized locally, can modulate the interactions of a cell with its environment. The matrix also serves as a reservoir for many extracellular signaling molecules that control cell growth and differentiation. In addition, the matrix provides a lattice through or on which cells can move, particularly in the early stages of tissue assembly. Morphogenesis—the later stage of embryonic development in which tissues, organs, and body parts are formed by cell movements and rearrangements—also is critically dependent on cell–matrix adhesion as well as cell–cell adhesion.

Diversity of Animal Tissues Depends on Evolution of Adhesion Molecules with Various Properties

Cell–cell adhesions and cell–matrix adhesions are responsible for the formation, composition, architecture, and function of animal tissues. Not surprisingly, adhesion molecules of animals are evolutionarily ancient and are some of the most highly conserved proteins among multicellular (metazoan) organisms. Sponges, the most primitive metazoans, express certain CAMs and multiadhesive ECM molecules whose structures are strikingly similar to those of the corresponding human proteins. The evolution of organisms with complex tissues and organs has depended on the evolution of diverse CAMs, adhesion receptors, and ECM molecules with novel properties and functions, whose levels of expression differ in different types of cells.

The diversity of adhesive molecules arises in large part from two phenomena that can generate numerous closely related proteins, called **isoforms,** that constitute a protein family. In some cases, the different members of a protein family are encoded by multiple genes that arose from a common ancestor by gene duplication and divergent evolution (Chapter 9). Analyses of gene and cDNA sequences can provide evidence for the existence of such a set of related genes, or gene family. In other cases, a single gene produces an RNA transcript that can undergo alternative splicing to yield multiple mRNAs, each encoding a distinct isoform (Chapter 4). Alternative splicing thus increases the number of proteins that can be expressed from one gene. Both of these phenomena contribute to the diversity of some protein families such as the cadherins. Particular isoforms of an adhesive protein are often expressed in some cell types but not others, accounting for their differential distribution in various tissues.

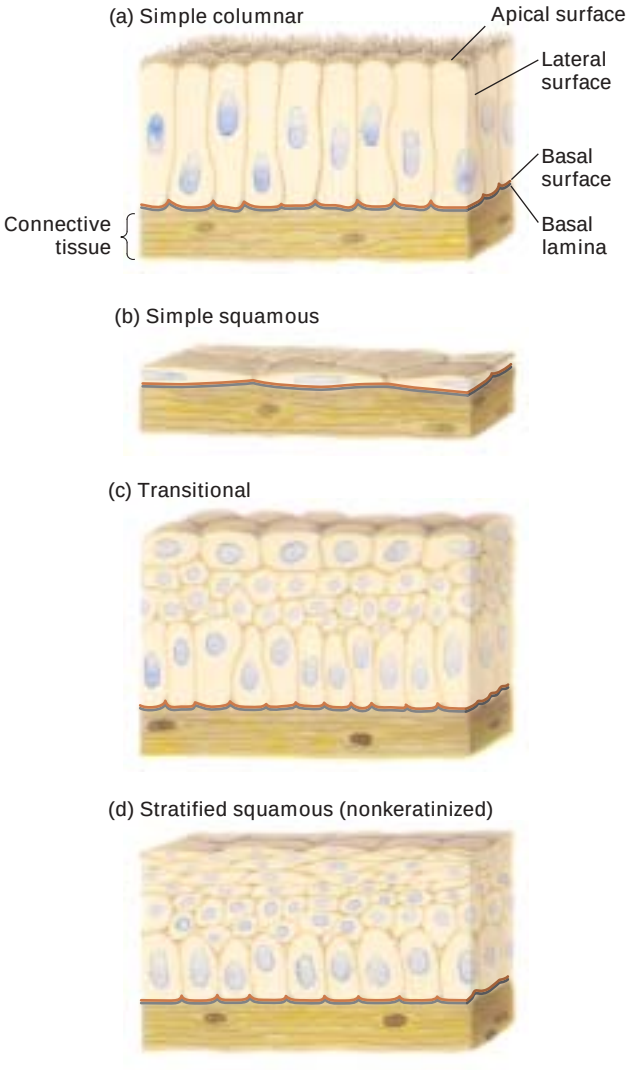
KEY CONCEPTS OF SECTION 6.1

Cell–Cell and Cell–Matrix Adhesion: An Overview

- Cell-adhesion molecules (CAMs) mediate direct cell–cell adhesions (homotypic and heterotypic), and cell-surface adhesion receptors mediate cell–matrix adhesions (see Figure 6-1). These interactions bind cells into tissues and facilitate communication between cells and their environments.
- The cytosolic domains of CAMs and adhesion receptors bind multifunctional adapter proteins that mediate interaction with cytoskeletal fibers and intracellular signaling proteins.
- The major families of cell-surface adhesion molecules are the cadherins, selectins, Ig-superfamily CAMs, and integrins (see Figure 6-2).
- Tight cell–cell adhesions entail both cis (lateral or intracellular) oligomerization of CAMs and trans (intercellular) interaction of like (homophilic) or different (heterophilic) CAMs (see Figure 6-3).
- The extracellular matrix (ECM) is a complex meshwork of proteins and polysaccharides that contributes to the structure and function of a tissue.
- The evolution of CAMs, adhesion receptors, and ECM molecules with specialized structures and functions permits cells to assemble into diverse classes of tissues with varying functions.

6.2 Sheetlike Epithelial Tissues: Junctions and Adhesion Molecules

In general, the external and internal surfaces of organs are covered by a sheetlike layer of epithelial tissue called an **epithelium.** Cells that form epithelial tissues are said to be *polarized* because their plasma membranes are organized into at least two discrete regions. Typically, the distinct surfaces of a polarized epithelial cell are called the **apical** (top), basal



▲ FIGURE 6-4 **Principal types of epithelium.** The apical and basolateral surfaces of epithelial cells exhibit distinctive characteristics. (a) Simple columnar epithelia consist of elongated cells, including mucus-secreting cells (in the lining of the stomach and cervical tract) and absorptive cells (in the lining of the small intestine). (b) Simple squamous epithelia, composed of thin cells, line the blood vessels (endothelial cells/endothelium) and many body cavities. (c) Transitional epithelia, composed of several layers of cells with different shapes, line certain cavities subject to expansion and contraction (e.g., the urinary bladder). (d) Stratified squamous (nonkeratinized) epithelia line surfaces such as the mouth and vagina; these linings resist abrasion and generally do not participate in the absorption or secretion of materials into or out of the cavity. The basal lamina, a thin fibrous network of collagen and other ECM components, supports all epithelia and connects them to the underlying connective tissue.

(base or bottom), and lateral (side) surfaces (Figure 6-4). The basal surface usually contacts an underlying extracellular matrix called the **basal lamina,** whose composition and function are discussed in Section 6.3. Often the basal and lateral surfaces are similar in composition and together are called the **basolateral** surface. The basolateral surfaces of most epithelia are usually on the side of the cell closest to the blood vessels. In animals with closed circulatory systems, blood flows through vessels whose inner lining is composed of flattened epithelial cells called endothelial cells. The apical side of endothelial cells, which faces the blood, is usually called the *luminal* surface, and the opposite basal side, the *abluminal* surface.

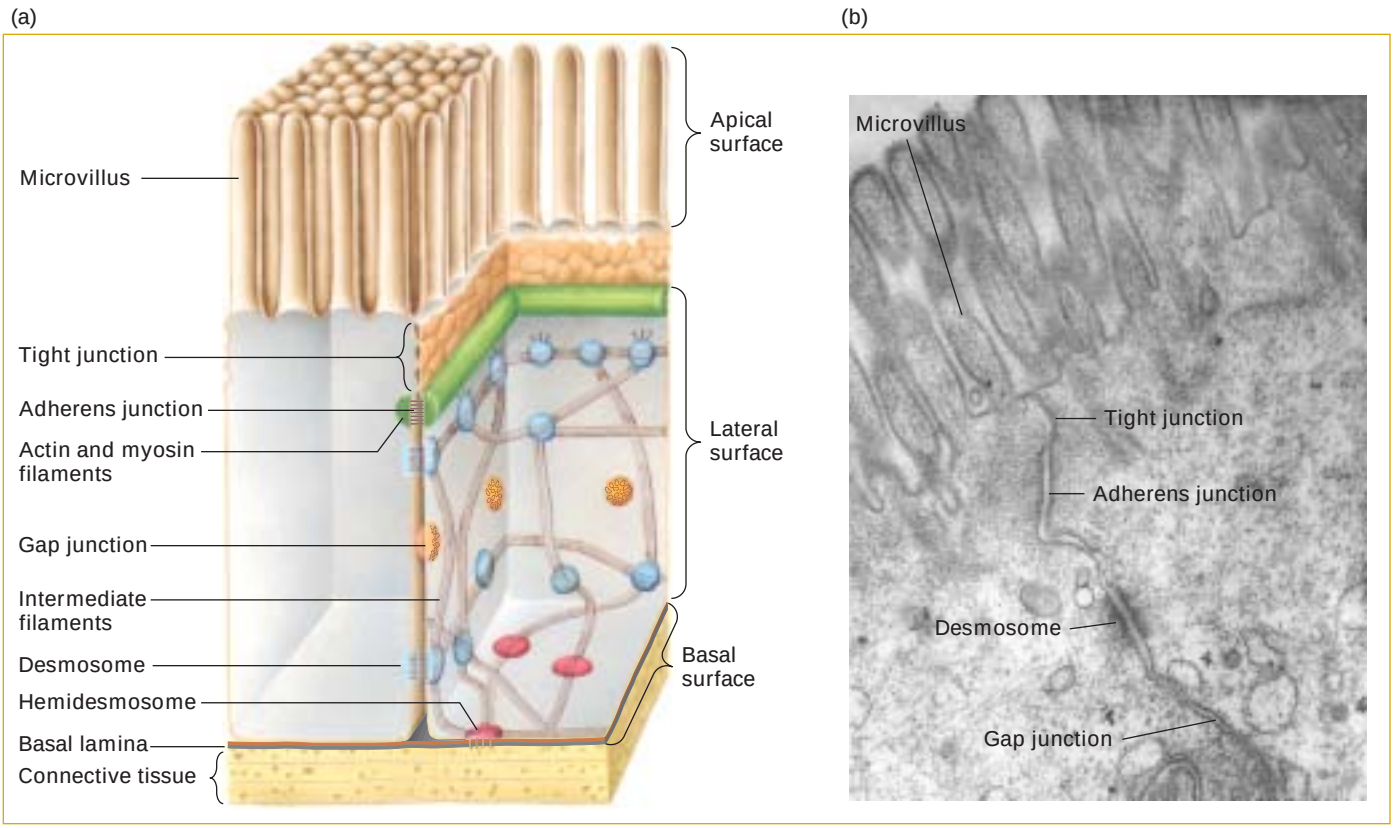
Epithelia in different body locations have characteristic morphologies and functions (see Figure 6-4). Stratified (multilayered) epithelia commonly serve as barriers and protective surfaces (e.g., the skin), whereas simple (single-layer) epithelia often selectively move ions and small molecules from one side of the layer to the other. For instance, the simple columnar epithelium lining the stomach secretes hydrochloric acid into the stomach lumen; a similar epithelium lining the small intestine transports products of digestion (e.g., glucose and amino acids) from the lumen of the intestine across the basolateral surface into the blood (Chapter 7). The simple columnar epithelium lining the small intestine has numerous fingerlike projections (100 nm in diameter) called **microvilli** (singular, microvillus) that extend from the luminal (apical) surface (see Figure 5-45). The upright orientation of a microvillus is maintained by numerous connections between the surrounding plasma membrane and a central bundle of actin microfilaments, which extend into the cell and interact with keratin intermediate filaments (see Figure 5-28). Microvilli greatly increase the area of the apical surface and thus the number of proteins that it can contain, enhancing the absorptive capacity of the intestinal epithelium.

Here we describe the various cell junctions and CAMs that play key roles in the assembly and functioning of epithelial sheets. In Section 6.3, we consider the components of the extracellular matrix intimately associated with epithelia.

Specialized Junctions Help Define the Structure and Function of Epithelial Cells

All epithelial cells in a sheet are connected to one another and the extracellular matrix by specialized cell junctions consisting of dense clusters of CAMs. Although hundreds of individual CAM-mediated interactions are sufficient to cause cells to adhere, junctions play special roles in imparting strength and rigidity to a tissue, transmitting information between the extracellular and the intracellular space, controlling the passage of ions and molecules across cell layers, and serving as conduits for the movement of ions and molecules from the cytoplasm of one cell to that of its immediate neighbor.

Three major classes of animal cell junctions are prominent features of the intestinal epithelium (Figure 6-5; see also Figure 6-1). **Anchoring junctions** and **tight junctions** perform the key task of holding cells together into tissues. These junctions are organized into three parts: adhesive proteins in the plasma membrane that connect one cell to another cell (CAMs) or to the extracellular matrix (adhesion receptors); adapter proteins, which connect the CAMs or adhesion re-



▲ FIGURE 6-5 **The principal types of cell junctions that connect the columnar epithelial cells lining the small intestine.** (a) Schematic cutaway drawing of intestinal epithelial cells. The basal surface of the cells rests on a basal lamina, and the apical surface is packed with fingerlike microvilli that project into the intestinal lumen. Tight junctions, lying just under the microvilli, prevent the diffusion of many substances between the intestinal lumen and the blood through the extracellular space between cells. Gap junctions allow the movement of small molecules and ions between the cytosols of adjacent cells. The remaining three types of junctions—adherens junctions, spot desmosomes, and hemidesmosomes—are critical to cell–cell and cell–matrix adhesion and signaling. (b) Electron micrograph of a thin section of intestinal epithelial cells, showing relative locations of the different junctions. [Part (b) C. Jacobson et al., 2001, *Journal Cell Biol.* **152**:435–450.]

ceptors to cytoskeletal filaments and signaling molecules; and the cytoskeletal filaments themselves. Tight junctions also control the flow of solutes between the cells forming an epithelial sheet. **Gap junctions** permit the rapid diffusion of small, water-soluble molecules between the cytoplasm of adjacent cells. Although present in epithelia, gap junctions are also abundant in nonepithelial tissues and structurally are very different from anchoring junctions and tight junctions; they also bear some resemblance to an important cell–cell junction in plants. For these reasons, we wait to consider gap junctions at the end of Section 6.5.

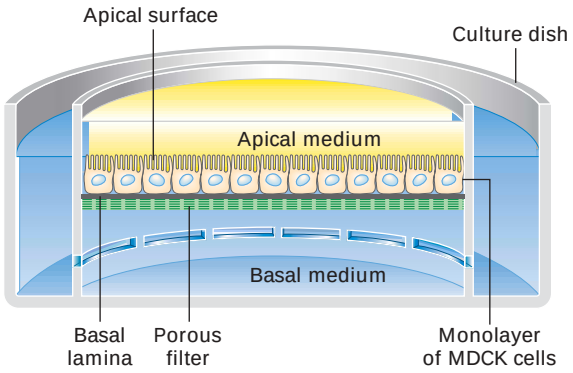
Of the three types of anchoring junctions present in epithelial cells, two participate in cell–cell adhesion, whereas the third participates in cell–matrix adhesion. *Adherens junctions*, which connect the lateral membranes of adjacent epithelial cells, are usually located near the apical surface, just below the tight junctions (see Figures 6-1 and 6-5). A circumferential belt of actin and myosin filaments in a complex with the adherens junction functions as a tension cable that can internally brace the cell and thereby control its shape. Epithelial and some other types of cells, such as smooth muscle, are also bound tightly together by *desmosomes*, button-like points of contact sometimes called spot desmosomes. *Hemidesmosomes*, found mainly on the basal surface of epithelial cells, anchor an epithelium to components of the underlying extracellular matrix, much like nails holding down a carpet. Bundles of intermediate filaments, running parallel to the cell surface or through the cell, rather than actin filaments, interconnect spot desmosomes and hemidesmosomes, imparting shape and rigidity to the cell.

Desmosomes and hemidesmosomes also transmit shear forces from one region of a cell layer to the epithelium as a whole, providing strength and rigidity to the entire epithelial cell layer. These junctions are especially important in maintaining the integrity of skin epithelia. For instance, mutations that interfere with hemidesmosomal anchoring in the skin can lead to blistering in which the epithelium becomes detached from its matrix foundation and extracellular fluid accumulates at the basolateral surface, forcing the skin to balloon outward.

Ca^{2+}-Dependent Homophilic Cell–Cell Adhesion in Adherens Junctions and Desmosomes Is Mediated by Cadherins

The primary CAMs in adherens junctions and desmosomes belong to the **cadherin** family. In vertebrates and invertebrates, this protein family of more than 100 members can be grouped into at least six subfamilies. The diversity of cadherins arises from the presence of multiple cadherin genes and alternative RNA splicing, which generates multiple mRNAs from one gene.

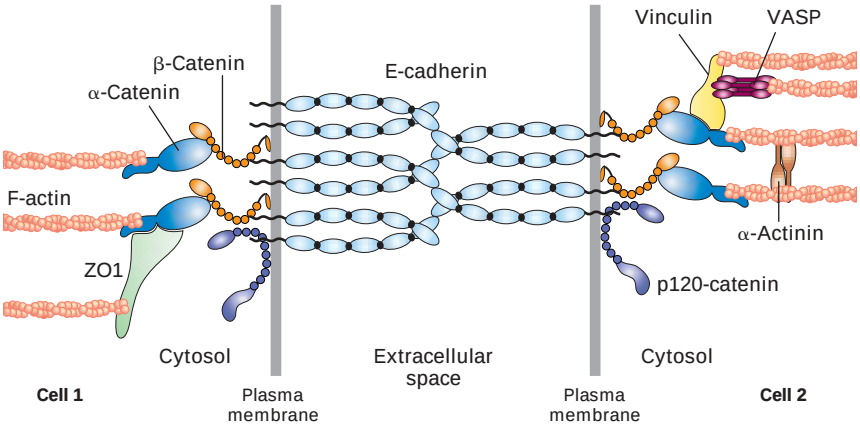
Cadherins are key molecules in cell–cell adhesion and cell signaling, and they play a critical role during tissue differentiation. The "classical" E-, P-, and N-cadherins are the most widely expressed, particularly during early differentiation. Sheets of polarized epithelial cells, such as those that line the small intestine or kidney tubules, contain abundant E-cadherin along their lateral surfaces. Although E-cadherin is concentrated in adherens junctions, it is present throughout the lateral surfaces where it is thought to link adjacent cell membranes. The brain expresses the largest number of different cadherins, presumably owing to the necessity of forming many very specific cell–cell contacts to help establish its complex wiring diagram.



▲ EXPERIMENTAL FIGURE 6-6 **Madin-Darby canine kidney (MDCK) cells grown in specialized containers provide a useful experimental system for studying epithelial cells.** MDCK cells form a polarized epithelium when grown on a porous membrane filter coated on one side with collagen and other components of the basal lamina. With the use of the special culture dish shown here, the medium on each side of the filter (apical and basal sides of the monolayer) can be experimentally manipulated and the movement of molecules across the layer monitored. Anchoring junctions and tight junctions form only if the growth medium contains sufficient Ca^{2+}.

Classical Cadherins The results of experiments with L cells, a line of cultured mouse fibroblasts grown in the laboratory, demonstrated that E-cadherin and P-cadherin preferentially mediate homophilic interactions. L cells express no cadherins and adhere poorly to themselves or to other types of cultured cells. When genes encoding either E-cadherin or P-cadherin were introduced into L cells with the use of techniques described in Chapter 9, the resulting engineered L cells expressed the encoded cadherin. These cadherin-expressing L cells were found to adhere preferentially to cells expressing the same type of cadherin molecules; that is, they mediate homophilic interactions. The L cells expressing E-cadherin also exhibited the polarized distribution of a membrane protein similar to that in epithelial cells, and they formed epithelial-like aggregates with one another and with epithelial cells isolated from lungs.

The adhesiveness of cadherins depends on the presence of extracellular Ca^{2+}, the property that gave rise to their name (*calcium adhering*). For example, the adhesion of engineered L cells expressing E-cadherin is prevented when the cells are bathed in a solution (growth medium) that is low in Ca^{2+}. The role of E-cadherin in adhesion can also be demonstrated



▲ FIGURE 6-7 **Protein constitutents of typical adherens junctions.** The exoplasmic domains of E-cadherin dimers clustered at adherens junctions on adjacent cells (1 and 2) form Ca^{+2}-dependent homophilic interactions. The cytosolic domains of the E-cadherins bind directly or indirectly to multiple adapter proteins that connect the junctions to actin filaments (F-actin) of the cytoskeleton and participate in intracellular signaling pathways (e.g., β-catenin). Somewhat different sets of adapter proteins are illustrated in the two cells shown to emphasize that a variety of adapters can interact with adherens junctions, which can thereby participate in diverse activities. [Adapted from V. Vasioukhin and E. Fuchs, 2001, *Curr. Opin. Cell Biol.***13**:76.]

in experiments with cultured cells called *Madin-Darby canine kidney (MDCK) cells*. When grown in specialized containers, these cells form a continuous one-cell-thick sheet (monolayer) of polarized kidneylike epithelial cells (Figure 6-6). In this experimental system, the addition of an antibody that binds to E-cadherin, preventing its homophilic interactions, blocks the Ca^{2+}-dependent attachment of suspended MDCK cells to a substrate and the subsequent formation of intercellular adherens junctions.

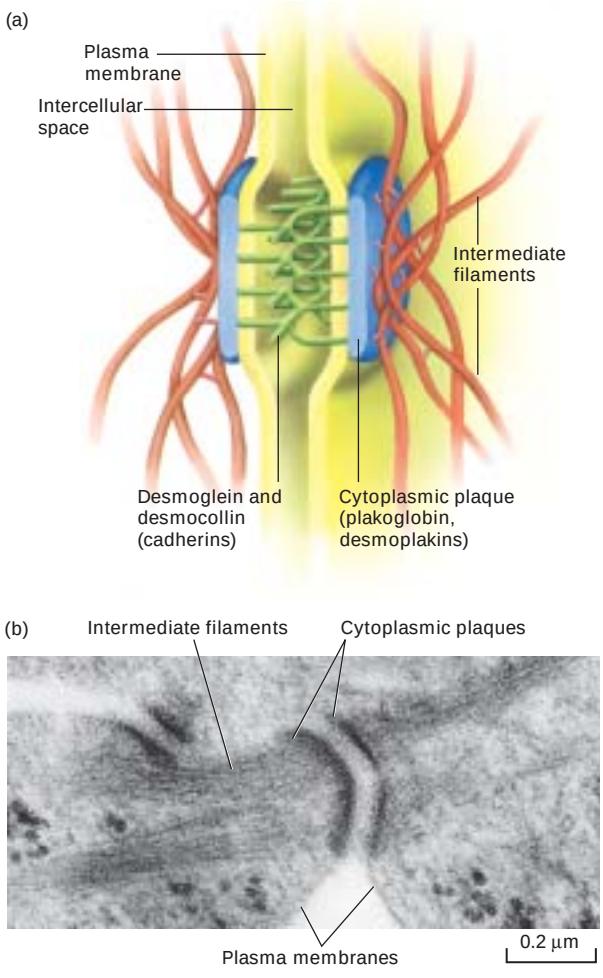
Each classical cadherin contains a single transmembrane domain, a relatively short C-terminal cytosolic domain, and five extracellular "cadherin" domains (see Figure 6-2). The extracellular domains are necessary for Ca^{2+} binding and cadherin-mediated cell–cell adhesion. Cadherin-mediated adhesion entails both lateral (intracellular) and trans (intercellular) molecular interactions as described previously (see Figure 6-3). The Ca^{2+}-binding sites, located between the cadherin repeats, serve to rigidify the cadherin oligomers. The cadherin oligomers subsequently form intercellular complexes to generate cell–cell adhesion and then additional lateral contacts, resulting in a "zippering up" of cadherins into clusters. In this way, multiple low-affinity interactions sum to produce a very tight intercellular adhesion.

The results of domain swap experiments, in which an extracellular domain of one kind of cadherin is replaced with the corresponding domain of a different cadherin, have indicated that the specificity of binding resides, at least in part, in the most distal extracellular domain, the N-terminal domain. In the past, cadherin-mediated adhesion was commonly thought to require only head-to-head interactions between the N-terminal domains of cadherin oligomers on adjacent cells, as depicted in Figure 6-3. However, the results of some experiments suggest that under some experimental conditions at least three cadherin domains from each molecule, not just the N-terminal domains, participate by interdigitation in trans associations.

The C-terminal cytosolic domain of classical cadherins is linked to the actin cytoskeleton by a number of cytosolic adapter proteins (Figure 6-7). These linkages are essential for strong adhesion, apparently owing primarily to their contributing to increased lateral associations. For example, disruption of the interactions between classical cadherins and α- or β-catenin—two common adapter proteins that link these cadherins to actin filaments—dramatically reduces cadherin-mediated cell–cell adhesion. This disruption occurs spontaneously in tumor cells, which sometimes fail to express α-catenin, and can be induced experimentally by depleting the cytosolic pool of accessible β-catenin. The cytosolic domains of cadherins also interact with intracellular signaling molecules such as β-catenin and p120-catenin. Interestingly, β-catenin not only mediates cytoskeletal attachment but can also translocate to the nucleus and alter gene transcription (see Figure 15-32).

Although E-cadherins exhibit primarily homophilic binding, some cadherins mediate heterophilic interactions. Importantly, each classical cadherin has a characteristic tissue distribution. In the course of differentiation, the amount or nature of the cell-surface cadherins changes, affecting many aspects of cell–cell adhesion and cell migration. For instance, the reorganization of tissues during morphogenesis is often accompanied by the conversion of nonmotile epithelial cells into motile precursor cells for other tissues (mesenchymal cells). Such epithelial-to-mesenchymal transitions are associated with a reduction in the expression of E-cadherin. The conversion of epithelial cells into cancerous melanoma cells also is marked by a loss of E-cadherin activity. The resulting decrease in cell–cell adhesion permits melanoma cells to invade the underlying tissue and spread throughout the body.

Desmosomal Cadherins Desmosomes (Figure 6-8) contain two specialized cadherin proteins, *desmoglein* and



▲ FIGURE 6-8 **Desmosomes.** (a) Schematic model showing components of a desmosome between epithelial cells and attachments to the sides of keratin intermediate filaments, which crisscross the interior of cells. The transmembrane CAMs, desmoglein and desmocollin, belong to the cadherin family. (b) Electron micrograph of a thin section of a desmosome connecting two cultured differentiated human keratinocytes. Bundles of intermediate filaments radiate from the two darkly staining cytoplasmic plaques that line the inner surface of the adjacent plasma membranes. [Part (a) see B. M. Gumbiner, 1993, *Neuron* **11**:551, and D. R. Garrod, 1993, *Curr. Opin. Cell Biol.* **5**:30. Part (b) courtesy of R. van Buskirk.]

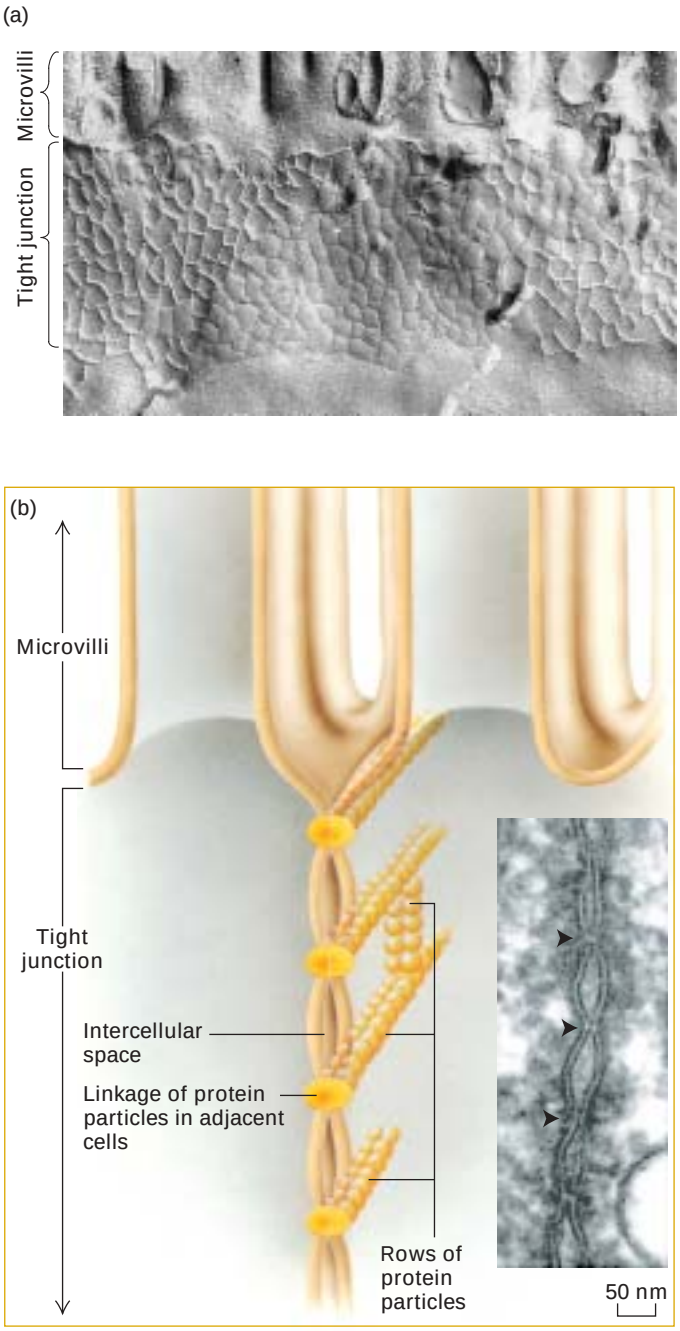
desmocollin, whose cytosolic domains are distinct from those in the classical cadherins. The cytosolic domains of desmosomal cadherins interact with plakoglobin (similar in structure to β-catenin) and the plakophilins. These adapter proteins, which form the thick cytoplasmic plaques characteristic of desmosomes, in turn interact with intermediate filaments. Thus desmosomes and adherens junctions are linked to different cytoskeletal fibers.

MEDICINE

The cadherin desmoglein was first identified by an unusual, but revealing, skin disease called *pemphigus vulgaris*, an autoimmune disease. Patients with autoimmune disorders synthesize antibodies that bind to a normal body protein. In this case, the autoantibodies disrupt adhesion between epithelial cells, causing blisters of the skin and mucous membranes. The predominant autoantibody was shown to be specific for desmoglein; indeed, the addition of such antibodies to normal skin induces the formation of blisters and disruption of cell adhesion.

Tight Junctions Seal Off Body Cavities and Restrict Diffusion of Membrane Components

For polarized epithelial cells to carry out their functions as barriers and mediators of selective transport, extracellular fluids surrounding their apical and basolateral membranes must be kept separate. The tight junctions between adjacent epithelial cells are usually located just below the apical surface and help establish and maintain cell polarity (see Figures 6-1 and 6-5). These specialized regions of the plasma membrane form a barrier that seals off body cavities such as the intestine, the stomach lumen, the blood (e.g., the blood–brain barrier), and the bile duct in the liver.



▶ FIGURE 6-9 **Tight junctions.** (a) Freeze-fracture preparation of tight junction zone between two intestinal epithelial cells. The fracture plane passes through the plasma membrane of one of the two adjacent cells. A honeycomb-like network of ridges and grooves below the microvilli constitutes the tight junction zone. (b) Schematic drawing shows how a tight junction might be formed by the linkage of rows of protein particles in adjacent cells. In the inset micrograph of an ultrathin sectional view of a tight junction, the adjacent cells can be seen in close contact where the rows of proteins interact. (c) As shown in these schematic drawings of the major proteins in tight junctions, both occludin and claudin-1 contain four transmembrane helices, whereas the junction adhesion molecule (JAM) has a single transmembrane domain and a large extracellular region. See text for discussion. [Part (a) courtesy of L. A. Staehelin. Drawing in part (b) adapted from L. A. Staehelin and B. E. Hull, 1978, *Sci. Am.* **238**(5):140, and D. Goodenough, 1999, *Proc. Nat'l. Acad. Sci. USA* **96**:319. Photograph in part (b) courtesy of S. Tsukita et al., 2001, *Nature Rev. Mol. Cell Biol.* **2**:285. Drawing in part (c) adapted from S. Tsukita et al., 2001, *Nature Rev. Mol. Cell Biol.* **2**:285.]

Tight junctions prevent the diffusion of macromolecules and to varying degrees impede the diffusion of small water-soluble molecules and ions across an epithelial sheet in the spaces between cells. They also maintain the polarity of epithelial cells by preventing the diffusion of membrane proteins and glycolipids (lipids with covalently attached sugars) between the apical and the basolateral regions of the plasma membrane, ensuring that these regions contain different membrane components. As a consequence, movement of many nutrients across the intestinal epithelium is in large part through the *transcellular pathway.* In this pathway, specific transport proteins in the apical membrane import small molecules from the intestinal lumen into cells; other transport proteins located in the basolateral membrane then export these molecules into the extracellular space. Such transcellular transport is covered in detail in Chapter 7.

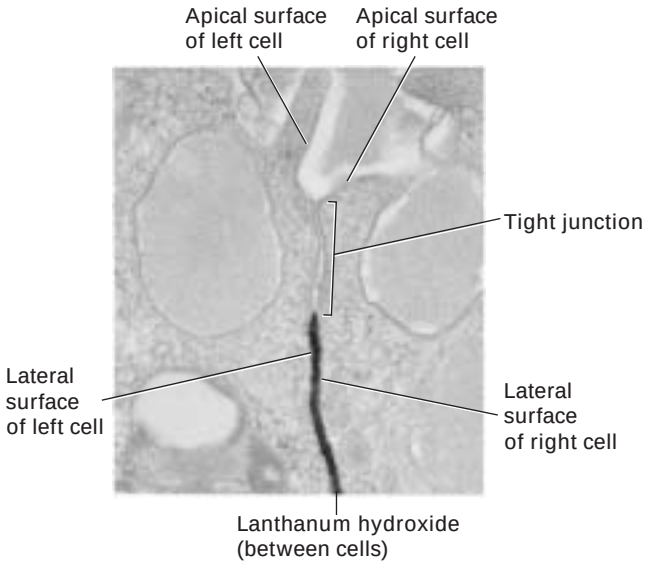
Tight junctions are composed of thin bands of plasma-membrane proteins that completely encircle a polarized cell and are in contact with similar thin bands on adjacent cells. When thin sections of cells are viewed in an electron microscope, the lateral surfaces of adjacent cells appear to touch each other at intervals and even to fuse in the zone just below the apical surface (see Figure 6-5b). In freeze-fracture preparations, tight junctions appear as an interlocking network of ridges in the plasma membrane (Figure 6-9a). More specifically, there appear to be ridges on the cytosolic face of the plasma membrane of each of the two contacting cells. Corresponding grooves are found on the exoplasmic face.

Very high magnification reveals that rows of protein particles 3–4 nm in diameter form the ridges seen in freeze-fracture micrographs of tight junctions. In the model shown in Figure 6-9b, the tight junction is formed by a double row of these particles, one row donated by each cell. The two principal integral-membrane proteins found in tight junctions are *occludin* and *claudin*. Initially, investigators thought that occludin was the only essential protein component of tight junctions. However, when investigators engineered mice with mutations inactivating the occludin gene, the mice still had morphologically distinct tight junctions. (This technique, called **gene knockout,** is described in Chapter 9.) Further analysis led to the discovery of claudin. Each of these proteins has four membrane-spanning α helices (Figure 6-9c). The claudin multigene family encodes numerous homologous proteins (isoforms) that exhibit distinct tissue-specific patterns of expression. Recently, a group of *junction adhesion molecules (JAMs)* have been found to contribute to homophilic adhesion and other functions of tight junctions. These molecules, which contain a single transmembrane α helix, belong to the Ig superfamily of CAMs. The extracellular domains of rows of occludin, claudin, and JAM proteins in the plasma membrane of one cell apparently form extremely tight links with similar rows of the same proteins in an adjacent cell, creating a tight seal. Treatment of an epithelium with the protease trypsin destroys the tight junctions, supporting the proposal that proteins are essential structural components of these junctions.

The long C-terminal cytosolic segment of occludin binds to PDZ domains in certain large cytosolic adapter proteins. These domains are found in various cytosolic proteins and mediate binding to the C-termini of particular plasma-membrane proteins. PDZ-containing adapter proteins associated with occludin are bound, in turn, to other cytoskeletal and signaling proteins and to actin fibers. These interactions appear to stabilize the linkage between occludin and claudin molecules that is essential for maintaining the integrity of tight junctions.

A simple experiment demonstrates the impermeability of certain tight junctions to many water-soluble substances. In this experiment, lanthanum hydroxide (an electron-dense colloid of high molecular weight) is injected into the pancreatic blood vessel of an experimental animal; a few minutes later, the pancreatic acinar cells, which are specialized epithelial cells, are fixed and prepared for microscopy. As shown in Figure 6-10, the lanthanum hydroxide diffuses from the blood into the space that separates the lateral surfaces of adjacent acinar cells, but cannot penetrate past the tight junction.

The importance of Ca^{2+} to the formation and integrity of tight junctions has been demonstrated in studies with MDCK cells in the experimental system described previously (see Figure 6-7). If the growth medium in the chamber contains very low concentrations of Ca^{2+}, MDCK cells form a monolayer in which the cells are not connected by tight junctions. As a result, fluids and salts flow freely across the cell layer. When sufficient Ca^{2+} is added to the medium, tight junctions form within an hour, and the cell layer becomes impermeable



▲ EXPERIMENTAL FIGURE 6-10 **Tight junctions prevent passage of large molecules through extracellular space between epithelial cells.** This experiment, described in the text, demonstrates the impermeability of tight junctions in the pancreas to the large water-soluble colloid lanthanum hydroxide. [Courtesy of D. Friend.]

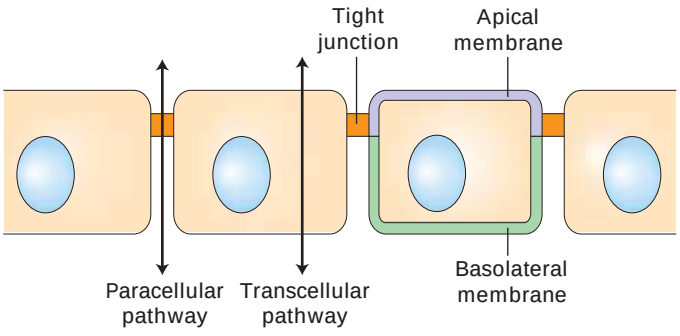
to fluids and salts. Thus Ca^{2+} is required for the formation of tight junctions as well as for cell–cell adhesion mediated by cadherins.

Plasma-membrane proteins cannot diffuse in the plane of the membrane past tight junctions. These junctions also restrict the lateral movement of lipids in the exoplasmic leaflet of the plasma membrane in the apical and basolateral regions of epithelial cells. Indeed, the lipid compositions of the exoplasmic leaflet in these two regions are distinct. Essentially all glycolipids are present in the exoplasmic face of the apical membrane, as are all proteins linked to the membrane by a glycosylphosphatidylinositol (GPI) anchor (see Figure 5-15). In contrast, lipids in the cytosolic leaflet in the apical and basolateral regions of epithelial cells have the same composition and can apparently diffuse laterally from one region of the membrane to the other.

Differences in Permeability of Tight Junctions Can Control Passage of Small Molecules Across Epithelia

The barrier to diffusion provided by tight junctions is not absolute. Owing at least in part to the varying properties of the different isoforms of claudin located in different tight junctions, their permeability to ions, small molecules, and water varies enormously among different epithelial tissues. In epithelia with "leaky" tight junctions, small molecules can move from one side of the cell layer to the other through the *paracellular pathway* in addition to the transcellular pathway (Figure 6-11).

The leakiness of tight junctions can be altered by intracellular signaling pathways, especially G protein–coupled pathways entailing cyclic AMP and protein kinase C (Chapter 13). The regulation of tight junction permeability is often studied by measuring ion flux (electrical resistance) or the movement of radioactive or fluorescent molecules across monolayers of MDCK cells.



▲ FIGURE 6-11 **Transcellular and paracellular pathways of transepithelial transport.** Transcellular transport requires the cellular uptake of molecules on one side and subsequent release on the opposite side by mechanisms discussed in Chapters 7 and 17. In paracellular transport, molecules move extracellularly through parts of tight junctions, whose permeability to small molecules and ions depends on the composition of the junctional components and the physiologic state of the epithelial cells. [Adapted from S. Tsukita et al., 2001, *Nature Rev. Mol. Cell Biol.* **2**:285.]

The importance of paracellular transport is illustrated in several human diseases. In hereditary hypomagnesemia, defects in the *claudin16* gene prevent the normal paracellular flow of magnesium through tight junctions in the kidney. This results in an abnormally low blood level of magnesium, which can lead to convulsions. Furthermore, a mutation in the *claudin14* gene causes hereditary deafness, apparently by altering transport around hair cells in the cochlea of the inner ear.

Toxins produced by *Vibrio cholerae*, which causes cholera, and several other enteric (gastrointestinal tract) bacteria alter the permeability barrier of the intestinal epithelium by altering the composition or activity of tight junctions. Other bacterial toxins can affect the ion-pumping activity of membrane transport proteins in intestinal epithelial cells. Toxin-induced changes in tight junction permeability (increased paracellular transport) and in protein-mediated ion-pumping proteins (increased transcellular transport) can result in massive loss of internal body ions and water into the gastrointestinal tract, which in turn leads to diarrhea and potentially lethal dehydration.

Many Cell–Matrix and Some Cell–Cell Interactions Are Mediated by Integrins

The **integrin** family comprises heterodimeric integral membrane proteins that function as adhesion receptors, mediating many cell–matrix interactions (see Figure 6-2). In vertebrates, at least 24 integrin heterodimers, composed of 18 types of α subunits and 8 types of β subunits in various combinations, are known. A single β chain can interact with any one of multiple α chains, forming integrins that bind different ligands. This phenomenon of *combinatorial diversity*, which is found throughout the biological world, allows a relatively small number of components to serve a large number of distinct functions.

In epithelial cells, integrin α6β4 is concentrated in hemidesmosomes and plays a major role in adhering cells to matrix in the underlying basal lamina, as discussed in detail in Section 6.3. Some integrins, particularly those expressed by certain blood cells, participate in heterophilic cell–cell interactions. The members of this large family play important roles in adhesion and signaling in both epithelial and nonepithelial tissues.

Integrins typically exhibit low affinities for their ligands with dissociation constants K_D between 10^{-6} and 10^{-8} mol/L. However, the multiple weak interactions generated by the binding of hundreds or thousands of integrin molecules to their ligands on cells or in the extracellular matrix allow a cell to remain firmly anchored to its ligand-expressing target. Moreover, the weakness of individual integrin-mediated interactions facilitates cell migration.

Parts of both the α and the β subunits of an integrin molecule contribute to the primary extracellular ligand-binding site (see Figure 6-2). Ligand binding to integrins also requires the simultaneous binding of divalent cations (positively charged ions). Like other cell-surface adhesive molecules, the cytosolic region of integrins interacts with adapter proteins that in turn bind to the cytoskeleton and intracellular signaling molecules. Although most integrins are linked to the actin cytoskeleton, the cytosolic domain of the β4 chain in the α6β4 integrin in hemidesmosomes, which is much longer than those of other β integrins, binds to specialized adapter proteins (e.g., plectin) that in turn interact with keratin-based intermediate filaments.

In addition to their adhesion function, integrins can mediate outside-in and inside-out transfer of information (signaling). In outside-in signaling, the engagement of integrins with their extracellular ligands can, through adapter proteins bound to the integrin cytosolic region, influence the cytoskeleton and intracellular signaling pathways. Conversely, in inside-out signaling, intracellular signaling pathways can alter, from the cytoplasm, the structure of integrins and consequently their abilities to adhere to their extracellular ligands and mediate cell–cell and cell–matrix interactions. Integrin-mediated signaling pathways influence processes as diverse as cell survival, cell proliferation, and programmed cell death (Chapter 22). Many cells express several different integrins that bind the same ligand. By selectively regulating the activity of each type of integrin, these cells can fine-tune their cell–cell and cell–matrix interactions and the associated signaling processes.

We will consider various integrins and the regulation of their activity in detail in Section 6.5.

KEY CONCEPTS OF SECTION 6.2

Sheetlike Epithelial Tissues: Junctions and Adhesion Molecules

- Polarized epithelial cells have distinct apical, basal, and lateral surfaces. Microvilli projecting from the apical surfaces of many epithelial cells considerably expand their surface areas.
- Three major classes of cell junctions—anchoring junctions, tight junctions, and gap junctions—assemble epithelial cells into sheets and mediate communication between them (see Figures 6-1 and 6-5).
- Adherens junctions and desmosomes are cadherin-containing anchoring junctions that bind the membranes of adjacent cells, giving strength and rigidity to the entire tissue. Hemidesmosomes are integrin-containing anchoring junctions that attach cells to elements of the underlying extracellular matrix.
- Cadherins are cell-adhesion molecules (CAMs) responsible for Ca^{2+}-dependent interactions between cells in epithelial and other tissues. They promote strong cell–cell adhesion by mediating both lateral and intercellular interactions.
- Adapter proteins that bind to the cytosolic domain of cadherins and other CAMs mediate the association of cytoskeletal and signaling molecules with the plasma membrane (see Figure 6-9). Strong cell–cell adhesion depends on the linkage of the interacting CAMs to the cytoskeleton.
- Tight junctions block the diffusion of proteins and some lipids in the plane of the plasma membrane, contributing to the polarity of epithelial cells. They also limit and regulate the extracellular (paracellular) flow of water and solutes from one side of the epithelium to the other (see Figure 6-11).
- Integrins are a large family of αβ heterodimeric cell-surface proteins that mediate both cell–cell and cell–matrix adhesions and inside-out and outside-in signaling in numerous tissues.

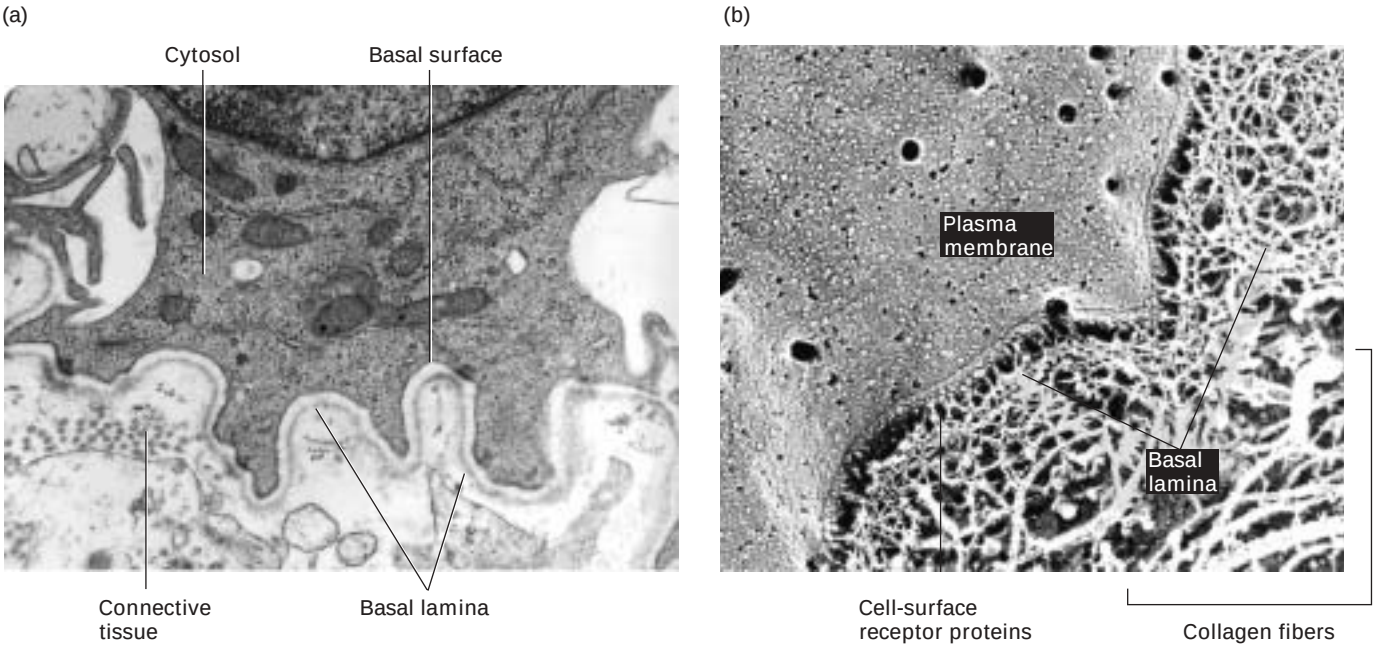
6.3 The Extracellular Matrix of Epithelial Sheets

In animals, the extracellular matrix helps organize cells into tissues and coordinates their cellular functions by activating intracellular signaling pathways that control cell growth, proliferation, and gene expression. Many functions of the matrix require transmembrane adhesion receptors that bind directly to ECM components and that also interact, through adapter proteins, with the cytoskeleton. The principal class of adhesion receptors that mediate cell–matrix adhesion are integrins, which were introduced in Section 6.2. However, other types of molecules also function as important adhesion receptors in some nonepithelial tissues.

Three types of molecules are abundant in the extracellular matrix of all tissues.

- Highly viscous **proteoglycans,** a group of glycoproteins that cushion cells and bind a wide variety of extracellular molecules
- **Collagen** fibers, which provide mechanical strength and resilience
- Soluble **multiadhesive matrix proteins,** which bind to and cross-link cell-surface adhesion receptors and other ECM components

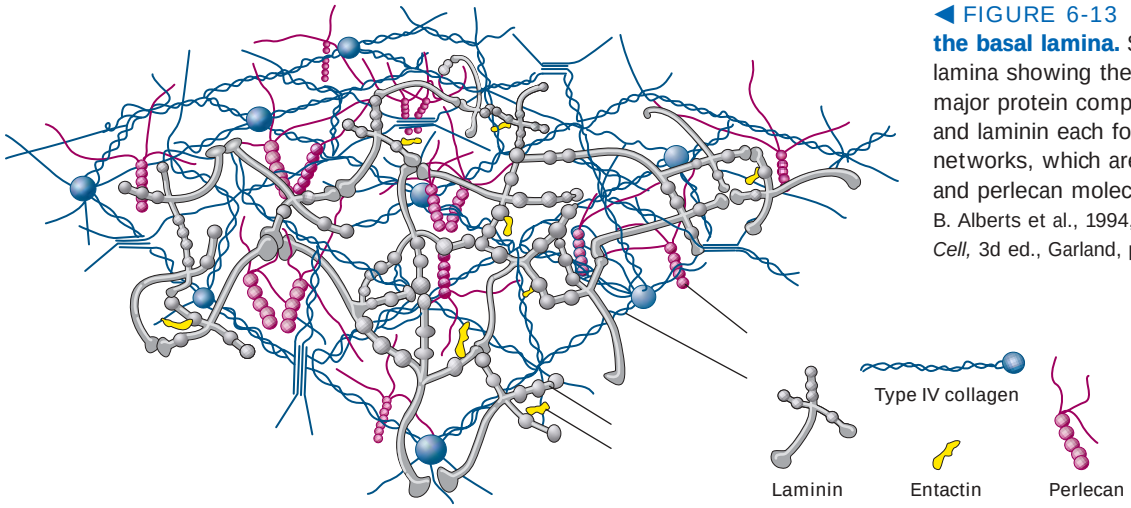
We begin our description of the structures and functions of these major ECM components in this section, focusing on the molecular components and organization of the basal lamina—the specialized extracellular matrix that helps determine the overall architecture of an epithelial tissue. In Section 6.4, we extend our discussion to specific ECM molecules that are commonly present in nonepithelial tissues.



▲ EXPERIMENTAL FIGURE 6-12 **The basal lamina separates epithelial cells and some other cells from connective tissue.** (a) Transmission electron micrograph of a thin section of cells (*top*) and underlying connective tissue (*bottom*). The electron-dense layer of the basal lamina can be seen to follow the undulation of the basal surface of the cells. (b) Electron micrograph of a quick-freeze deep-etch preparation of skeletal muscle showing the relation of the plasma membrane, basal lamina, and surrounding connective tissue. In this preparation, the basal lamina is revealed as a meshwork of filamentous proteins that associate with the plasma membrane and the thicker collagen fibers of the connective tissue. [Part (a) courtesy of P. FitzGerald. Part (b) from D. W. Fawcett, 1981, *The Cell,* 2d ed., Saunders/Photo Researchers; courtesy of John Heuser.]

The Basal Lamina Provides a Foundation for Epithelial Sheets

In animals, epithelia and most organized groups of cells are underlain or surrounded by the basal lamina, a sheetlike meshwork of ECM components usually no more than 60–120 nm thick (Figure 6-12; see also Figures 6-1 and 6-4). The basal lamina is structured differently in different tissues. In columnar and other epithelia (e.g., intestinal lining, skin), it is a foundation on which only one surface of the cells rests. In other tissues, such as muscle or fat, the basal lamina surrounds each cell. Basal laminae play important roles in regeneration after tissue damage and in embryonic development. For instance, the basal lamina helps



◀ FIGURE 6-13 **Major components of the basal lamina.** Schematic model of basal lamina showing the organization of the major protein components. Type IV collagen and laminin each form two-dimensional networks, which are cross-linked by entactin and perlecan molecules. [Adapted from B. Alberts et al., 1994, *Molecular Biology of the Cell,* 3d ed., Garland, p. 991.]

four- and eight-celled embryos adhere together in a ball. In the development of the nervous system, neurons migrate along ECM pathways that contain basal lamina components. Thus the basal lamina is important not only for organizing cells into tissues but also for tissue repair and for guiding migrating cells during tissue formation.

Most of the ECM components in the basal lamina are synthesized by the cells that rest on it. Four ubiquitous protein components are found in basal laminae (Figure 6-13):

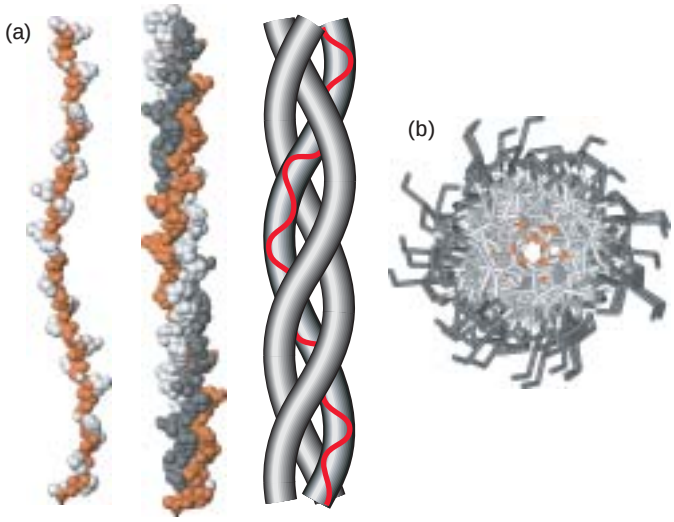
- *Type IV collagen*, trimeric molecules with both rodlike and globular domains that form a two-dimensional network
- *Laminins*, a family of multiadhesive proteins that form a fibrous two-dimensional network with type IV collagen and that also bind to integrins
- *Entactin* (also called nidogen), a rodlike molecule that cross-links type IV collagen and laminin and helps incorporate other components into the ECM
- *Perlecan*, a large multidomain proteoglycan that binds to and cross-links many ECM components and cell-surface molecules

As depicted in Figure 6-1, one side of the basal lamina is linked to cells by adhesion receptors, including α6β4 integrin that binds to laminin in the basal lamina. The other side of the basal lamina is anchored to the adjacent connective tissue by a layer of fibers of collagen embedded in a proteoglycan-rich matrix. In stratified squamous epithelia (e.g., skin), this linkage is mediated by anchoring fibrils of type VII collagen. Together, the basal lamina and this collagen-containing layer (see the micrograph on page 197) form the structure called the *basement membrane*.

Sheet-Forming Type IV Collagen Is a Major Structural Component in Basal Laminae

Type IV collagen, the principal component of all basal lamina, is one of more than 20 types of collagen that participate in the formation of the extracellular matrix in various tissues. Although they differ in certain structural features and tissue distribution, all collagens are trimeric proteins made from three polypeptides called collagen α chains. All three α chains can be identical (homotrimeric) or different (heterotrimeric). A trimeric collagen molecule contains one or more three-stranded segments, each with a similar triple-helical structure (Figure 6-14a). Each strand contributed by one of the α chains is twisted into a left-handed helix, and three such strands from the three α chains wrap around each other to form a right-handed triple helix.

The collagen triple helix can form because of an unusual abundance of three amino acids: glycine, proline, and a modified form of proline called hydroxyproline (see Figure 3-12). They make up the characteristic repeating motif Gly-X-Y, where X and Y can be any amino

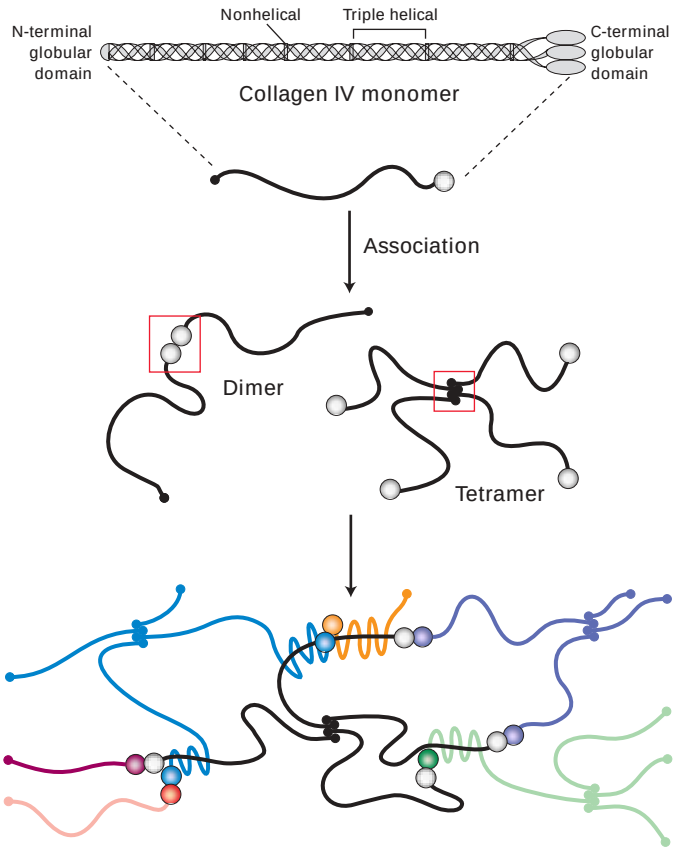


▲ FIGURE 6-14 **The collagen triple helix.** (a) (*Left*) Side view of the crystal structure of a polypeptide fragment whose sequence is based on repeating sets of three amino acids, Gly-X-Y, characteristic of collagen α chains. (*Center*) Each chain is twisted into a left-handed helix, and three chains wrap around each other to form a right-handed triple helix. The schematic model (*right*) clearly illustrates the triple helical nature of the structure. (b) View down the axis of the triple helix. The proton side chains of the glycine residues (orange) point into the very narrow space between the polypeptide chains in the center of the triple helix. In mutations in collagen in which other amino acids replace glycine, the proton in glycine is replaced by larger groups that disrupt the packing of the chains and destablize the triple-helical structure. [Adapted from R. Z. Kramer et al., 2001, *J. Mol. Biol.* **311**(1):131.]

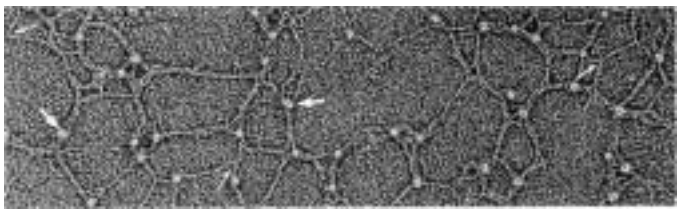
acid but are often proline and hydroxyproline and less often lysine and hydroxylysine. Glycine is essential because its small side chain, a hydrogen atom, is the only one that can fit into the crowded center of the three-stranded helix (Figure 6-14b). Hydrogen bonds help hold the three chains together. Although the rigid peptidyl-proline and peptidyl-hydroxyproline linkages are not compatible with formation of a classic single-stranded α helix, they stabilize the distinctive three-stranded collagen helix. The hydroxyl group in hydroxyproline helps hold its ring in a conformation that stabilizes the three-stranded helix.

The unique properties of each type of collagen are due mainly to differences in (1) the number and lengths of the collagenous, triple-helical segments; (2) the segments that flank or interrupt the triple-helical segments and that fold into other kinds of three-dimensional structures; and (3) the covalent modification of the α chains (e.g., hydroxylation, glycosylation, oxidation, cross-linking). For example, the chains in type IV collagen, which is unique to basal laminae, are designated IVα chains. Mammals express six homologous IVα chains, which assemble into a series of type IV

(a)



(b) Type IV network



▲ FIGURE 6-15 **Structure and assembly of type IV collagen.** (a) Schematic representation of type IV collagen. This 400-nm-long molecule has a small noncollagenous globular domain at the N-terminus and a large globular domain at the C-terminus. The triple helix is interrupted by nonhelical segments that introduce flexible kinks in the molecule. Lateral interactions between triple helical segments, as well as head-to-head and tail-to-tail interactions between the globular domains, form dimers, tetramers, and higher-order complexes, yielding a sheetlike network. (b) Electron micrograph of type IV collagen network formed in vitro. The lacy appearance results from the flexibility of the molecule, the side-to-side binding between triple-helical segments (thin arrows), and the interactions between C-terminal globular domains (thick arrows). [Part (a) adapted from A. Boutaud, 2000, *J. Biol. Chem.* **275**:30716. Part (b) courtesy of P. Yurchenco; see P. Yurchenco and G. C. Ruben, 1987, *J. Cell Biol.* **105**:2559.]

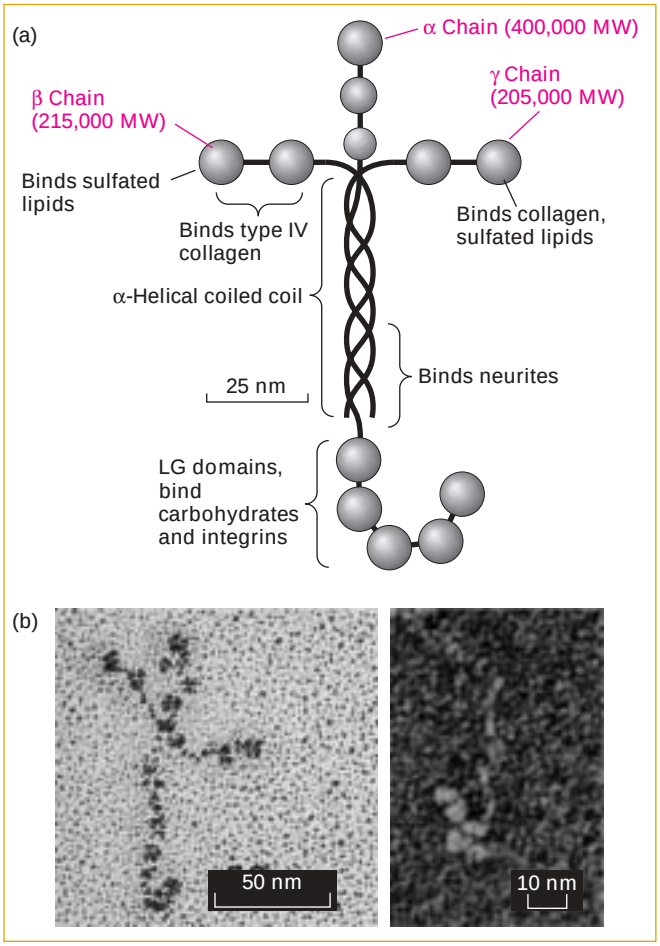
collagens with distinct properties. All subtypes of type IV collagen, however, form a 400-nm-long triple helix that is interrupted about 24 times with nonhelical segments and flanked by large globular domains at the C-termini of the chains and smaller globular domains at the N-termini. The nonhelical regions introduce flexibility into the molecule. Through both lateral associations and interactions entailing the globular N- and C-termini, type IV collagen molecules assemble into a branching, irregular two-dimensional fibrous network that forms the lattice on which the basal lamina is built (Figure 6-15).

MEDICINE In the kidney, a double basal lamina, the glomerular basement membrane, separates the epithelium that lines the urinary space from the endothelium that lines the surrounding blood-filled capillaries. Defects in this structure, which is responsible for ultrafiltration of the blood and initial urine formation, can lead to renal failure. For instance, mutations that alter the C-terminal globular domain of certain IVα chains are associated with progressive renal failure as well as sensorineural hearing loss and ocular abnormalities, a condition known as *Alport's syndrome*. In *Goodpasture's syndrome,* a relatively rare autoimmune disease, self-attacking, or "auto," antibodies bind to the α3 chains of type IV collagen found in the glomerular basement membrane and lungs. This binding sets off an immune response that causes cellular damage resulting in progressive renal failure and pulmonary hemorrhage.

Laminin, a Multiadhesive Matrix Protein, Helps Cross-link Components of the Basal Lamina

Multiadhesive matrix proteins are long, flexible molecules that contain multiple domains responsible for binding various types of collagen, other matrix proteins, polysaccharides, cell-surface adhesion receptors, and extracellular signaling molecules (e.g., growth factors and hormones). These proteins are important for organizing the other components of the extracellular matrix and for regulating cell–matrix adhesion, cell migration, and cell shape in both epithelial and nonepithelial tissues.

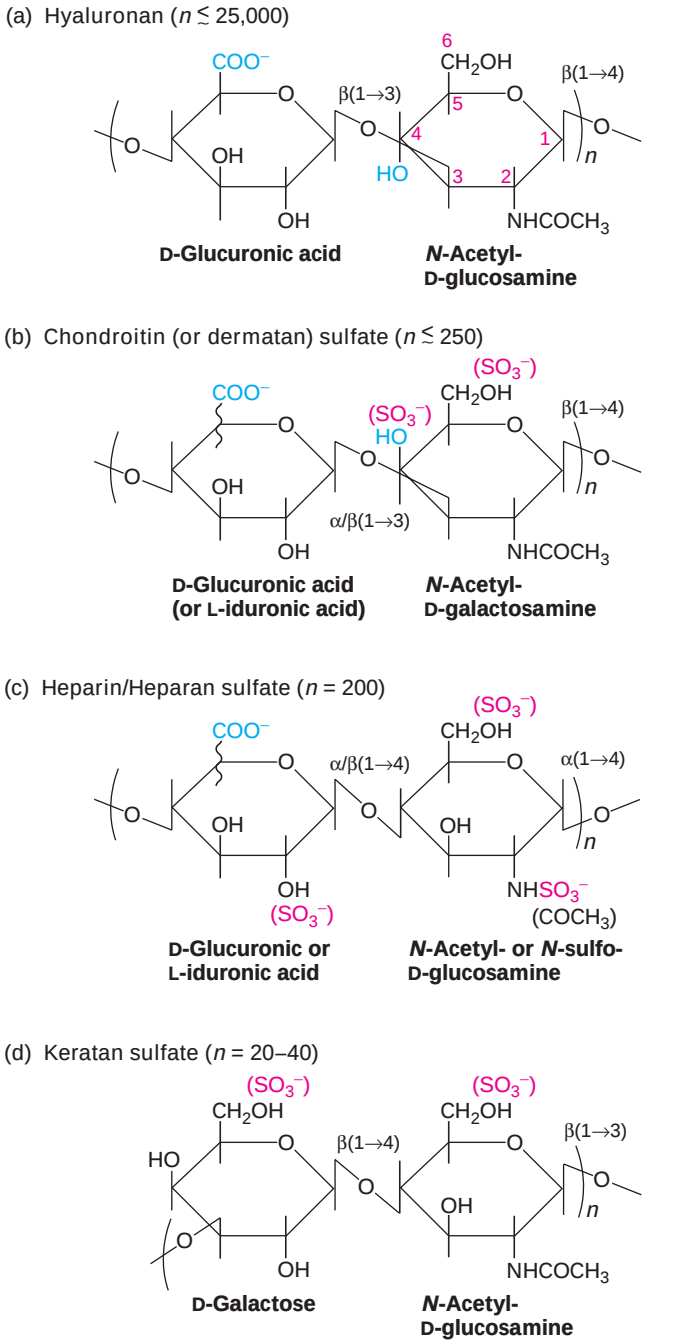
Laminin, the principal multiadhesive matrix protein in basal laminae, is a heterotrimeric, cross-shaped protein with a total molecular weight of 820,000 (Figure 6-16). Many laminin isoforms, containing slightly different polypeptide chains, have been identified. Globular *LG domains* at the C-terminus of the laminin α subunit mediate Ca^{2+}-dependent binding to specific carbohydrates on certain cell-surface molecules such as syndecan and dystroglycan. LG domains are found in a wide variety of proteins and can mediate binding to steroids and proteins as well as carbohydrates. For example, LG domains in the α chain of laminin can mediate binding to certain integrins, including α6β4 integrin on epithelial cells.



▲ FIGURE 6-16 **Laminin, a heterotrimeric multiadhesive matrix protein found in all basal laminae.** (a) Schematic model showing the general shape, location of globular domains, and coiled-coil region in which laminin's three chains are covalently linked by several disulfide bonds. Different regions of laminin bind to cell-surface receptors and various matrix components. (b) Electron micrographs of intact laminin molecule, showing its characteristic cross appearance (*left*) and the carbohydrate-binding LG domains near the C-terminus (*right*). [Part (a) adapted from G. R. Martin and R. Timpl, 1987, *Ann. Rev. Cell Biol.* **3**:57, and K. Yamada, 1991, *J. Biol. Chem.* **266**:12809. Part (b) from R. Timpl et al., 2000, *Matrix Biol.* **19**:309; photograph at right courtesy of Jürgen Engel.]

Secreted and Cell-Surface Proteoglycans Are Expressed by Many Cell Types

Proteoglycans are a subset of glycoproteins containing covalently linked specialized polysaccharide chains called **glycosaminoglycans (GAGs),** which are long linear polymers of specific repeating disaccharides. Usually one sugar is either a uronic acid (D-glucuronic acid or L-iduronic acid) or D-galactose; the other sugar is *N*-acetylglucosamine or *N*-acetylgalactosamine (Figure 6-17). One or both of the sugars contain at least one anionic group (carboxylate or sulfate). Thus each GAG chain bears many negative charges.

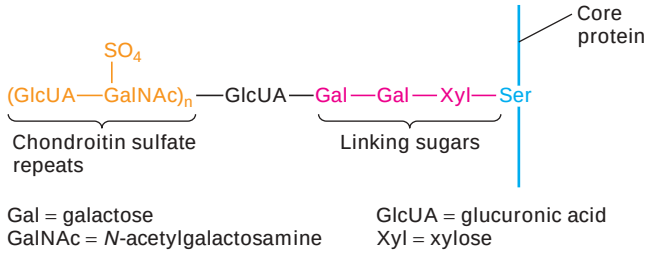


▲ FIGURE 6-17 **The repeating disaccharides of glycosaminoglycans (GAGs), the polysaccharide components of proteoglycans.** Each of the four classes of GAGs is formed by polymerization of monomer units into repeats of a particular disaccharide and subsequent modifications, including addition of sulfate groups and inversion (epimerization) of the carboxyl group on carbon 5 of D-glucuronic acid to yield L-iduronic acid. Heparin is generated by hypersulfation of heparan sulfate, whereas hyaluronan is unsulfated. The number *(n)* of disaccharides typically found in each glycosaminoglycan chain is given. The squiggly lines represent covalent bonds that are oriented either above (D-glucuronic acid) or below (L-iduronic acid) the ring.

GAGs are classified into several major types based on the nature of the repeating disaccharide unit: heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, and hyaluronan. A hypersulfated form of heparan sulfate called heparin, produced mostly by mast cells, plays a key role in allergic reactions. It is also used medically as an anticlotting drug because of its ability to activate a natural clotting inhibitor called antithrombin III.

As we will see in later chapters, complex signaling pathways direct the emergence of various cell types in the proper position and at the proper time in normal embryonic development. Laboratory generation and analysis of mutants with defects in proteoglycan production in *Drosophila melanogaster* (fruit fly), *C. elegans* (roundworm), and mice have clearly shown that proteoglycans play critical roles in development, most likely as modulators of various signaling pathways.

Biosynthesis of Proteoglycans With the exception of hyaluronan, which is discussed in the next section, all the major GAGs occur naturally as components of proteoglycans. Like other secreted and transmembrane glycoproteins, proteoglycan core proteins are synthesized on the endoplasmic reticulum (Chapter 16). The GAG chains are assembled on these cores in the Golgi complex. To generate heparan or chondroitin sulfate chains, a three-sugar "linker" is first attached to the hydroxyl side chains of certain serine residues in a core protein (Figure 6-18). In contrast, the linkers for the addition of keratan sulfate chains are oligosaccharide chains attached to asparagine residues; such ***N*-linked oligosaccharides** are present in most glycoproteins, although only a subset carry GAG chains. All GAG chains are elongated by the alternating addition of sugar monomers to form the disaccharide repeats characteristic of a particular GAG; the chains are often modified subsequently by the covalent linkage of small molecules such as sulfate. The mechanisms responsible for determining which proteins are modified with GAGs, the sequence of disaccharides to be added, the sites to be sulfated, and the lengths of the GAG chains are unknown. The ratio of polysaccharide to protein in all proteoglycans is much higher than that in most other glycoproteins.

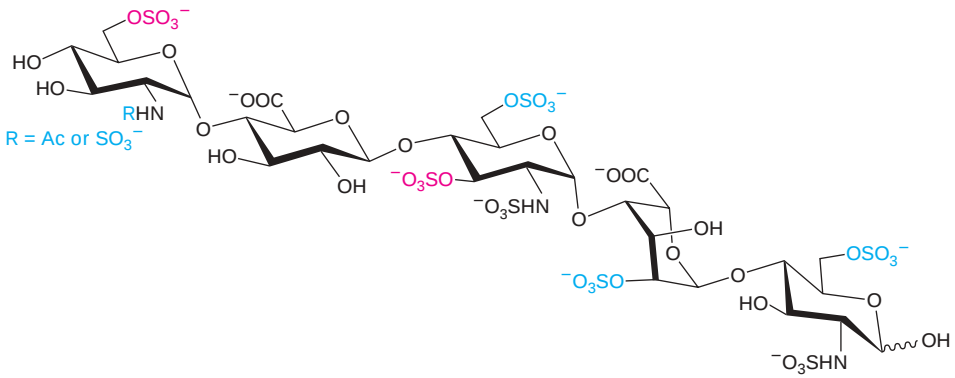


▲ FIGURE 6-18 **Biosynthesis of heparan and chondroitin sulfate chains in proteoglycans.** Synthesis of a chondroitin sulfate chain (shown here) is initiated by transfer of a xylose residue to a serine residue in the core protein, most likely in the Golgi complex, followed by sequential addition of two galactose residues. Glucuronic acid and *N*-acetylgalactosamine residues are then added sequentially to these linking sugars, forming the chondroitin sulfate chain. Heparan sulfate chains are connected to core proteins by the same three-sugar linker.

Diversity of Proteoglycans The proteoglycans constitute a remarkably diverse group of molecules that are abundant in the extracellular matrix of all animal tissues and are also expressed on the cell surface. For example, of the five major classes of heparan sulfate proteoglycans, three are located in the extracellular matrix (perlecan, agrin, and type XVIII collagen) and two are cell-surface proteins. The latter include integral membrane proteins (syndecans) and GPI-anchored proteins (glypicans); the GAG chains in both types of cell-surface proteoglycans extend into the extracellular space. The sequences and lengths of proteoglycan core proteins vary considerably, and the number of attached GAG chains ranges from just a few to more than 100. Moreover, a core protein is often linked to two different types of GAG chains (e.g., heparan sulfate and chondroitin sulfate), generating a "hybrid" proteoglycan. Thus, the molecular weight and charge density of a population of proteoglycans can be expressed only as an average; the composition and sequence of individual molecules can differ considerably.

Perlecan, the major secreted proteoglycan in basal laminae, consists of a large multidomain core protein (≈400 kDa) with three or four specialized GAG chains. Both the protein and the GAG components of perlecan contribute to its ability to incorporate into and define the structure and function of basal laminae. Because of its multiple domains with distinctive binding properties, perlecan can cross-link not only ECM components to one another but also certain cell-surface molecules to ECM components.

Syndecans are expressed by epithelial cells and many other cell types. These cell-surface proteoglycans bind to collagens and multiadhesive matrix proteins such as the fibronectins, which are discussed in Section 6.4. In this way, cell-surface proteoglycans can anchor cells to the extracellular matrix. Like that of many integral membrane proteins, the cytosolic domain of syndecan interacts with the actin cytoskeleton and in some cases with intracellular regulatory molecules. In addition, cell-surface proteoglycans bind many protein growth factors and other external signaling molecules, thereby helping to regulate cellular metabolism and function. For instance, syndecans in the hypothalamic region of the brain modulate feeding behavior in response to food deprivation (fasted state). They do so by participating in the binding of antisatiety peptides to cell-surface receptors that help control feeding behavior. In the fed state, the syndecan extracellular domain decorated with heparan sulfate chains is released from the surface by proteolysis, thus suppressing the activity of the antisatiety peptides and feeding behavior. In mice engineered to overexpress the syndecan-1 gene in the hypothalamic region of the brain and other tissues, normal control of feeding by antisatiety peptides is disrupted and the animals overeat and become obese. Other examples of proteoglycans interacting with external signaling molecules are described in Chapter 14.



◀ FIGURE 6-19 **Pentasaccharide GAG sequence that regulates the activity of antithrombin III (ATIII).** Sets of modified five-residue sequences in heparin with the composition shown here bind to ATIII and activate it, thereby inhibiting blood clotting. The sulfate groups in red type are essential for this heparin function; the modifications in blue type may be present but are not essential. Other sets of modified GAG sequences are thought to regulate the activity of other target proteins. [Courtesy of Robert Rosenberg and Balagurunathan Kuberan.]

Modifications in Glycosaminoglycan (GAG) Chains Can Determine Proteoglycan Functions

As is the case with the sequence of amino acids in proteins, the arrangement of the sugar residues in GAG chains and the modification of specific sugars (e.g., addition of sulfate) in the chains can determine their function and that of the proteoglycans containing them. For example, groupings of certain modified sugars in the GAG chains of heparin sulfate proteoglycans can control the binding of growth factors to certain receptors, the activities of proteins in the blood-clotting cascade, and the activity of lipoprotein lipase, a membrane-associated enzyme that hydrolyzes triglycerides to fatty acids (Chapter 18).

For years, the chemical and structural complexity of proteoglycans posed a daunting barrier to an analysis of their structures and an understanding of their many diverse functions. In recent years, investigators employing classical and new state-of-the-art biochemical techniques (e.g., capillary high-pressure liquid chromatography), mass spectrometry, and genetics have begun to elucidate the detailed structures and functions of these ubiquitous ECM molecules. The results of ongoing studies suggest that sets of sugar-residue sequences containing some modifications in common, rather than single unique sequences, are responsible for specifying distinct GAG functions. A case in point is a set of five-residue (pentasaccharide) sequences found in a subset of heparin GAGs that control the activity of antithrombin III (ATIII), an inhibitor of the key blood-clotting protease thrombin. When these pentasaccharide sequences in heparin are sulfated at two specific positions, heparin can activate ATIII, thereby inhibiting clot formation (Figure 6-19). Several other sulfates can be present in the active pentasaccharide in various combinations, but they are not essential for the anticlotting activity of heparin. The rationale for generating sets of similar active sequences rather than a single unique sequence and the mechanisms that control GAG biosynthetic pathways, permitting the generation of such active sequences, are not well understood.

KEY CONCEPTS OF SECTION 6.3

The Extracellular Matrix of Epithelial Sheets

- The basal lamina, a thin meshwork of extracellular matrix (ECM) molecules, separates most epithelia and other organized groups of cells from adjacent connective tissue. Together, the basal lamina and collagenous reticular lamina form a structure called the basement membrane.
- Four ECM proteins are found in all basal laminae (see Figure 6-13): type IV collagen, laminin (a multiadhesive matrix protein), entactin (nidogen), and perlecan (a proteoglycan).
- Cell-surface adhesion receptors (e.g., α6β4 integrin in hemidesmosomes) anchor cells to the basal lamina, which in turn is connected to other ECM components (see Figure 6-1).
- Repeating sequences of Gly-X-Y give rise to the collagen triple-helical structure (see Figure 6-14). Different collagens are distinguished by the length and chemical modifications of their α chains and by the segments that interrupt or flank their triple-helical regions.
- The large, flexible molecules of type IV collagen interact end to end and laterally to form a meshlike scaffold to which other ECM components and adhesion receptors can bind (see Figure 6-15).
- Laminin and other multiadhesive matrix proteins are multidomain molecules that bind multiple adhesion receptors and ECM components.
- Proteoglycans consist of membrane-associated or secreted core proteins covalently linked to one or more glycosaminoglycan (GAG) chains, which are linear polymers of sulfated disaccharides.
- Perlecan, a large secreted proteoglycan present primarily in the basal lamina, binds many ECM components and adhesion receptors.
- Cell-surface proteoglycans such as the syndecans facilitate cell–matrix interactions and help present certain external signaling molecules to their cell-surface receptors.

6.4 The Extracellular Matrix of Nonepithelial Tissues

We have seen how diverse CAMs and adhesion receptors participate in the assembly of animal cells into epithelial sheets that rest on and adhere to a well-defined ECM structure, the basal lamina. The same or similar molecules mediate and control cell–cell and cell–matrix interactions in connective, muscle, and neural tissues and between blood cells and the surrounding vessels. In this section, we consider some of the ECM molecules characteristic of these nonepithelial tissues. We also describe the synthesis of fibrillar collagens, which are the most abundant proteins in animals. The interactions entailing CAMs and adhesion receptors expressed by various nonepithelial cells, which serve a wide variety of distinctive functions, are covered in Section 6.5.

TABLE 6-1 Selected Collagens

Type	Molecule Composition	Structural Features	Representative Tissues
FIBRILLAR COLLAGENS			
I	$[\alpha1(I)]_2[\alpha2(I)]$	300-nm-long fibrils	Skin, tendon, bone, ligaments, dentin, interstitial tissues
II	$[\alpha1(II)]_3$	300-nm-long fibrils	Cartilage, vitreous humor
III	$[\alpha1(III)]_3$	300-nm-long fibrils; often with type I	Skin, muscle, blood vessels
V	$[\alpha1(V)_2\ \alpha2(V)]$, $[\alpha1(V)_3]$	390-nm-long fibrils with globular N-terminal extension; often with type I	Cornea, teeth, bone, placenta, skin, smooth muscle
FIBRIL-ASSOCIATED COLLAGENS			
VI	$[\alpha1(VI)][\alpha2(VI)]$	Lateral association with type I; periodic globular domains	Most interstitial tissues
IX	$[\alpha1(IX)][\alpha2(IX)][\alpha3(IX)]$	Lateral association with type II; N-terminal globular domain; bound GAG	Cartilage, vitreous humor
SHEET-FORMING AND ANCHORING COLLAGENS			
IV	$[\alpha1(IV)]_2[\alpha2(IV)]$	Two-dimensional network	All basal laminae
VII	$[\alpha1(VII)]_3$	Long fibrils	Below basal lamina of the skin
XV	$[\alpha1(XV)]_3$	Core protein of chondroitin sulfate proteoglycan	Widespread; near basal lamina in muscle
TRANSMEMBRANE COLLAGENS			
XIII	$[\alpha1(XIII)]_3$	Integral membrane protein	Hemidesmosomes in skin
XVII	$[\alpha1(XVII)]_3$	Integral membrane protein	Hemidesmosomes in skin
HOST DEFENSE COLLAGENS			
Collectins		Oligomers of triple helix; lectin domains	Blood, alveolar space
C1q		Oligomers of triple helix	Blood (complement)
Class A scavenger receptors		Homotrimeric membrane proteins	Macrophages

SOURCES: K. Kuhn, 1987, in R. Mayne and R. Burgeson, eds., *Structure and Function of Collagen Types,* Academic Press, p. 2; and M. van der Rest and R. Garrone, 1991, *FASEB J.* **5**:2814.

Fibrillar Collagens Are the Major Fibrous Proteins in the Extracellular Matrix of Connective Tissues

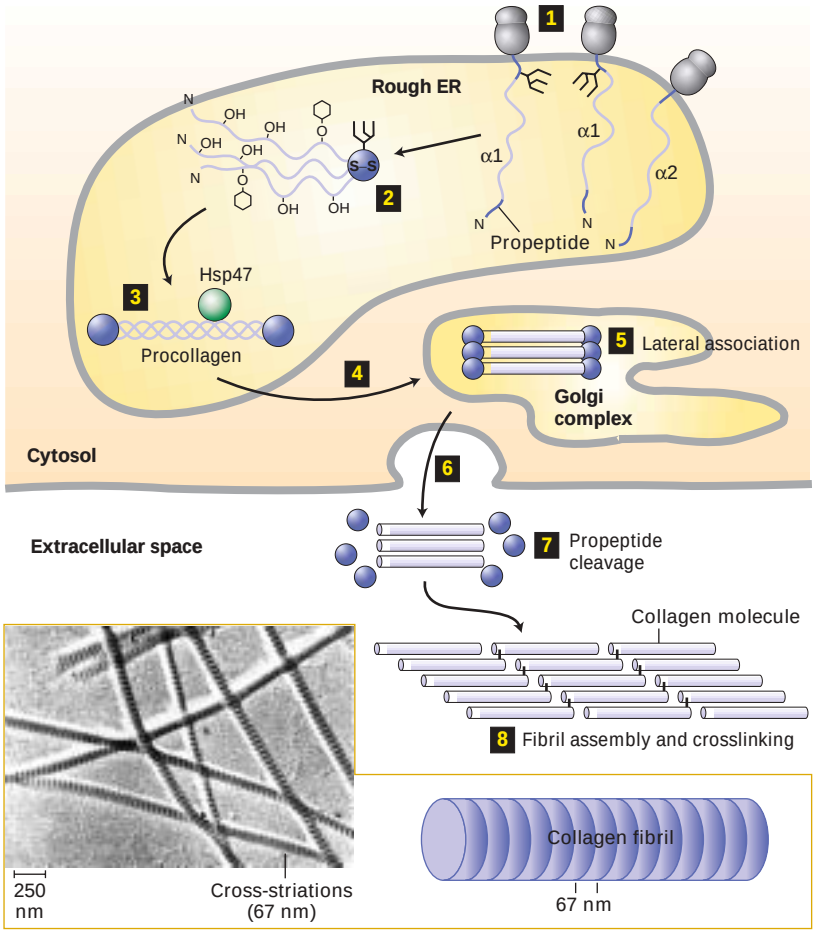
Connective tissue, such as tendon and cartilage, differs from other solid tissues in that most of its volume is made up of extracellular matrix rather than cells. This matrix is packed with insoluble protein fibers and contains proteoglycans, various multiadhesive proteins, and **hyaluronan,** a very large, nonsulfated GAG. The most abundant fibrous protein in connective tissue is collagen. Rubberlike elastin fibers, which can be stretched and relaxed, also are present in deformable sites (e.g., skin, tendons, heart). As discussed later, the fibronectins, a family of multiadhesive matrix proteins, form their own distinct fibrils in the matrix of some connective tissues. Although several types of cells are found in connective tissues, the various ECM components are produced largely by cells called **fibroblasts.**

About 80–90 percent of the collagen in the body consists of types I, II, and III collagens, located primarily in connective tissues. Because of its abundance in tendon-rich tissue such as rat tail, type I collagen is easy to isolate and was the first collagen to be characterized. Its fundamental structural unit is a long (300-nm), thin (1.5-nm-diameter) triple helix consisting of two α1(I) chains and one α2(I) chain, each precisely 1050 amino acids in length (see Figure 6-14). The triple-stranded molecules associate into higher-order polymers called collagen *fibrils,* which in turn often aggregate into larger bundles called collagen *fibers.*

The minor classes of collagen include *fibril-associated collagens,* which link the fibrillar collagens to one another or to other ECM components; *sheet-forming and anchoring collagens,* which form two-dimensional networks in basal laminae (type IV) and connect the basal lamina in skin to the underlying connective tissue (type VII); *transmembrane collagens,* which function as adhesion receptors; and *host defense collagens,* which help the body recognize and eliminate pathogens. Table 6-1 lists specific examples in the various classes of collagens. Interestingly, several collagens (e.g., types XVIII and XV) function as core proteins in proteoglycans.

Formation of Collagen Fibrils Begins in the Endoplasmic Reticulum and Is Completed Outside the Cell

Collagen biosynthesis and secretion follow the normal pathway for a secreted protein, which is described in detail in Chapters 16 and 17. The collagen α chains are synthesized as longer precursors, called pro-α chains, by ribosomes attached to the endoplasmic reticulum (ER). The pro-α chains undergo a series of covalent modifications and fold into triple-helical *procollagen* molecules before their release from cells (Figure 6-20).



◀ FIGURE 6-20 **Major events in biosynthesis of fibrillar collagens.** Step 1: Procollagen α chains are synthesized on ribosomes associated with the endoplasmic reticulum (ER) membrane, and asparagine-linked oligosaccharides are added to the C-terminal propeptide. Step 2: Propeptides associate to form trimers and are covalently linked by disulfide bonds, and selected residues in the Gly-X-Y triplet repeats are covalently modified [certain prolines and lysines are hydroxylated, galactose (Gal) or galactose-glucose (hexagons) is attached to some hydroxylysines, prolines are cis → trans isomerized]. Step 3: The modifications facilitate zipperlike formation, stabilization of triple helices, and binding by the chaperone protein Hsp47 (Chapter 16), which may stabilize the helices or prevent premature aggregation of the trimers or both. Steps 4 and 5: The folded procollagens are transported to and through the Golgi apparatus, where some lateral association into small bundles takes place. The chains are then secreted (step 6),the N- and C-terminal propeptides are removed (step 7), and the trimers assemble into fibrils and are covalently cross-linked (step 8). The 67-nm staggering of the trimers gives the fibrils a striated appearance in electron micrographs (*inset*). [Adapted from A. V. Persikov and B. Brodsky, 2002, *Proc. Nat'l. Acad. Sci. USA* **99**(3):1101–1103.]

After the secretion of procollagen from the cell, extracellular peptidases (e.g., bone morphogenetic protein-1) remove the N-terminal and C-terminal propeptides. In regard to fibrillar collagens, the resulting molecules, which consist almost entirely of a triple-stranded helix, associate laterally to generate fibrils with a diameter of 50–200 nm. In fibrils, adjacent collagen molecules are displaced from one another by 67 nm, about one-quarter of their length. This staggered array produces a striated effect that can be seen in electron micrographs of collagen fibrils (see Figure 6-20, *inset*). The unique properties of the fibrous collagens (e.g., types I, II, III) are mainly due to the formation of fibrils.

Short non-triple-helical segments at either end of the collagen α chains are of particular importance in the formation of collagen fibrils. Lysine and hydroxylysine side chains in these segments are covalently modified by extracellular lysyl oxidases to form aldehydes in place of the amine group at the end of the side chain. These reactive aldehyde groups form covalent cross-links with lysine, hydroxylysine, and histidine residues in adjacent molecules. These cross-links stabilize the side-by-side packing of collagen molecules and generate a strong fibril. The removal of the propeptides and covalent cross-linking take place in the extracellular space to prevent the potentially catastrophic assembly of fibrils within the cell.

MEDICINE

The post-translational modifications of pro-α chains are crucial for the formation of mature collagen molecules and their assembly into fibrils. Defects in these modifications have serious consequences, as ancient mariners frequently experienced. For example, ascorbic acid (vitamin C) is an essential cofactor for the hydroxylases responsible for adding hydroxyl groups to proline and lysine residues in pro-α chains. In cells deprived of ascorbate, as in the disease *scurvy*, the pro-α chains are not hydroxylated sufficiently to form stable triple-helical procollagen at normal body temperature, and the procollagen that forms cannot assemble into normal fibrils. Without the structural support of collagen, blood vessels, tendons, and skin become fragile. Because fresh fruit in the diet can supply sufficient vitamin C to support the formation of normal collagen, early British sailors were provided with limes to prevent scurvy, leading to their being called "limeys."

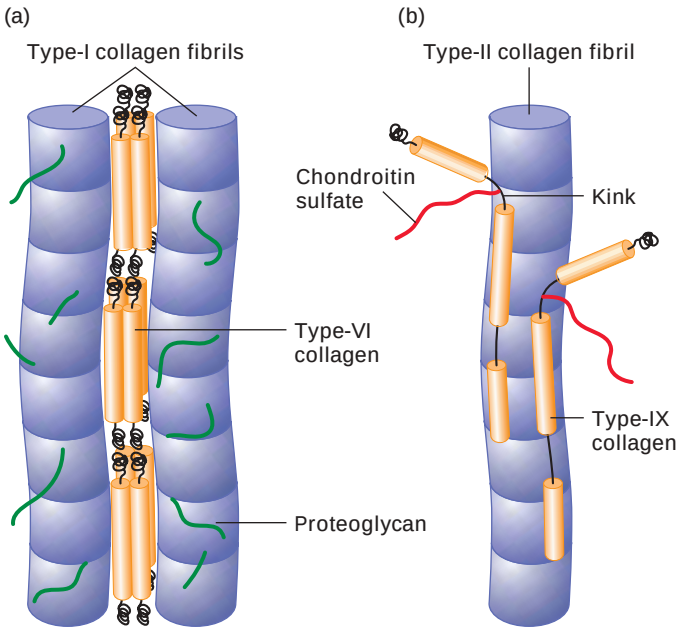
Rare mutations in lysyl hydroxylase genes cause Bruck syndrome and one form of Ehlers-Danlos syndrome. Both disorders are marked by connective-tissue defects, although their clinical symptoms differ. ▮

Type I and II Collagens Form Diverse Structures and Associate with Different Nonfibrillar Collagens

Collagens differ in their ability to form fibers and to organize the fibers into networks. In tendons, for instance, long type I collagen fibrils are packed side by side in parallel bundles, forming thick collagen fibers. Tendons connect muscles to bones and must withstand enormous forces. Because type I collagen fibers have great tensile strength, tendons can be stretched without being broken. Indeed, gram for gram, type I collagen is stronger than steel. Two quantitatively minor fibrillar collagens, type V and type XI, coassemble into fibers with type I collagen, thereby regulating the structures and properties of the fibers. Incorporation of type V collagen, for example, results in smaller-diameter fibers.

Type I collagen fibrils are also used as the reinforcing rods in the construction of bone. Bones and teeth are hard and strong because they contain large amounts of dahllite, a crystalline calcium- and phosphate-containing mineral. Most bones are about 70 percent mineral and 30 percent protein, the vast majority of which is type I collagen. Bones form when certain cells (chondrocytes and osteoblasts) secrete collagen fibrils that are then mineralized by deposition of small dahllite crystals.

In many connective tissues, type VI collagen and proteoglycans are noncovalently bound to the sides of type I fibrils and may bind the fibrils together to form thicker collagen fibers (Figure 6-21a). Type VI collagen is unusual in that the molecule consists of a relatively short triple helix with glob-



▲ FIGURE 6-21 **Interactions of fibrous collagens with nonfibrous fibril-associated collagens.** (a) In tendons, type I fibrils are all oriented in the direction of the stress applied to the tendon. Proteoglycans and type VI collagen bind noncovalently to fibrils, coating the surface. The microfibrils of type VI collagen, which contain globular and triple-helical segments, bind to type I fibrils and link them together into thicker fibers. (b) In cartilage, type IX collagen molecules are covalently bound at regular intervals along type II fibrils. A chondroitin sulfate chain, covalently linked to the α2(IX) chain at the flexible kink, projects outward from the fibril, as does the globular N-terminal region. [Part (a), see R. R. Bruns et al., 1986, *J. Cell Biol.* **103**:393. Part (b), see L. M. Shaw and B. Olson, 1991, *Trends Biochem. Sci.* **18**:191]

ular domains at both ends. The lateral association of two type VI monomers generates an "antiparallel" dimer. The end-to-end association of these dimers through their globular domains forms type VI "microfibrils." These microfibrils have a beads-on-a-string appearance, with about 60-nm-long triple-helical regions separated by 40-nm-long globular domains.

The fibrils of type II collagen, the major collagen in cartilage, are smaller in diameter than type I fibrils and are oriented randomly in a viscous proteoglycan matrix. The rigid collagen fibrils impart a strength and compressibility to the matrix and allow it to resist large deformations in shape. This property allows joints to absorb shocks. Type II fibrils are cross-linked to matrix proteoglycans by type IX collagen, another fibril-associated collagen. Type IX collagen and several related types have two or three triple-helical segments connected by flexible kinks and an N-terminal globular segment (Figure 6-22b). The globular N-terminal segment of type IX collagen extends from the fibrils at the end of one of its helical segments, as does a GAG chain that is sometimes linked to one of the type IX chains. These protruding nonhelical structures are thought to anchor the type II fibril to proteoglycans and other components of the matrix. The interrupted triple-helical structure of type IX and related collagens prevents them from assembling into fibrils, although they can associate with fibrils formed from other collagen types and form covalent cross-links to them.

Certain mutations in the genes encoding collagen α1(I) or α2(I) chains, which form type I collagen, lead to *osteogenesis imperfecta*, or brittle-bone disease. Because every third position in a collagen α chain must be a glycine for the triple helix to form (see Figure 6-14), mutations of glycine to almost any other amino acid are deleterious, resulting in poorly formed and unstable helices. Only one defective α chain of the three in a collagen molecule can disrupt the whole molecule's triple-helical structure and function. A mutation in a single copy (allele) of either the α1(I) gene or the α2(I) gene, which are located on nonsex chromosomes (autosomes), can cause this disorder. Thus it normally shows autosomal dominant inheritance (Chapter 9). ▮

Hyaluronan Resists Compression and Facilitates Cell Migration

Hyaluronan, also called hyaluronic acid (HA) or hyaluronate, is a nonsulfated GAG formed as a disaccharide repeat composed of glucuronic acid and *N*-acetylglucosamine (see Figure 6-17a) by a plasma-membrane-bound enzyme (HA synthase) and is directly secreted into the extracellular space. It is a major component of the extracellular matrix that surrounds migrating and proliferating cells, particularly in embryonic tissues. In addition, as will be described shortly, hyaluronan forms the backbone of complex proteoglycan aggregates found in many extracellular matrices, particularly cartilage. Because of its remarkable physical properties, hyaluronan imparts stiffness and resilience as well as a lubricating quality to many types of connective tissue such as joints.

Hyaluronan molecules range in length from a few disaccharide repeats to ≈25,000. The typical hyaluronan in joints such as the elbow has 10,000 repeats for a total mass of 4×10^6 Da and length of 10 μm (about the diameter of a small cell). Individual segments of a hyaluronan molecule fold into a rodlike conformation because of the β glycosidic linkages between the sugars and extensive intrachain hydrogen bonding. Mutual repulsion between negatively charged carboxylate groups that protrude outward at regular intervals also contributes to these local rigid structures. Overall, however, hyaluronan is not a long, rigid rod as is fibrillar collagen; rather, in solution it is very flexible, bending and twisting into many conformations, forming a random coil.

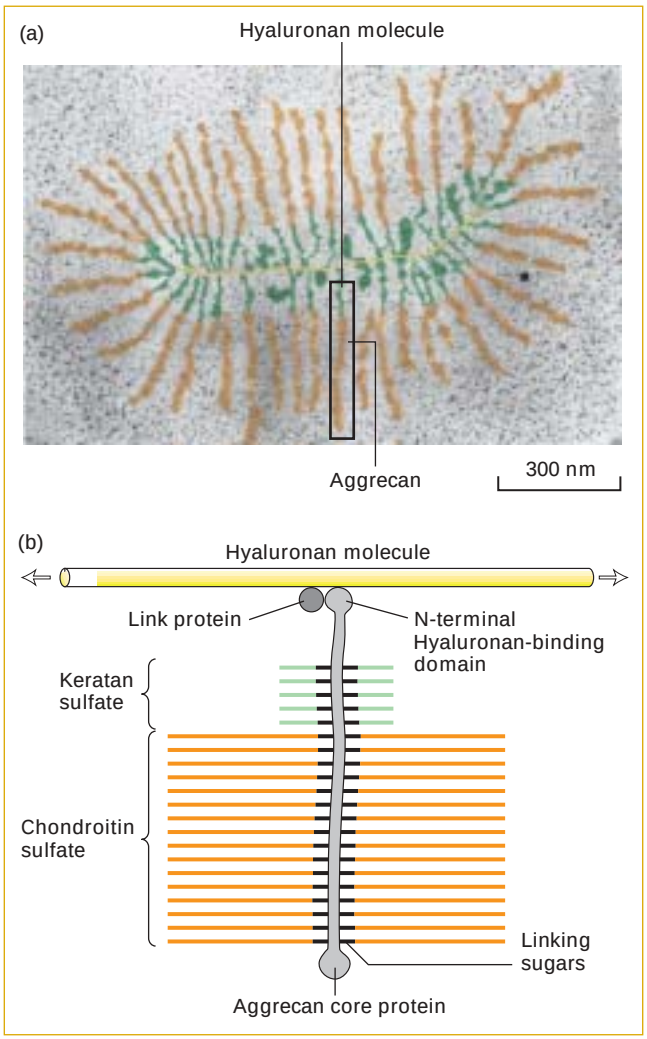
Because of the large number of anionic residues on its surface, the typical hyaluronan molecule binds a large amount of water and behaves as if it were a large hydrated sphere with a diameter of ≈500 nm. As the concentration of hyaluronan increases, the long chains begin to entangle, forming a viscous gel. Even at low concentrations, hyaluronan forms a hydrated gel; when placed in a confining space, such as in a matrix between two cells, the long hyaluronan molecules will tend to push outward. This outward pushing creates a swelling, or *turgor pressure*, within the extracellular space. In addition, the binding of cations by COO^- groups on the surface of hyaluronan increases the concentration of ions and thus the osmotic pressure in the gel. As a result, large amounts of water are taken up into the matrix, contributing to the turgor pressure. These swelling forces give connective tissues their ability to resist compression forces, in contrast with collagen fibers, which are able to resist stretching forces.

Hyaluronan is bound to the surface of many migrating cells by a number of adhesion receptors (e.g., one called CD44) containing HA-binding domains, each with a similar three-dimensional conformation. Because of its loose, hydrated, porous nature, the hyaluronan "coat" bound to cells appears to keep cells apart from one another, giving them the freedom to move about and proliferate. The cessation of cell movement and the initiation of cell–cell attachments are frequently correlated with a decrease in hyaluronan, a decrease in HA-binding cell-surface molecules, and an increase in the extracellular enzyme hyaluronidase, which degrades hyaluronan in the matrix. These functions of hyaluronan are particularly important during the many cell migrations that facilitate differentiation and in the release of a mammalian egg cell (oocyte) from its surrounding cells after ovulation.

Association of Hyaluronan and Proteoglycans Forms Large, Complex Aggregates

The predominant proteoglycan in cartilage, called *aggrecan*, assembles with hyaluronan into very large aggregates, illustrative of the complex structures that proteoglycans sometimes form. The backbone of the cartilage proteoglycan

aggregate is a long molecule of hyaluronan to which multiple aggrecan molecules are bound tightly but noncovalently (Figure 6-22a). A single aggrecan aggregate, one of the largest macromolecular complexes known, can be more than 4 mm long and have a volume larger than that of a bacterial cell.



▲ FIGURE 6-22 **Structure of proteoglycan aggregate from cartilage.** (a) Electron micrograph of an aggrecan aggregate from fetal bovine epiphyseal cartilage. Aggrecan core proteins are bound at ≈40-nm intervals to a molecule of hyaluronan. (b) Schematic representation of an aggrecan monomer bound to hyaluronan. In aggrecan, both keratan sulfate and chondroitin sulfate chains are attached to the core protein. The N-terminal domain of the core protein binds noncovalently to a hyaluronan molecule. Binding is facilitated by a link protein, which binds to both the hyaluronan molecule and the aggrecan core protein. Each aggrecan core protein has 127 Ser-Gly sequences at which GAG chains can be added. The molecular weight of an aggrecan monomer averages 2×10^6. The entire aggregate, which may contain upward of 100 aggrecan monomers, has a molecular weight in excess of 2×10^8. [Part (a) from J. A. Buckwalter and L. Rosenberg, 1983, *Coll. Rel. Res.* **3**:489; courtesy of L. Rosenberg.]

These aggregates give cartilage its unique gel-like properties and its resistance to deformation, essential for distributing the load in weight-bearing joints.

The aggrecan core protein (≈250,000 MW) has one N-terminal globular domain that binds with high affinity to a specific decasaccharide sequence within hyaluronan. This specific sequence is generated by covalent modification of some of the repeating disaccharides in the hyaluronan chain. The interaction between aggrecan and hyaluronan is facilitated by a link protein that binds to both the aggrecan core protein and hyaluronan (Figure 6-22b). Aggrecan and the link protein have in common a "link" domain, ≈100 amino acids long, that is found in numerous matrix and cell-surface hyaluronan-binding proteins in both cartilaginous and noncartilaginous tissues. Almost certainly these proteins arose in the course of evolution from a single ancestral gene that encoded just this domain.

MEDICINE

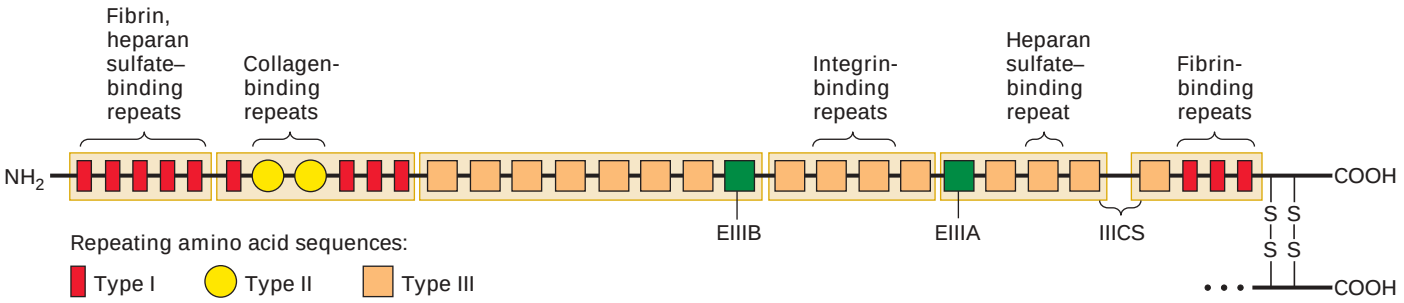
The importance of the GAG chains that are part of various matrix proteoglycans is illustrated by the rare humans who have a genetic defect in one of the enzymes required for synthesis of the GAG dermatan sulfate. These persons have many defects in their bones, joints, and muscles; do not grow to normal height; and have wrinkled skin, giving them a prematurely aged appearance. ▮

Fibronectins Connect Many Cells to Fibrous Collagens and Other Matrix Components

Many different cell types synthesize **fibronectin,** an abundant multiadhesive matrix protein found in all vertebrates. The discovery that fibronectin functions as an adhesive molecule stemmed from observations that it is present on the surfaces of normal fibroblastic cells, which adhere tightly to petri dishes in laboratory experiments, but is absent from the surfaces of tumorigenic cells, which adhere weakly. The 20 or so isoforms of fibronectin are generated by alternative splicing of the RNA transcript produced from a single gene (see Figure 4-15). Fibronectins are essential for the migration and differentiation of many cell types in embryogenesis. These proteins are also important for wound healing because they promote blood clotting and facilitate the migration of macrophages and other immune cells into the affected area.

Fibronectins help attach cells to the extracellular matrix by binding to other ECM components, particularly fibrous collagens and heparan sulfate proteoglycans, and to cell-surface adhesion receptors such as integrins (see Figure 6-2). Through their interactions with adhesion receptors (e.g., α5β1 integrin), fibronectins influence the shape and movement of cells and the organization of the cytoskeleton. Conversely, by regulating their receptor-mediated attachments to fibronectin and other ECM components, cells can sculpt the immediate ECM environment to suit their needs.

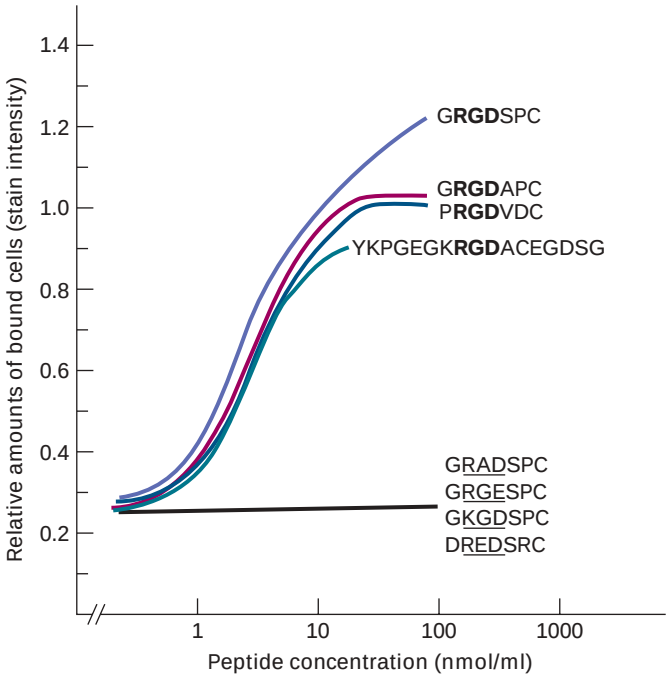
Fibronectins are dimers of two similar polypeptides linked at their C-termini by two disulfide bonds; each chain is about 60–70 nm long and 2–3 nm thick. Partial digestion



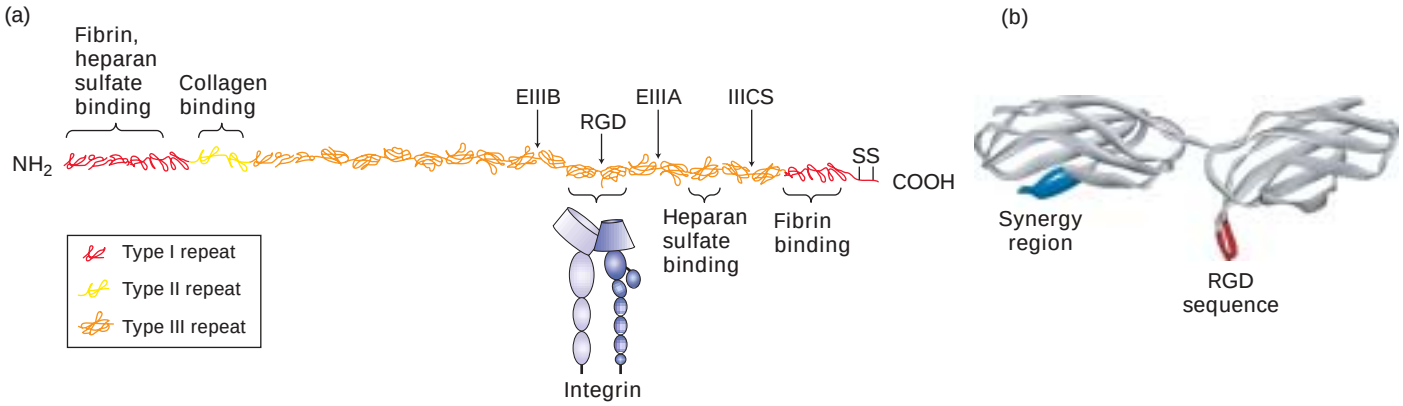
▲ FIGURE 6-23 **Organization of fibronectin chains.** Only one of the two chains present in the dimeric fibronectin molecule is shown; both chains have very similar sequences. Each chain contains about 2446 amino acids and is composed of three types of repeating amino acid sequences. Circulating fibronectin lacks one or both of the type III repeats designated EIIIA and EIIIB owing to alternative mRNA splicing (see Figure 4-15). At least five different sequences may be present in the IIICS region as a result of alternative splicing. Each chain contains six domains (tan boxes), some of which contain specific binding sites for heparan sulfate, fibrin (a major constituent of blood clots), collagen, and cell-surface integrins. The integrin-binding domain is also known as the cell-binding domain. [Adapted from G. Paolella, M. Barone, and F. Baralle, 1993, in M. Zern and L. Reid, eds., *Extracellular Matrix,* Marcel Dekker, pp. 3–24.]

of fibronectin with low amounts of proteases and analysis of the fragments showed that each chain comprises six functional regions with different ligand-binding specificities (Figure 6-23). Each region, in turn, contains multiple copies of certain sequences that can be classified into one of three types. These classifications are designated fibronectin type I, II, and III repeats, on the basis of similarities in amino acid sequence, although the sequences of any two repeats of a given type are not always identical. These linked repeats give the molecule the appearance of beads on a string. The combination of different repeats composing the regions, another example of combinatorial diversity, confers on fibronectin its ability to bind multiple ligands.

One of the type III repeats in the cell-binding region of fibronectin mediates binding to certain integrins. The results of studies with synthetic peptides corresponding to parts of this repeat identified the tripeptide sequence Arg-Gly-Asp, usually called the *RGD sequence,* as the minimal sequence within this repeat required for recognition by those integrins. In one study, heptapeptides containing the RGD sequence or a variation of this sequence were tested for their ability to mediate the adhesion of rat kidney cells to a culture dish. The results showed that heptapeptides containing the RGD sequence mimicked intact fibronectin's ability to stimulate integrin-mediated adhesion, whereas variant heptapeptides lacking this sequence were ineffective (Figure 6-24).

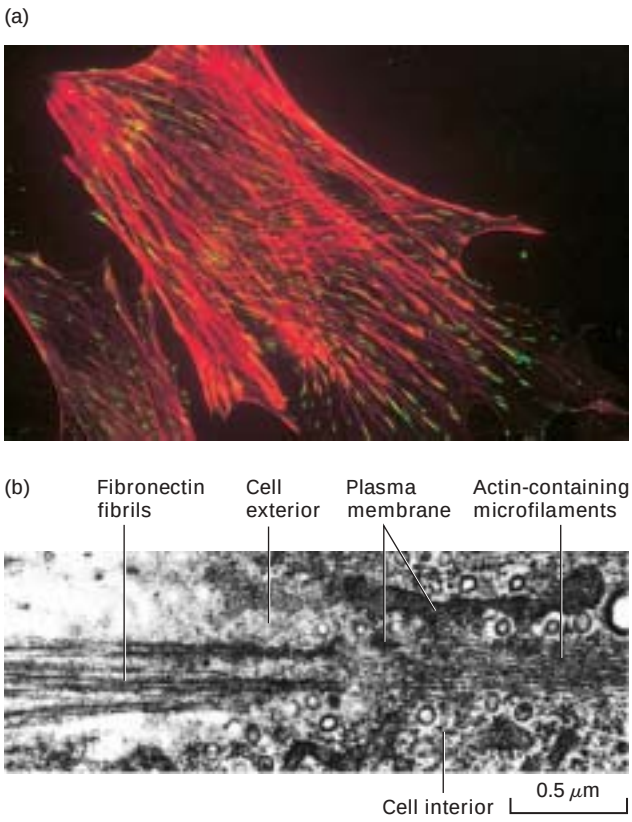


◀ EXPERIMENTAL FIGURE 6-24 **A specific tripeptide sequence (RGD) in the cell-binding region of fibronectin is required for adhesion of cells.** The cell-binding region of fibronectin contains an integrin-binding heptapeptide sequence, GRDSPC in the single-letter amino acid code (see Figure 2-13). This heptapeptide and several variants were synthesized chemically. Different concentrations of each synthetic peptide were added to polystyrene dishes that had the protein immunoglobulin G (IgG) firmly attached to their surfaces; the peptides were then chemically cross-linked to the IgG. Subsequently, cultured normal rat kidney cells were added to the dishes and incubated for 30 minutes to allow adhesion. After the nonbound cells were washed away, the relative amounts of cells that had adhered firmly were determined by staining the bound cells with a dye and measuring the intensity of the staining with a spectrophotometer. The plots shown here indicate that cell adhesion increased above the background level with increasing peptide concentration for those peptides containing the RGD sequence but not for the variants lacking this sequence (modification underlined). [From M. D. Pierschbacher and E. Ruoslahti, 1984, *Proc. Nat'l. Acad. Sci. USA* **81**:5985.]



▲ FIGURE 6-25 **Model of fibronectin binding to integrin through its RGD-containing type III repeat.** (a) Scale model of fibronectin is shown docked by two type III repeats to the extracellular domains of integrin. Structures of fibronectin's domains were determined from fragments of the molecule. The EIIIA, EIIIB, and IIICS domains (not shown; see Figure 6-23) are variably spliced into the structure at locations indicated by arrows. (b) A high-resolution structure shows that the RGD binding sequence (red) extends outward in a loop from its compact type III domain on the same side of fibronectin as the synergy region (blue), which also contributes to high-affinity binding to integrins. [Adapted from D. J. Leahy et al., 1996, *Cell* **84**:161]

A three-dimensional model of fibronectin binding to integrin based on structures of parts of both fibronectin and integrin has been assembled (Figure 6-25a). In a high-resolution structure of the integrin-binding fibronectin type III repeat and its neighboring type III domain, the RGD sequence is at the apex of a loop that protrudes outward from the molecule, in a position facilitating binding to integrins (Figure 6-25a, b). Although the RGD sequence is required for binding to several integrins, its affinity for integrins is substantially less than that of intact fibronectin or of the entire cell-binding region in fibronectin. Thus structural features near to the RGD sequence in fibronectins (e.g., parts of adjacent repeats, such as the synergy region; see Figure 6-25b) and in other RGD-containing proteins enhance their binding to certain integrins. Moreover, the simple soluble dimeric forms of fibronectin produced by the liver or fibroblasts are initially in a nonfunctional closed conformation that binds poorly to integrins because the RGD sequence is not readily accessible. The adsorption of fibronectin to a col-



▶ EXPERIMENTAL FIGURE 6-26 **Integrins mediate linkage between fibronectin in the extracellular matrix and the cytoskeleton.** (a) Immunofluorescent micrograph of a fixed cultured fibroblast showing colocalization of the α5β1 integrin and actin-containing stress fibers. The cell was incubated with two types of monoclonal antibody: an integrin-specific antibody linked to a green fluorescing dye and an actin-specific antibody linked to a red fluorescing dye. Stress fibers are long bundles of actin microfilaments that radiate inward from points where the cell contacts a substratum. At the distal end of these fibers, near the plasma membrane, the coincidence of actin (red) and fibronectin-binding integrin (green) produces a yellow fluorescence. (b) Electron micrograph of the junction of fibronectin and actin fibers in a cultured fibroblast. Individual actin-containing 7-nm microfilaments, components of a stress fiber, end at the obliquely sectioned cell membrane. The microfilaments appear in close proximity to the thicker, densely stained fibronectin fibrils on the outside of the cell. [Part (a) from J. Duband et al., 1988, *J. Cell Biol.* **107**:1385. Part (b) from I. J. Singer, 1979, *Cell* **16**:675; courtesy of I. J. Singer; copyright 1979, MIT.]

lagen matrix or the basal lamina or, experimentally, to a plastic tissue-culture dish results in a conformational change that enhances its ability to bind to cells. Most likely, this conformational change increases the accessibility of the RGD sequence for integrin binding.

Microscopy and other experimental approaches (e.g., biochemical binding experiments) have demonstrated the role of integrins in cross-linking fibronectin and other ECM components to the cytoskeleton. For example, the colocalization of cytoskeletal actin filaments and integrins within cells can be visualized by fluorescence microscopy (Figure 6-26a). The binding of cell-surface integrins to fibronectin in the matrix induces the actin cytoskeleton–dependent movement of some integrin molecules in the plane of the membrane. The ensuing mechanical tension due to the relative movement of different integrins bound to a single fibronectin dimer stretches the fibronectin. This stretching promotes self-association of the fibronectin into multimeric fibrils.

The force needed to unfold and expose functional self-association sites in fibronectin is much less than that needed to disrupt fibronectin–integrin binding. Thus fibronectin molecules remain bound to integrin while cell-generated mechanical forces induce fibril formation. In effect, the integrins through adapter proteins transmit the intracellular forces generated by the actin cytoskeleton to extracellular fibronectin. Gradually, the initially formed fibronectin fibrils mature into highly stable matrix components by covalent cross-linking. In some electron micrographic images, exterior fibronectin fibrils appear to be aligned in a seemingly continuous line with bundles of actin fibers within the cell (Figure 6-26b). These observations and the results from other studies provided the first example of a molecularly well defined adhesion receptor (i.e., an integrin) forming a bridge between the intracellular cytoskeleton and the extracellular matrix components—a phenomenon now known to be widespread.

KEY CONCEPTS OF SECTION 6.4

The Extracellular Matrix of Nonepithelial Tissues

- Connective tissue, such as tendon and cartilage, differs from other solid tissues in that most of its volume is made up of extracellular matrix (ECM) rather than cells.
- The synthesis of fibrillar collagen (e.g., types I, II, and III) begins inside the cell with the chemical modification of newly made α chains and their assembly into triple-helical procollagen within the endoplasmic reticulum. After secretion, procollagen molecules are cleaved, associate laterally, and are covalently cross-linked into bundles called fibrils, which can form larger assemblies called fibers (see Figure 6-20).
- The various collagens are distinguished by the ability of their helical and nonhelical regions to associate into fibrils, to form sheets, or to cross-link other collagen types (see Table 6-1).
- Hyaluronan, a highly hydrated GAG, is a major component of the ECM of migrating and proliferating cells. Certain cell-surface adhesion receptors bind hyaluronan to cells.
- Large proteoglycan aggregates containing a central hyaluronan molecule noncovalently bound to the core protein of multiple proteoglycan molecules (e.g., aggrecan) contribute to the distinctive mechanical properties of the matrix (see Figure 6-22).
- Fibronectins are abundant multiadhesive matrix proteins that play a key role in migration and cellular differentiation. They contain binding sites for integrins and ECM components (collagens, proteoglycans) and can thus attach cells to the matrix (see Figure 6-23).
- The tripeptide RGD sequence (Arg-Gly-Asp), found in fibronectins and some other matrix proteins, is recognized by several integrins.

6.5 Adhesive Interactions and Nonepithelial Cells

After adhesive interactions in epithelia form during differentiation, they often are very stable and can last throughout the life span of epithelial cells or until the cells undergo differentiation into loosely associated nonpolarized mesenchymal cells, the epithelial–mesenchymal transition. Although such long-lasting (nonmotile) adhesion also exists in nonepithelial tissues, some nonepithelial cells must be able to crawl across or through a layer of extracellular matrix or other cells. In this section, we describe various cell-surface structures in nonepithelial cells that mediate long-lasting adhesion and transient adhesive interactions that are especially adapted for the movement of cells. The detailed intracellular mechanisms used to generate the mechanical forces that propel cells and modify their shapes are covered in Chapter 19.

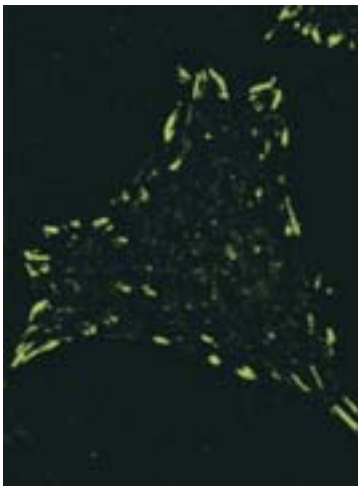
Integrin-Containing Adhesive Structures Physically and Functionally Connect the ECM and Cytoskeleton in Nonepithelial Cells

As already discussed in regard to epithelia, integrin-containing hemidesmosomes connect epithelial cells to the basal lamina and, through adapter proteins, to intermediate filaments of the cytoskeleton (see Figure 6-1). In nonepithelial cells, integrins in the plasma membrane also are clustered with other molecules in various adhesive structures called focal adhesions, focal contacts, focal complexes, 3D adhesions, and fibrillar adhesions and in circular adhesions called podosomes (Chapter 14). These structures are readily observed by fluorescence microscopy with the use of antibodies that recognize integrins or other coclustered molecules (Figure 6-27). Like cell–matrix anchoring junctions in epithelial cells, the various adhesive structures attach nonepithelial cells to the extracellular matrix;

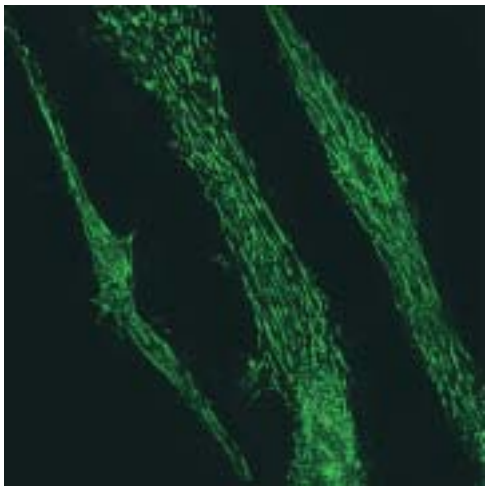
▶ EXPERIMENTAL FIGURE 6-27

Integrins cluster into adhesive structures with various morphologies in nonepithelial cells. Immunofluorescence methods were used to detect adhesive structures (green) on cultured cells. Shown here are focal adhesions (a) and 3D adhesions (b) on the surfaces of human fibroblasts. Cells were grown directly on the flat surface of a culture dish (a) or on a three-dimensional matrix of ECM components (b). The shape, distribution, and composition of the integrin-based adhesions formed by cells vary, depending on culture conditions. [Part (a) from B. Geiger et al., 2001, *Nature Rev. Mol. Cell Biol.* **2:**793. Part (b) courtesy of K. Yamada and E. Cukierman; see E. Cukierman et al., 2001, *Science* **294**:1708–12.]

(a) Focal adhesion



(b) 3D adhesion



they also contain dozens of intracellular adapter and associated proteins that mediate attachment to cytoskeletal actin filaments and activate adhesion-dependent signals for cell growth and cell motility.

Although found in many nonepithelial cells, integrin-containing adhesive structures have been studied most frequently in fibroblasts grown in cell culture on flat glass or plastic surfaces (substrata). These conditions only poorly approximate the three-dimensional ECM environment that normally surrounds such cells in vivo. When fibroblasts are cultured in three-dimensional ECM matrices derived from cells or tissues, they form adhesions to the three-dimensional ECM substratum, called 3D adhesions. These structures differ somewhat in composition, shape, distribution, and activity from the focal or fibrillar adhesions seen in cells growing on the flat substratum typically used in cell-culture experiments (see Figure 6-27). Cultured fibroblasts with these "more natural" anchoring junctions display greater adhesion and mobility, increased rates of cell proliferation, and spindle-shaped morphologies more like those of fibroblasts in tissues than do cells cultured on flat surfaces. These observations indicate that the topological, compositional, and mechanical (e.g., flexibility) properties of the extracellular matrix all play a role in controlling the shape and activity of a cell. Tissue-specific differences in these matrix characteristics probably contribute to the tissue-specific properties of cells.

TABLE 6-2 Selected Vertebrate Integrins*

Subunit Composition	Primary Cellular Distribution	Ligands
α1β1	Many types	Mainly collagens; also laminins
α2β1	Many types	Mainly collagens; also laminins
α4β1	Hematopoietic cells	Fibronectin; VCAM-1
α5β1	Fibroblasts	Fibronectin
αLβ2	T lymphocytes	ICAM-1, ICAM-2
αMβ2	Monocytes	Serum proteins (e.g., C3b, fibrinogen, factor X); ICAM-1
αIIbβ3	Platelets	Serum proteins (e.g., fibrinogen, von Willebrand factor, vitronectin); fibronectin
α6β4	Epithelial cells	Laminin

*The integrins are grouped into subfamilies having a common β subunit. Ligands shown in red are CAMs; all others are ECM or serum proteins. Some subunits can have multiply spliced isoforms with different cytosolic domains.

SOURCE: R. O. Hynes, 1992, *Cell* **69**:11.

Diversity of Ligand–Integrin Interactions Contributes to Numerous Biological Processes

Although most cells express several distinct integrins that bind the same ligand or different ligands, many integrins are expressed predominantly in certain types of cells. Table 6-2 lists a few of the numerous integrin-mediated interactions with ECM components or CAMs or both. Not only do many integrins bind more than one ligand, but several of their ligands bind to multiple integrins.

All integrins appear to have evolved from two ancient general subgroups: those that bind RGD-containing molecules (e.g., fibronectin) and those that bind laminin. For example, α5β1 integrin binds fibronectin, whereas the widely expressed α1β1 and α2β1 integrins, as well as the α6β4 integrin expressed by epithelial cells, bind laminin. The α1, α2, and several other integrin α subunits contain a distinctive inserted domain, the *I-domain*. The I-domain in some integrins (e.g., α1β1 and α2β1) mediates binding to various collagens. Other integrins containing α subunits with I-domains are expressed exclusively on leukocytes and hematopoietic cells; these integrins recognize cell-adhesion molecules on other cells, including members of the Ig superfamily (e.g., ICAMs, VCAMs), and thus participate in cell–cell adhesion.

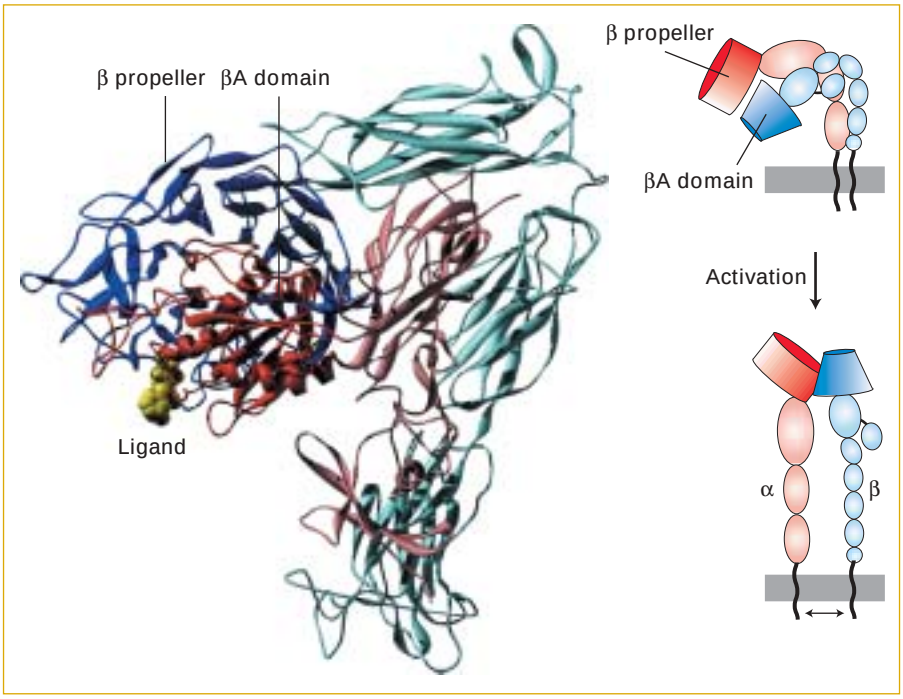
The diversity of integrins and their ECM ligands enables integrins to participate in a wide array of key biological processes, including the migration of cells to their correct locations in the formation the body plan of an embryo (morphogenesis) and in the inflammatory response. The importance of integrins in diverse processes is highlighted by the defects exhibited by knockout mice engineered to have mutations in each of almost all of the integrin subunit genes. These defects include major abnormalities in development, blood vessel formation, leukocyte function, the response to infection (inflammation), bone remodeling, and hemostasis.

Cell–Matrix Adhesion Is Modulated by Changes in the Binding Activity and Numbers of Integrins

Cells can exquisitely control the strength of integrin-mediated cell–matrix interactions by regulating the ligand-binding activity of integrins or their expression or both. Such regulation is critical to the role of these interactions in cell migration and other functions.

Many, if not all, integrins can exist in two conformations: a low-affinity (inactive) form and a high-affinity (active) form (Figure 6-28). The results of structural studies and experiments investigating the binding of ligands by integrins have provided a model of the changes that take place when integrins are activated. In the inactive state, the αβ heterodimer is bent, the conformation of the ligand-binding site at the tip of the molecule allows only low-affinity ligand binding, and the cytoplasmic C-terminal tails of the two subunits are closely bound together. In the "straight," active state, alterations in the conformation of the domains that form the binding site permit tighter (high-affinity) ligand binding, and the cytoplasmic tails separate.

These structural models also provide an attractive explanation for the ability of integrins to mediate outside-in and inside-out signaling. The binding of certain ECM molecules or CAMs on other cells to the bent, low-affinity structure would force the molecule to straighten and consequently separate the cytoplasmic tails. Intracellular adapters could "sense" the separation of the tails and, as a result, either bind or dissociate from the tails. The changes in these adapters



◀ FIGURE 6-28 **Model for integrin activation.** (*Left*) The molecular model is based on the x-ray crystal structure of the extracellular region of αvβ3 integrin in its inactive, low-affinity ("bent") form, with the α subunit in shades of blue and the β subunit in shades of red. The major ligand-binding sites are at the tip of the molecule where the β propeller domain (dark blue) and βA domain (dark red) interact. An RGD peptide ligand is shown in yellow. (*Right*) Activation of integrins is thought to be due to conformational changes that include straightening of the molecule, key movements near the β propeller and βA domains, which increases the affinity for ligands, and separation of the cytoplasmic domains, resulting in altered interactions with adapter proteins. See text for further discussion. [Adapted from M. Arnaout et al., 2002, *Curr. Opin. Cell Biol.* **14**:641, and R. O. Hynes, 2002, *Cell* **110**:673.]

could then alter the cytoskeleton and activate or inhibit intracellular signaling pathways. Conversely, changes in the metabolic state of the cells (e.g., changes in the platelet cytoskeleton that accompany platelet activation; see Figure 19-5) could cause intracellular adapters to bind to the tails or to dissociate from them and thus force the tails to either separate or associate. As a consequence, the integrin would either bend (inactivate) or straighten (activate), thereby altering its interaction with the ECM or other cells.

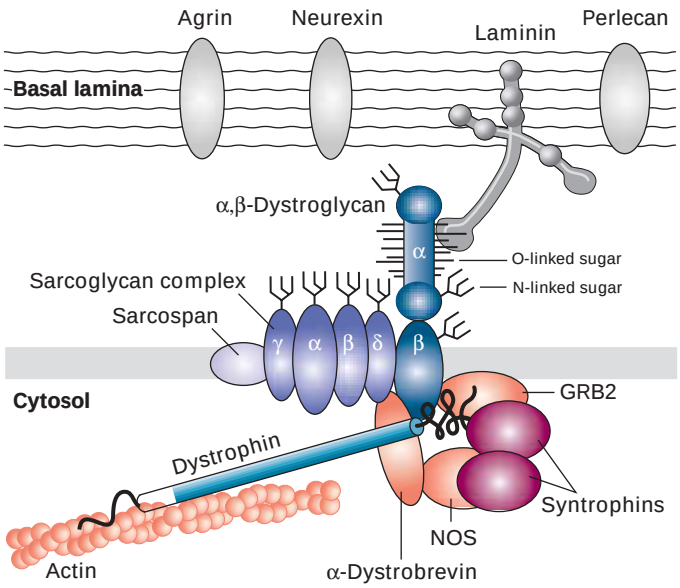
Platelet function provides a good example of how cell–matrix interactions are modulated by controlling integrin binding activity. In its basal state, the αIIbβ3 integrin present on the plasma membranes of platelets normally cannot bind tightly to its protein ligands (e.g., fibrinogen, fibronectin), all of which participate in the formation of a blood clot, because it is in the inactive (bent) conformation. The binding of a platelet to collagen or thrombin in a forming clot induces from the cytoplasm an activating conformational change in αIIbβ3 integrin that permits it to tightly bind clotting proteins and participate in clot formation. Persons with genetic defects in the β3 integrin subunit are prone to excessive bleeding, attesting to the role of this integrin in the formation of blood clots.

The attachment of cells to ECM components can also be modulated by altering the number of integrin molecules exposed on the cell surface. The α4β1 integrin, which is found on many hematopoietic cells (precursors of red and white blood cells), offers an example of this regulatory mechanism. For these hematopoietic cells to proliferate and differentiate, they must be attached to fibronectin synthesized by supportive ("stromal") cells in the bone marrow. The α4β1 integrin on hematopoietic cells binds to a Glu-Ile-Leu-Asp-Val (EILDV) sequence in fibronectin, thereby anchoring the cells to the matrix. This integrin also binds to a sequence in a CAM called vascular CAM-1 (VCAM-1), which is present on stromal cells of the bone marrow. Thus hematopoietic cells directly contact the stromal cells, as well as attach to the matrix. Late in their differentiation, hematopoietic cells decrease their expression of α4β1 integrin; the resulting reduction in the number of α4β1 integrin molecules on the cell surface is thought to allow mature blood cells to detach from the matrix and stromal cells in the bone marrow and subsequently enter the circulation.

Molecular Connections Between the ECM and Cytoskeleton Are Defective in Muscular Dystrophy

The importance of the adhesion receptor–mediated linkage between ECM components and the cytoskeleton is highlighted by a set of hereditary muscle-wasting diseases, collectively called muscular dystrophies. Duchenne muscular dystrophy (DMD), the most common type, is a sex-linked disorder, affecting 1 in 3300 boys, that results in cardiac or respiratory failure in the late teens or early twenties. The first clue to understanding the molecular basis of this disease came from the discovery that persons with DMD carry mutations in the gene encoding a protein named *dystrophin*. This very large protein was found to be a cytosolic adapter protein, binding to actin filaments and to an adhesion receptor called *dystroglycan*.

Dystroglycan is synthesized as a large glycoprotein precursor that is proteolytically cleaved into two subunits. The α subunit is a peripheral membrane protein, and the β subunit is a transmembrane protein whose extracellular domain associates with the α subunit (Figure 6-29). Multiple ***O*-linked oligosaccharides** are attached covalently to side-chain hydroxyl groups of serine and threonine residues in the α subunit. These *O*-linked oligosaccharides bind to various basal lamina components, including the multiadhesive matrix protein laminin and the proteoglycans perlecan and



▲ FIGURE 6-29 **Schematic model of the dystrophin glycoprotein complex (DGC) in skeletal muscle cells.** The DGC comprises three subcomplexes: the α,β dystroglycan subcomplex; the sarcoglycan/sarcospan subcomplex of integral membrane proteins; and the cytosolic adapter subcomplex comprising dystrophin, other adapter proteins, and signaling molecules. Through its *O*-linked sugars, β-dystroglycan binds to components of the basal lamina, such as laminin. Dystrophin—the protein defective in Duchenne muscular dystrophy—links β-dystroglycan to the actin cytoskeleton, and α-dystrobrevin links dystrophin to the sarcoglycan/sarcospan subcomplex. Nitric oxide synthase (NOS) produces nitric oxide, a gaseous signaling molecule, and GRB2 is a component of signaling pathways activated by certain cell-surface receptors (Chapter 14). See text for further discussion. [Adapted from S. J. Winder, 2001, *Trends Biochem. Sci.* **26**:118, and D. E. Michele and K. P. Campbell, 2003, *J. Biol. Chem.*]

agrin. The neurexins, a family of adhesion molecules expressed by neurons, also are bound by the α subunit.

The transmembrane segment of the dystroglycan β subunit associates with a complex of integral membrane proteins; its cytosolic domain binds dystrophin and other adapter proteins, as well as various intracellular signaling proteins. The resulting large, heterogeneous assemblage, the *dystrophin glycoprotein complex (DGC),* links the extracellular matrix to the cytoskeleton and signaling pathways within muscle cells (see Figure 6-29). For instance, the signaling enzyme nitric oxide synthase (NOS) is associated through syntrophin with the cytosolic dystrophin subcomplex in skeletal muscle. The rise in intracellular Ca^{2+} during muscle contraction activates NOS to produce nitric oxide (NO), which diffuses into smooth muscle cells surrounding nearby blood vessels. By a signaling pathway described in Chapter 13, NO promotes smooth muscle relaxation, leading to a local rise in the flow of blood supplying nutrients and oxygen to the skeletal muscle.

Mutations in dystrophin, other DGC components, laminin, or enzymes that add the *O*-linked sugars to dystroglycan disrupt the DGC-mediated link between the exterior and the interior of muscle cells and cause muscular dystrophies. In addition, dystroglycan mutations have been shown to greatly reduce the clustering of acetylcholine receptors on muscle cells at the neuromuscular junctions (Chapter 7), which also is dependent on the basal lamina proteins laminin and agrin. These and possibly other effects of DGC defects apparently lead to a cumulative weakening of the mechanical stability of muscle cells as they undergo contraction and relaxation, resulting in deterioration of the cells and muscular dystrophy.

Ca^{2+}-Independent Cell–Cell Adhesion in Neuronal and Other Tissues Is Mediated by CAMs in the Immunoglobulin Superfamily

Numerous transmembrane proteins characterized by the presence of multiple immunoglobulin domains (repeats) in their extracellular regions constitute the Ig superfamily of CAMs, or **IgCAMs.** The Ig domain is a common protein motif, containing 70–110 residues, that was first identified in antibodies, the antigen-binding immunoglobulins. The human, *D. melanogaster,* and *C. elegans* genomes include about 765, 150, and 64 genes, respectively, that encode proteins containing Ig domains. Immunoglobin domains are found in a wide variety of cell-surface proteins including T-cell receptors produced by lymphocytes and many proteins that take part in adhesive interactions. Among the IgCAMs are neural CAMs; intercellular CAMs (ICAMs), which function in the movement of leukocytes into tissues; and junction adhesion molecules (JAMs), which are present in tight junctions.

As their name implies, neural CAMs are of particular importance in neural tissues. One type, the NCAMs, primarily mediate homophilic interactions. First expressed during morphogenesis, NCAMs play an important role in the differentiation of muscle, glial, and nerve cells. Their role in cell adhesion has been directly demonstrated by the inhibition of adhesion with anti-NCAM antibodies. Numerous NCAM isoforms, encoded by a single gene, are generated by alternative mRNA splicing and by differences in glycosylation. Other neural CAMs (e.g., L1-CAM) are encoded by different genes. In humans, mutations in different parts of the L1-CAM gene cause various neuropathologies (e.g., mental retardation, congenital hydrocephalus, and spasticity).

An NCAM comprises an extracellular region with five Ig repeats and two fibronectin type III repeats, a single membrane-spanning segment, and a cytosolic segment that interacts with the cytoskeleton (see Figure 6-2). In contrast, the extracellular region of L1-CAM has six Ig repeats and four fibronectin type III repeats. As with cadherins, cis (intracellular) interactions and trans (intercellular) interactions probably play key roles in IgCAM-mediated adhesion (see Figure 6-3).

The covalent attachment of multiple chains of sialic acid, a negatively charged sugar derivative, to NCAMs alters their adhesive properties. In embryonic tissues such as brain, polysialic acid constitutes as much as 25 percent of the mass of NCAMs. Possibly because of repulsion between the many negatively charged sugars in these NCAMs, cell–cell contacts are fairly transient, being made and then broken, a property necessary for the development of the nervous system. In contrast, NCAMs from adult tissues contain only one-third as much sialic acid, permitting more stable adhesions.

Movement of Leukocytes into Tissues Depends on a Precise Sequence of Combinatorially Diverse Sets of Adhesive Interactions

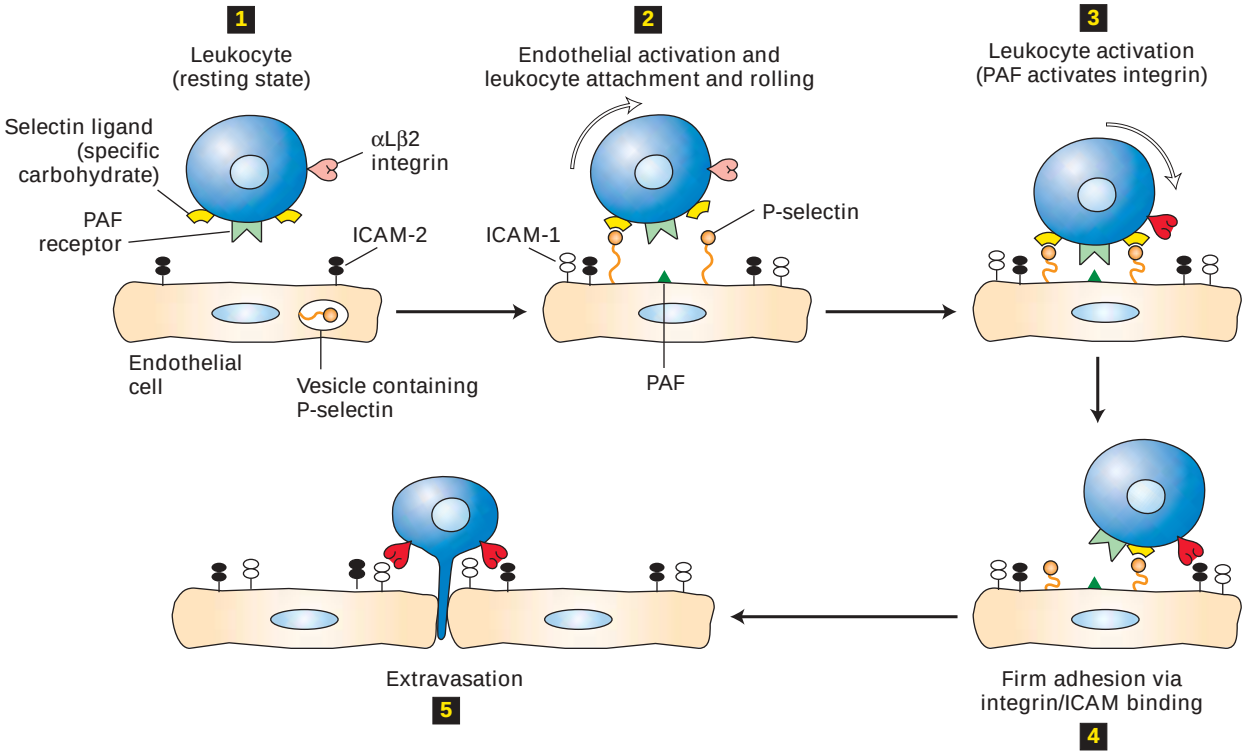
In adult organisms, several types of white blood cells (leukocytes) participate in the defense against infection caused by foreign invaders (e.g., bacteria and viruses) and tissue damage due to trauma or inflammation. To fight infection and clear away damaged tissue, these cells must move rapidly from the blood, where they circulate as unattached, relatively quiescent cells, into the underlying tissue at sites of infection, inflammation, or damage. We know a great deal about the movement into tissue, termed *extravasation,* of four types of leukocytes: neutrophils, which release several antibacterial proteins; monocytes, the precursors of macrophages, which can engulf and destroy foreign particles; and T and B lymphocytes, the antigen-recognizing cells of the immune system.

Extravasation requires the successive formation and breakage of cell–cell contacts between leukocytes in the blood and endothelial cells lining the vessels. Some of these contacts are mediated by **selectins,** a family of CAMs that mediate leukocyte–vascular cell interactions. A key player in these interactions is *P-selectin,* which is localized to the blood-facing surface of endothelial cells. All selectins contain

a Ca^{2+}-dependent *lectin domain,* which is located at the distal end of the extracellular region of the molecule and recognizes oligosaccharides in glycoproteins or glycolipids (see Figure 6-2). For example, the primary ligand for P- and E-selectins is an oligosaccharide called the *sialyl Lewis-x antigen,* a part of longer oligosaccharides present in abundance on leukocyte glycoproteins and glycolipids.

Figure 6-30 illustrates the basic sequence of cell–cell interactions leading to the extravasation of leukocytes. Various inflammatory signals released in areas of infection or inflammation first cause activation of the endothelium. P-selectin exposed on the surface of activated endothelial cells mediates the weak adhesion of passing leukocytes. Because of the force of the blood flow and the rapid "on" and "off" rates of P-selectin binding to its ligands, these "trapped" leukocytes are slowed but not stopped and literally roll along the surface of the endothelium. Among the signals that promote activation of the endothelium are *chemokines,* a group of small secreted proteins (8–12 kDa) produced by a wide variety of cells, including endothelial cells and leukocytes.

For tight adhesion to occur between activated endothelial cells and leukocytes, β2-containing integrins on the surfaces of leukocytes also must be activated by chemokines or other local activation signals such as platelet-activating factor (PAF). Platelet-activating factor is unusual in that it is a phospholipid, rather than a protein; it is exposed on the surface of activated endothelial cells at the same time that P-selectin is exposed. The binding of PAF or other activators to their receptors on leukocytes leads to activation of the leukocyte integrins to their high-affinity form (see Figure 6-28). (Most of the receptors for chemokines and PAF are members of the G protein–coupled receptor superfamily discussed in Chapter 13.) Activated integrins on leukocytes then bind to each of two distinct IgCAMs on the surface of en-



▲ FIGURE 6-30 **Sequence of cell–cell interactions leading to tight binding of leukocytes to activated endothelial cells and subsequent extravasation.** Step 1: In the absence of inflammation or infection, leukocytes and endothelial cells lining blood vessels are in a resting state. Step 2: Inflammatory signals released only in areas of inflammation or infection or both activate resting endothelial cells to move vesicle-sequestered selectins to the cell surface. The exposed selectins mediate loose binding of leukocytes by interacting with carbohydrate ligands on leukocytes. Activation of the endothelium also causes synthesis of platelet-activating factor (PAF) and ICAM-1, both expressed on the cell surface. PAF and other usually secreted activators, including chemokines, then induce changes in the shapes of the leukocytes and activation of leukocyte integrins such as αLβ2, which is expressed by T lymphocytes (3). The subsequent tight binding between activated integrins on leukocytes and CAMs on the endothelium (e.g., ICAM-2 and ICAM-1) results in firm adhesion (4) and subsequent movement (extravasation) into the underlying tissue (5).See text for further discussion. [Adapted from R. O. Hynes and A. Lander, 1992, *Cell* **68**:303.]

MEDIA CONNECTIONS
Focus Animation: Cell–Cell Adhesion in Leukocyte Extravasation

dothelial cells: ICAM-2, which is expressed constitutively, and ICAM-1. ICAM-1, whose synthesis along with that of E-selectin and P-selectin is induced by activation, does not usually contribute substantially to leukocyte endothelial cell adhesion immediately after activation, but rather participates at later times in cases of chronic inflammation. The resulting tight adhesion mediated by the Ca^{2+}-independent integrin–ICAM interactions leads to the cessation of rolling and to the spreading of leukocytes on the surface of the endothelium; soon the adhered cells move between adjacent endothelial cells and into the underlying tissue.

The selective adhesion of leukocytes to the endothelium near sites of infection or inflammation thus depends on the sequential appearance and activation of several different CAMs on the surfaces of the interacting cells. Different types of leukocytes express specific integrins containing the β2 subunit: for example, αLβ2 by T lymphocytes and αMβ2 by monocytes, the circulating precursors of tissue macrophages. Nonetheless, all leukocytes move into tissues by the same general mechanism depicted in Figure 6-30.

Many of the CAMs used to direct leukocyte adhesion are shared among different types of leukocytes and target tissues. Yet often only a particular type of leukocyte is directed to a particular tissue. A three-step model has been proposed to account for the cell-type specificity of such leukocyte–endothelial cell interactions. First, endothelium activation promotes initial relatively weak, transient, and reversible binding (e.g., the interaction of selectins and their carbohydrate ligands). Without additional local activation signals, the leukocyte will quickly move on. Second, cells in the immediate vicinity of the site of infection or inflammation release or express on their surfaces chemical signals (e.g., chemokines, PAF) that activate only special subsets of the transiently attached leukocytes. Third, additional activation-dependent CAMs (e.g., integrins) engage their binding partners, leading to strong sustained adhesion. Only if the proper combination of CAMs, binding partners, and activation signals are engaged in the right order at a specific site will a given leukocyte adhere strongly. This additional example of combinatorial diversity and cross talk allows parsimonious exploitation of a small set of CAMs for diverse functions throughout the body.

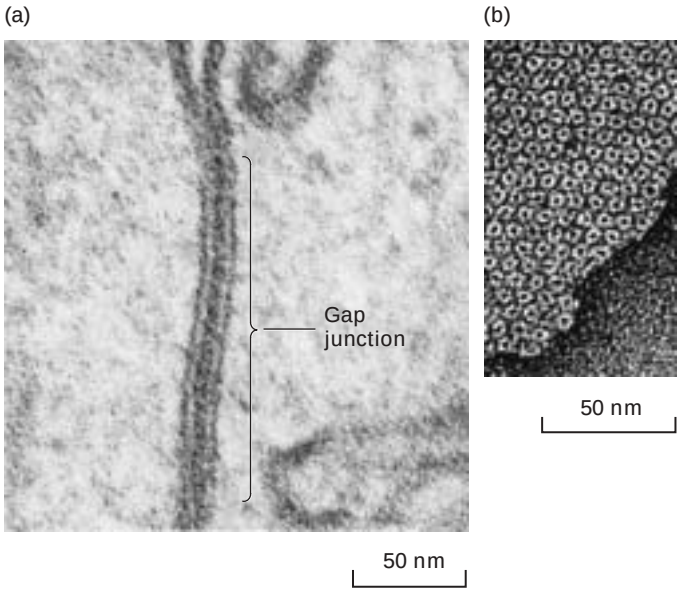
Leukocyte-adhesion deficiency is caused by a genetic defect in the synthesis of the integrin β2 subunit. Persons with this disorder are susceptible to repeated bacterial infections because their leukocytes cannot extravasate properly and thus fight the infection within the tissue.

Some pathogenic viruses have evolved mechanisms to exploit for their own purposes cell-surface proteins that participate in the normal response to inflammation. For example, many of the RNA viruses that cause the common cold (rhinoviruses) bind to and enter cells through ICAM-1, and chemokine receptors can be important entry sites for human immunodeficiency virus (HIV), the cause of AIDS. ▮

Gap Junctions Composed of Connexins Allow Small Molecules to Pass Between Adjacent Cells

Early electron micrographs of virtually all animal cells that were in contact revealed sites of cell–cell contact with a characteristic intercellular gap (Figure 6-31a). This feature prompted early morphologists to call these regions gap junctions. In retrospect, the most important feature of these junctions is not the gap itself but a well-defined set of cylindrical particles that cross the gap and compose pores connecting the cytoplasms of adjacent cells—hence their alternate name of *intercytoplasmic* junctions. In epithelia, gap junctions are distributed along the lateral surfaces of adjacent cells (see Figures 6-1 and 6-5).

In many tissues (e.g., the liver), large numbers of individual cylindrical particles cluster together in patches. This property has enabled researchers to separate gap junctions from other components of the plasma membrane. When the plasma membrane is purified and then sheared into small fragments, some pieces mainly containing patches of gap junctions are generated. Owing to their relatively high protein content, these fragments have a higher density than that of the bulk of the plasma membrane and can be purified on an equilibrium density gradient (see Figure 5-37). When these



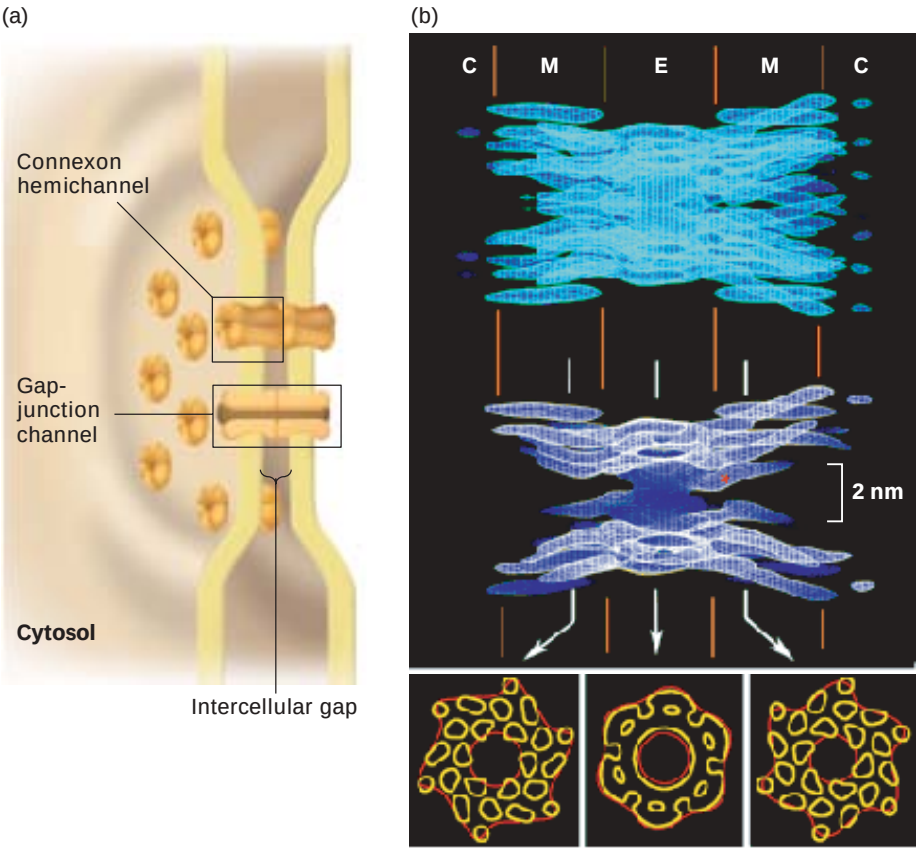
▲ EXPERIMENTAL FIGURE 6-31 **Gap junctions have a characteristic appearance in electron micrographs.** (a) In this thin section through a gap junction connecting two mouse liver cells, the two plasma membranes are closely associated for a distance of several hundred nanometers, separated by a "gap" of 2–3 nm. (b) Numerous roughly hexagonal particles are visible in this perpendicular view of the cytosolic face of a region of plasma membrane enriched in gap junctions. Each particle aligns with a similar particle on an adjacent cell, forming a channel connecting the two cells. [Part (a) courtesy of D. Goodenough. Part (b) courtesy of N. Gilula.]

preparations are viewed in cross section, the gap junctions appear as arrays of hexagonal particles that enclose water-filled channels (Figure 6-31b). Such pure preparations of gap junctions have permitted the detailed biophysical and functional analysis of these structures.

The effective pore size of gap junctions can be measured by injecting a cell with a fluorescent dye covalently linked to molecules of various sizes and observing with a fluorescence microscope whether the dye passes into neighboring cells. Gap junctions between mammalian cells permit the passage of molecules as large as 1.2 nm in diameter. In insects, these junctions are permeable to molecules as large as 2 nm in diameter. Generally speaking, molecules smaller than 1200 Da pass freely, and those larger than 2000 Da do not pass; the passage of intermediate-sized molecules is variable and limited. Thus ions, many low-molecular-weight precursors of cellular macromolecules, products of intermediary metabolism, and small intracellular signaling molecules can pass from cell to cell through gap junctions.

In nervous tissue, some neurons are connected by gap junctions through which ions pass rapidly, thereby allowing very rapid transmission of electrical signals. Impulse transmission through these connections, called electrical synapses, is almost a thousandfold as rapid as at chemical synapses (Chapter 7). Gap junctions are also present in many non-neuronal tissues where they help to integrate the electrical and metabolic activities of many cells. In the heart, for instance, gap junctions rapidly pass ionic signals among muscle cells and thus contribute to the electrically stimulated coordinate contraction of cardiac muscle cells during a beat. As discussed in Chapter 13, some extracellular hormonal signals induce the production or release of small intracellular signaling molecules called **second messengers** (e.g., cyclic AMP and Ca^{2+}) that regulate cellular metabolism. Because second messengers can be transferred between cells through gap junctions, hormonal stimulation of one cell can trigger a coordinated response by that same cell and many of its neighbors. Such gap junction–mediated signaling plays an important role, for example, in the secretion of digestive enzymes by the pancreas and in the coordinated muscular contractile waves (peristalsis) in the intestine. Another vivid example of gap junction–mediated transport is the phenomenon of *metabolic coupling*, or *metabolic cooperation*, in which a cell transfers nutrients or intermediary metabolites to a neighboring cell that is itself unable to synthesize them. Gap junctions play critical roles in the development of egg cells in the ovary by mediating the movement of both metabolites and signaling molecules between an oocyte and its surrounding granulosa cells as well as between neighboring granulosa cells.

A current model of the structure of the gap junction is shown in Figure 6-32. Vertebrate gap junctions are composed



▶ FIGURE 6-32 **Molecular structure of gap junctions.** (a) Schematic model of a gap junction, which comprises a cluster of channels between two plasma membranes separated by a gap of about 2–3 nm. Both membranes contain connexon hemichannels, cylinders of six dumbbell-shaped connexin molecules. Two connexons join in the gap between the cells to form a gap-junction channel, 1.5–2.0 nm in diameter, that connects the cytosols of the two cells. (b) Electron density of a recombinant gap-junction channel determined by electron crystallography. Shown here are side views of the complete structure (*top*) and the same structure with several chains removed to show the channel's interior (*center*); on the bottom are perpendicular cross sections through the gap junction within and between the membrane bilayers. There appear to be 24 transmembrane α helices per connexon hemichannel, consistent with each of the six connexin subunits having four α helices. The narrowest part of the channel is ≈1.5 nm in diameter. M = membrane bilayer; E = extracellular gap; C = cytosol. [Part (b) from V. M. Unger et al., 1999, *Science* **283**:1176.]

of **connexins,** a family of structurally related transmembrane proteins with molecular weights between 26,000 and 60,000. A completely different family of proteins, the innexins, forms the gap junctions in invertebrates. Each vertebrate hexagonal particle consists of 12 connexin molecules: 6 of the molecules are arranged in a connexon hemichannel—a hexagonal cylinder in one plasma membrane—and joined to a connexon hemichannel in the adjacent cell membrane, forming the continuous aqueous channel between the cells. Each connexin molecule spans the plasma membrane four times; one conserved transmembrane α helix from each subunit apparently lines the aqueous channel.

There are probably more than 20 different connexin genes in vertebrates, and different sets of connexins are expressed in different cell types. Some cells express a single connexin; consequently their gap-junction channels are homotypic, consisting of identical connexons. Most cells, however, express at least two connexins; these different proteins assemble into hetero-oligomeric connexons, which in turn form heterotypic gap-junction channels. This diversity in channel composition leads to differences in the permeability of channels to various molecules. For example, channels made from a 43-kDa connexin isoform, Cx43, are more than a hundredfold as permeable to ADP and ATP as those made from Cx32 (32 kDa). Moreover, the permeability of gap junctions can be altered by changes in the intracellular pH and Ca^{2+} concentration, as well as by the phosphorylation of connexin, providing numerous mechanisms for regulating transport through them.

The generation of mutant mice with inactivating mutations in connexin genes has highlighted the importance of connexins in a wide variety of cellular systems. For instance, Cx43-defective mice exhibit numerous defects including defective oocyte maturation due to decreased gap-junctional communication between granulosa cells in the ovary.

Mutations in several connexin genes are related to human diseases, including neurosensory deafness (Cx26 and Cx31), cataract or heart malformations (Cx43, Cx46, and Cx50), and the X-linked form of Charcot-Marie-Tooth disease (Cx32), which is marked by progressive degeneration of peripheral nerves. ▮

KEY CONCEPTS OF SECTION 6.5

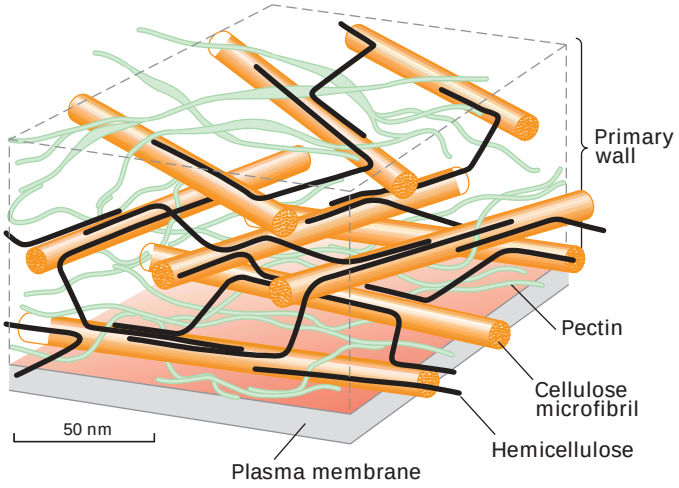
Adhesive Interactions and Nonepithelial Cells

- Many nonepithelial cells have integrin-containing aggregates (e.g., focal adhesions, 3D adhesions, podosomes) that physically and functionally connect cells to the extracellular matrix and facilitate inside-out and outside-in signaling.
- Integrins exist in two conformations that differ in the affinity for ligands and interactions with cytosolic adapter proteins (see Figure 6-28).
- Dystroglycan, an adhesion receptor expressed by muscle cells, forms a large complex with dystrophin, other adapter proteins, and signaling molecules (see Figure 6-29). This complex links the actin cytoskeleton to the surrounding matrix, providing mechanical stability to muscle. Mutations in various components of this complex cause different types of muscular dystrophy.
- Neural cell-adhesion molecules (CAMs), which belong to the immunoglobulin (Ig) family of CAMs, mediate Ca^{2+}-independent cell–cell adhesion, predominantly in neural tissue and muscle.
- The combinatorial and sequential interaction of several types of CAMs (e.g., selectins, integrins, and ICAMs) is critical for the specific and tight adhesion of different types of leukocytes to endothelial cells in response to local signals induced by infection or inflammation (see Figure 6-30).
- Gap junctions are constructed of multiple copies of connexin proteins, assembled into a transmembrane channel that interconnects the cytoplasm of two adjacent cells (see Figure 6-32). Small molecules and ions can pass through gap junctions, permitting metabolic and electrical coupling of adjacent cells.

6.6 Plant Tissues

We turn now to the assembly of plant cells into tissues. The overall structural organization of plants is generally simpler than that of animals. For instance, plants have only four broad types of cells, which in mature plants form four basic classes of tissue: *dermal tissue* interacts with the environment; *vascular tissue* transports water and dissolved substances (e.g., sugars, ions); space-filling *ground tissue* constitutes the major sites of metabolism; and *sporogenous tissue* forms the reproductive organs. Plant tissues are organized into just four main organ systems: *stems* have support and transport functions; *roots* provide anchorage and absorb and store nutrients; *leaves* are the sites of photosynthesis; and *flowers* enclose the reproductive structures. Thus at the cell, tissue, and organ levels, plants are generally less complex than most animals.

Moreover, unlike animals, plants do not replace or repair old or damaged cells or tissues; they simply grow new organs. Most importantly for this chapter and in contrast with animals, few cells in plants directly contact one another through molecules incorporated into their plasma membranes. Instead, plant cells are typically surrounded by a rigid **cell wall** that contacts the cell walls of adjacent cells (Figure 6-33). Also in contrast with animal cells, a plant cell rarely changes its position in the organism relative to other cells. These features of plants and their organization have determined the distinctive molecular mechanisms by which their cells are incorporated into tissues. ▮



▲ FIGURE 6-33 **Schematic representation of the cell wall of an onion.** Cellulose and hemicellulose are arranged into at least three layers in a matrix of pectin polymers. The size of the polymers and their separations are drawn to scale. To simplify the diagram, most of the hemicellulose cross-links and other matrix constituents (e.g., extensin, lignin) are not shown. [Adapted from M. McCann and K. R. Roberts, 1991, in C. Lloyd, ed., *The Cytoskeletal Basis of Plant Growth and Form,* Academic Press, p. 126.]

The Plant Cell Wall Is a Laminate of Cellulose Fibrils in a Matrix of Glycoproteins

The plant cell wall is ≈0.2 μm thick and completely coats the outside of the plant cell's plasma membrane. This structure serves some of the same functions as those of the extracellular matrix produced by animal cells, even though the two structures are composed of entirely different macromolecules and have a different organization. Like the extracellular matrix, the plant cell wall connects cells into tissues, signals a plant cell to grow and divide, and controls the shape of plant organs. Just as the extracellular matrix helps define the shapes of animal cells, the cell wall defines the shapes of plant cells. When the cell wall is digested away from plant cells by hydrolytic enzymes, spherical cells enclosed by a plasma membrane are left. In the past, the plant cell wall was viewed as an inanimate rigid box, but it is now recognized as a dynamic structure that plays important roles in controlling the differentiation of plant cells during embryogenesis and growth.

Because a major function of a plant cell wall is to withstand the osmotic turgor pressure of the cell, the cell wall is built for lateral strength. It is arranged into layers of **cellulose** microfibrils—bundles of long, linear, extensively hydrogen-bonded polymers of glucose in β glycosidic linkages. The cellulose microfibrils are embedded in a matrix composed of *pectin*, a polymer of D-galacturonic acid and other monosaccharides, and *hemicellulose*, a short, highly branched polymer of several five- and six-carbon monosaccharides. The mechanical strength of the cell wall depends on cross-linking of the microfibrils by hemicellulose chains (see Figure 6-33). The layers of microfibrils prevent the cell wall from stretching laterally. Cellulose microfibrils are synthesized on the exoplasmic face of the plasma membrane from UDP-glucose and ADP-glucose formed in the cytosol. The polymerizing enzyme, called *cellulose synthase*, moves within the plane of the plasma membrane as cellulose is formed, in directions determined by the underlying microtubule cytoskeleton.

Unlike cellulose, pectin and hemicellulose are synthesized in the Golgi apparatus and transported to the cell surface where they form an interlinked network that helps bind the walls of adjacent cells to one another and cushions them. When purified, pectin binds water and forms a gel in the presence of Ca^{2+} and borate ions—hence the use of pectins in many processed foods. As much as 15 percent of the cell wall may be composed of *extensin*, a glycoprotein that contains abundant hydroxyproline and serine. Most of the hydroxyproline residues are linked to short chains of arabinose (a five-carbon monosaccharide), and the serine residues are linked to galactose. Carbohydrate accounts for about 65 percent of extensin by weight, and its protein backbone forms an extended rodlike helix with the hydroxyl or *O*-linked carbohydrates protruding outward. *Lignin*—a complex, insoluble polymer of phenolic residues—associates with cellulose and is a strengthening material. Like cartilage proteoglycans, lignin resists compression forces on the matrix.

The cell wall is a selective filter whose permeability is controlled largely by pectins in the wall matrix. Whereas water and ions diffuse freely across cell walls, the diffusion of large molecules, including proteins larger than 20 kDa, is limited. This limitation may account for why many plant hormones are small, water-soluble molecules, which can diffuse across the cell wall and interact with receptors in the plasma membrane of plant cells.

Loosening of the Cell Wall Permits Elongation of Plant Cells

Because the cell wall surrounding a plant cell prevents the cell from expanding, its structure must be loosened when the cell grows. The amount, type, and direction of plant cell growth are regulated by small-molecule hormones (e.g., indoleacetic acid) called *auxins*. The auxin-induced weakening of the cell wall permits the expansion of the intracellular vacuole by uptake of water, leading to elongation of the cell. We can grasp the magnitude of this phenomenon by considering that, if all cells in a redwood tree were reduced to the size of a typical liver cell, the tree would have a maximum height of only 1 meter.

The cell wall undergoes its greatest changes at the **meristem** of a root or shoot tip. These sites are where cells divide and expand. Young meristematic cells are connected by thin primary cell walls, which can be loosened and stretched to

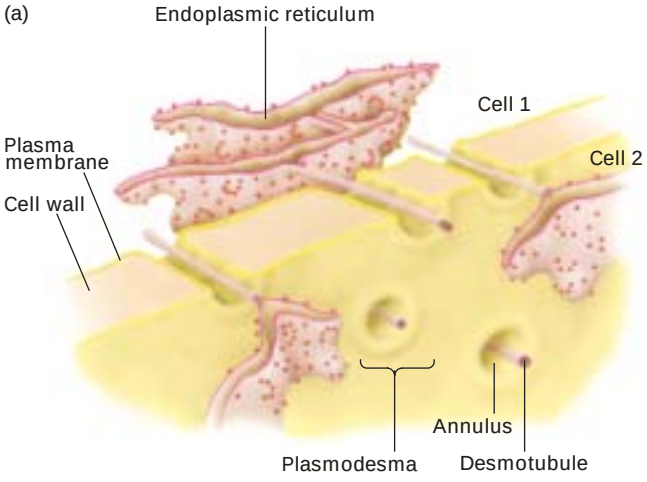
allow subsequent cell elongation. After cell elongation ceases, the cell wall is generally thickened, either by the secretion of additional macromolecules into the primary wall or, more usually, by the formation of a secondary cell wall composed of several layers. Most of the cell eventually degenerates, leaving only the cell wall in mature tissues such as the xylem—the tubes that conduct salts and water from the roots through the stems to the leaves (see Figure 8-45). The unique properties of wood and of plant fibers such as cotton are due to the molecular properties of the cell walls in the tissues of origin.

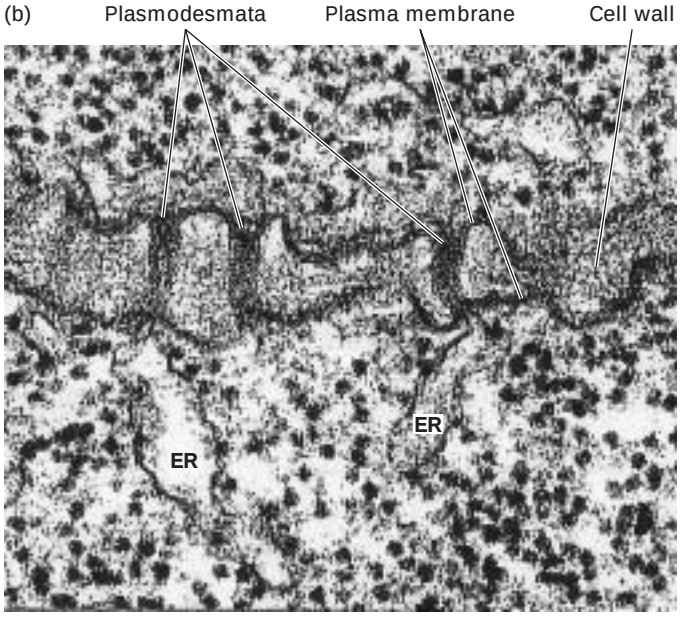
Plasmodesmata Directly Connect the Cytosols of Adjacent Cells in Higher Plants

Plant cells can communicate directly through specialized cell–cell junctions called **plasmodesmata,** which extend through the cell wall. Like gap junctions, plasmodesmata are open channels that connect the cytosol of a cell with that of an adjacent cell. The diameter of the cytosol-filled channel is about 30–60 nm, and plasmodesmata can traverse cell walls as much as 90 nm thick. The density of plasmodesmata varies depending on the plant and cell type, and even the smallest meristematic cells have more than 1000 interconnections with their neighbors.

Molecules smaller than about 1000 Da, including a variety of metabolic and signaling compounds, generally can diffuse through plasmodesmata. However, the size of the channel through which molecules pass is highly regulated. In some circumstances, the channel is clamped shut; in others, it is dilated sufficiently to permit the passage of molecules larger than 10,000 Da. Among the factors that affect the permeability of plasmodesmata is the cytosolic Ca^{2+} concentration, with an increase in cytosolic Ca^{2+} reversibly inhibiting movement of molecules through these structures.

Although plasmodesmata and gap junctions resemble each other functionally, their structures differ in two significant ways (Figure 6-34). The plasma membranes of the adjacent plant cells merge to form a continuous channel, the *annulus,* at each plasmodesma, whereas the membranes of cells at a gap junction are not continuous with each other. In addition, an extension of the endoplasmic reticulum called a *desmotubule* passes through the annulus, which connects the cytosols of adjacent plant cells. Many types of molecules spread from cell to cell through plasmodesmata, including proteins, nucleic acids, metabolic products, and plant viruses. Soluble molecules pass through the cytosolic annulus, whereas membrane-bound molecules can pass from cell to cell through the desmotubule.





▲ FIGURE 6-34 **Structure of a plasmodesma.**
(a) Schematic model of a plasmodesma showing the desmotubule, an extension of the endoplasmic reticulum, and the annulus, a plasma membrane–lined channel filled with cytosol that interconnects the cytosols of adjacent cells. Not shown is a gating complex that fills the channel and controls the transport of materials through the plasmodesma.
(b) Electron micrograph of thin section of plant cell and cell wall containing multiple plasmodesmata. [E. H. Newcomb and W. P. Wergin/Biological Photo Service.]

Only a Few Adhesive Molecules Have Been Identified in Plants

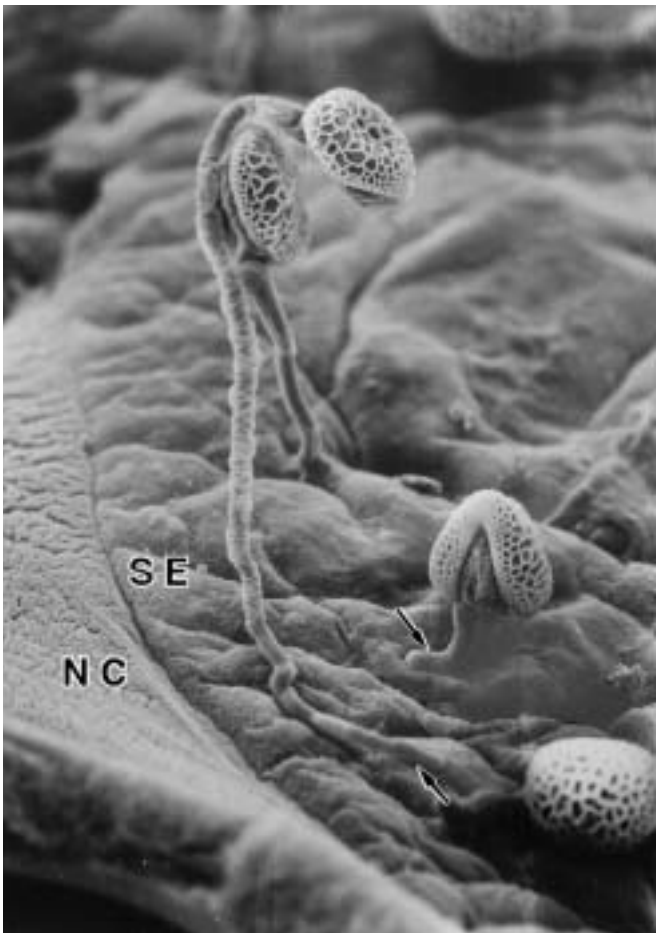
Systematic analysis of the *Arabidopsis* genome and biochemical analysis of other plant species provide no evidence for the existence of plant homologs of most animal CAMs, adhesion receptors, and ECM components. This finding is not surprising, given the dramatically different nature of cell–cell and cell–matrix/wall interactions in animals and plants.

Among the adhesive-type proteins apparently unique to plants are five wall-associated kinases (WAKs) and WAK-like proteins expressed in the plasma membrane of *Arabidopsis* cells. The extracellular regions in all these proteins contain multiple epidermal growth factor (EGF) repeats, which may directly participate in binding to other molecules. Some WAKs have been shown to bind to glycine-rich proteins in the cell wall, thereby mediating membrane–wall contacts. These *Arabidopsis* proteins have a single transmembrane domain and an intracellular cytosolic tyrosine kinase domain, which may participate in signaling pathways somewhat like the receptor tyrosine kinases discussed in Chapter 14.

The results of in vitro binding assays combined with in vivo studies and analyses of plant mutants have identified several macromolecules in the ECM that are important for adhesion. For example, normal adhesion of pollen, which contains sperm cells, to the stigma or style in the female reproductive organ of the Easter lily requires a cysteine-rich protein called stigma/stylar cysteine-rich adhesin (SCA) and a specialized pectin that can bind to SCA (Figure 6-35).

Disruption of the gene encoding glucuronyltransferase 1, a key enzyme in pectin biosynthesis, has provided a striking illustration of the importance of pectins in intercellular adhesion in plant meristems. Normally, specialized pectin molecules help hold the cells in meristems tightly together. When grown in culture as a cluster of relatively undifferentiated cells, called a callus, normal meristematic cells adhere tightly and can differentiate into chlorophyll-producing cells, giving the callus a green color. Eventually the callus will generate shoots. In contrast, mutant cells with an inactivated glucuronyltransferase 1 gene are large, associate loosely with each other, and do not differentiate normally, forming a yellow callus. The introduction of a normal glucuronyltransferase 1 gene into the mutant cells by methods discussed in Chapter 9 restores their ability to adhere and differentiate normally.

The paucity of plant adhesive molecules identified to date, in contrast with the many well-defined animal adhesive molecules, may be due to the technical difficulties in working with the ECM/cell wall of plants. Adhesive interactions are often likely to play different roles in plant and animal biology, at least in part because of their differences in development and physiology.



▲ EXPERIMENTAL FIGURE 6-35 **An in vitro assay used to identify molecules required for adherence of pollen tubes to the stylar matrix.** In this assay, extracellular stylar matrix collected from lily styles (SE) or an artificial matrix is dried onto nitrocellulose membranes (NC). Pollen tubes containing sperm are then added and their binding to the dried matrix is assessed. In this scanning electron micrograph, the tips of pollen tubes (arrows) can be seen binding to dried stylar matrix. This type of assay has shown that pollen adherence depends on stigma/stylar cysteine-rich adhesin (SCA) and a pectin that binds to SCA. [From G. Y. Jauh et al., 1997, *Sex Plant Reprod.* **10**:173.]

KEY CONCEPTS OF SECTION 6.6

Plant Tissues

- The integration of cells into tissues in plants is fundamentally different from the assembly of animal tissues, primarily because each plant cell is surrounded by a relatively rigid cell wall.
- The plant cell wall comprises layers of cellulose microfibrils embedded within a matrix of hemicellulose, pectin, extensin, and other less abundant molecules.
- Cellulose, a large, linear glucose polymer, assembles spontaneously into microfibrils stabilized by hydrogen bonding.
- The cell wall defines the shapes of plant cells and restricts their elongation. Auxin-induced loosening of the cell wall permits elongation.

- Adjacent plant cells can communicate through plasmodesmata, which allow small molecules to pass between the cells (see Figure 6-34).
- Plants do not produce homologs of the common adhesive molecules found in animals. Only a few adhesive molecules unique to plants have been well documented to date.

6.7 Growth and Use of Cultured Cells

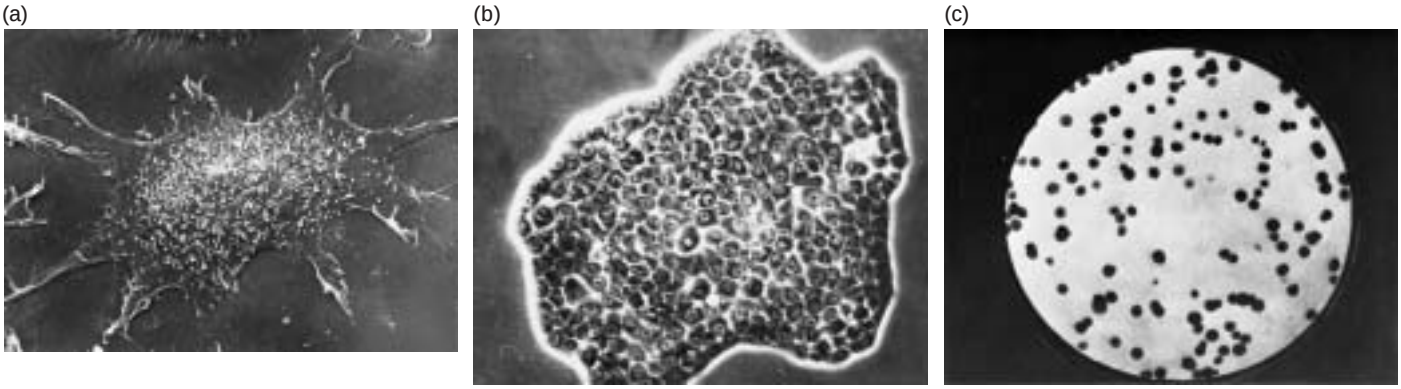
Many technical constraints hamper studies on specific cells or subsets of cells in intact animals and plants. One alternative is the use of intact organs that are removed from animals and perfused with an appropriately buffered solution to maintain their physiologic integrity and function. Such organ perfusion systems have been widely used by physiologists. However, the organization of organs, even isolated ones, is sufficiently complex to pose numerous problems for research on many fundamental aspects of cell biology. Thus molecular cell biologists often conduct experimental studies on cells isolated from an organism and maintained in conditions that permit their survival and growth, a procedure known as *culturing*.

Cultured cells have several advantages over intact organisms for cell biology research. First, most animal and plant tissues consist of a variety of different types of cells, whereas cells of a single specific type with homogeneous properties can be grown in culture. Second, experimental conditions (e.g., composition of the extracellular environment) can be controlled far better in culture than in an intact organism. Third, in many cases a single cell can be readily grown into a colony of many identical cells, a process called cell cloning, or simply cloning (Figure 6-36). The resulting strain of cells, which is genetically homogeneous, is called a **clone.** This simple technique, which is commonly used with many bacteria, yeasts, and mammalian cell types, makes it easy to isolate genetically distinct clones of cells.

A major disadvantage of cultured cells is that they are not in their normal environment and hence their activities are not regulated by the other cells and tissues as they are in an intact organism. For example, insulin produced by the pancreas has an enormous effect on liver glucose metabolism; however, this normal regulatory mechanism does not operate in a purified population of liver cells (called hepatocytes) grown in culture. In addition, as already described, the three-dimensional distribution of cells and extracellular matrix around a cell influences its shape and behavior. Because the immediate environment of cultured cells differs radically from this "normal" environment, their properties may be affected in various ways. Thus care must always be exercised in drawing conclusions about the normal properties of cells in complex tissues and organisms only on the basis of experiments with isolated, cultured cells.

Culture of Animal Cells Requires Nutrient-Rich Media and Special Solid Surfaces

In contrast with most bacterial cells, which can be cultured quite easily, animal cells require many specialized nutrients and often specially coated dishes for successful culturing. To permit the survival and normal function of cultured tissues or cells, the temperature (37 °C for mammalian cells), pH, ionic strength, and access to essential nutrients must simulate as closely as possible the conditions within an intact organism. Isolated animal cells are typically placed in a nutrient-rich liquid, the culture medium, within specially treated plastic dishes or flasks. The cultures are kept in incubators in which the temperature, atmosphere, and humidity can be controlled. To reduce the chances of bacterial or fungal contamination, antibiotics are often added to the culture medium. To further guard against contamination, investigators usually transfer cells between dishes, add reagents to the culture medium, and otherwise manipulate the specimens within special cabinets



▲ FIGURE 6-36 **Cultured mammalian cells viewed at three magnifications.** (a) A single mouse cell attached to a plastic petri dish, viewed through a scanning electron microscope. (b) A single colony of human HeLa cells about 1 mm in diameter, produced from a single cell after growth for 2 weeks. (c) After cells initially introduced into a 6-cm-diameter petri dish have grown for several days and then been stained, individual colonies can easily be seen and counted. All the cells in a colony are progeny of a single precursor cell and thus genetically identical. [Part (a) courtesy of N. K. Weller. Parts (b) and (c) courtesy of T. T. Puck.]

containing circulating air that is filtered to remove microorganisms and other airborne contaminants.

Media for culturing animal cells must supply histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine; no cells in adult vertebrate animals can synthesize these nine essential amino acids. In addition, most cultured cells require three other amino acids (cysteine, glutamine, and tyrosine) that are synthesized only by specialized cells in intact animals. The other necessary components of a medium for culturing animal cells are vitamins, various salts, fatty acids, glucose, and serum—the fluid remaining after the noncellular part of blood (plasma) has been allowed to clot. Serum contains various protein factors that are needed for the proliferation of mammalian cells in culture. These factors include the polypeptide hormone insulin; transferrin, which supplies iron in a bioaccessible form; and numerous growth factors. In addition, certain cell types require specialized protein growth factors not present in serum. For instance, hematopoietic cells require erythropoietin, and T lymphocytes require interleukin 2 (Chapter 14). A few mammalian cell types can be grown in a chemically defined, serum-free medium containing amino acids, glucose, vitamins, and salts plus certain trace minerals, specific protein growth factors, and other components.

Unlike bacterial and yeast cells, which can be grown in suspension, most animal cells will grow only on a solid surface. This highlights the importance of cell adhesion molecules. Many types of cells can grow on glass or on specially treated plastics with negatively charged groups on the surface (e.g., SO_3^{2-}). The cells secrete ECM components, which adhere to these surfaces, and then attach and grow on the secreted matrix. A single cell cultured on a glass or a plastic dish proliferates to form a visible mass, or *colony,* containing thousands of genetically identical cells in 4–14 days, depending on the growth rate (see Figure 6-36c). Some specialized blood cells and tumor cells can be maintained or grown in suspension as single cells.

Primary Cell Cultures and Cell Strains Have a Finite Life Span

Normal animal tissues (e.g., skin, kidney, liver) or whole embryos are commonly used to establish *primary cell cultures.* To prepare tissue cells for a primary culture, the cell–cell and cell–matrix interactions must be broken. To do so, tissue fragments are treated with a combination of a protease (e.g., trypsin or the collagen-hydrolyzing enzyme collagenase or both) and a divalent cation chelator (e.g., EDTA) that depletes the medium of usable Ca^{2+} or Mg^{2+}. The released cells are then placed in dishes in a nutrient-rich, serum-supplemented medium, where they can adhere to the surface and one another. The same protease/chelator solution is used to remove adherent cells from a culture dish for biochemical studies or subculturing (transfer to another dish).

Often connective tissue fibroblasts divide in culture more rapidly than other cells in a tissue, eventually becoming the predominant type of cells in the primary culture, unless special precautions are taken to remove them when isolating other types of cells. Certain cells from blood, spleen, or bone marrow adhere poorly, if at all, to a culture dish but nonetheless grow well. In the body, such *nonadherent* cells are held in suspension (in the blood) or they are loosely adherent (in the bone marrow and spleen). Because these cells often come from immature stages in the development of differentiated blood cells, they are very useful for studying normal blood cell differentiation and the abnormal development of leukemias.

When cells removed from an embryo or an adult animal are cultured, most of the adherent ones will divide a finite number of times and then cease growing (cell senescence, Figure 6-38a). For instance, human fetal fibroblasts divide about 50 times before they cease growth. Starting with 10^6 cells, 50 doublings can produce $10^6 \times 2^{50}$, or more than 10^{20} cells, which is equivalent to the weight of about 10^5 people. Normally, only a very small fraction of these cells are used in any one experiment. Thus, even though its lifetime is limited, a single culture, if carefully maintained, can be studied through many generations. Such a lineage of cells originating from one initial primary culture is called a **cell strain.**

Cell strains can be frozen in a state of suspended animation and stored for extended periods at liquid nitrogen temperature, provided that a preservative that prevents the formation of damaging ice crystals is used. Although some cells do not survive thawing, many do survive and resume growth. Research with cell strains is simplified by the ability to freeze and successfully thaw them at a later time for experimental analysis.

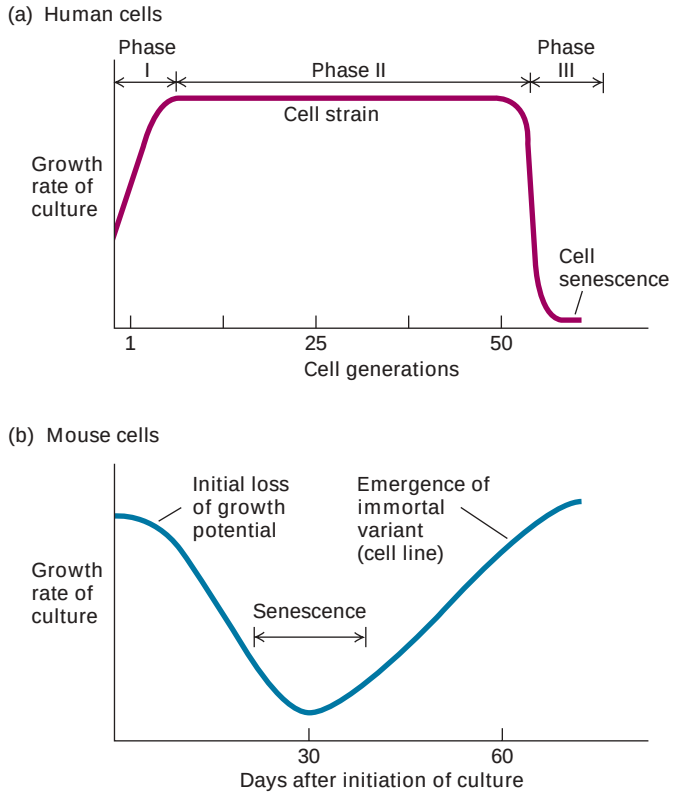
Transformed Cells Can Grow Indefinitely in Culture

To be able to clone individual cells, modify cell behavior, or select mutants, biologists often want to maintain cell cultures for many more than 100 doublings. Such prolonged growth is exhibited by cells derived from some tumors. In addition, rare cells in a population of primary cells that undergo certain spontaneous genetic changes, called oncogenic **transformation**, are able to grow indefinitely. These cells are said to be oncogenically transformed or simply *transformed.* A culture of cells with an indefinite life span is considered immortal and is called a **cell line.**

The HeLa cell line, the first human cell line, was originally obtained in 1952 from a malignant tumor (carcinoma) of the uterine cervix. Although primary cell cultures of normal human cells rarely undergo transformation into a cell line, rodent cells commonly do. After rodent cells are grown in culture for several generations, the culture goes into senescence (Figure 6-37b). During this period, most of the cells stop growing, but often a rapidly dividing transformed cell arises spontaneously and takes over, or overgrows, the culture. A cell line derived from such a

transformed variant will grow indefinitely if provided with the necessary nutrients.

Regardless of the source, cells in immortalized lines often have chromosomes with abnormal structures. In addition, the number of chromosomes in such cells is usually greater than that in the normal cell from which they arose, and the chromosome number expands and contracts as the cells continue to divide in culture. A noteworthy exception is the Chinese hamster ovary (CHO) line and its derivatives, which have fewer chromosomes than their hamster progenitors. Cells with an abnormal number of chromosomes are said to be *aneuploid*.



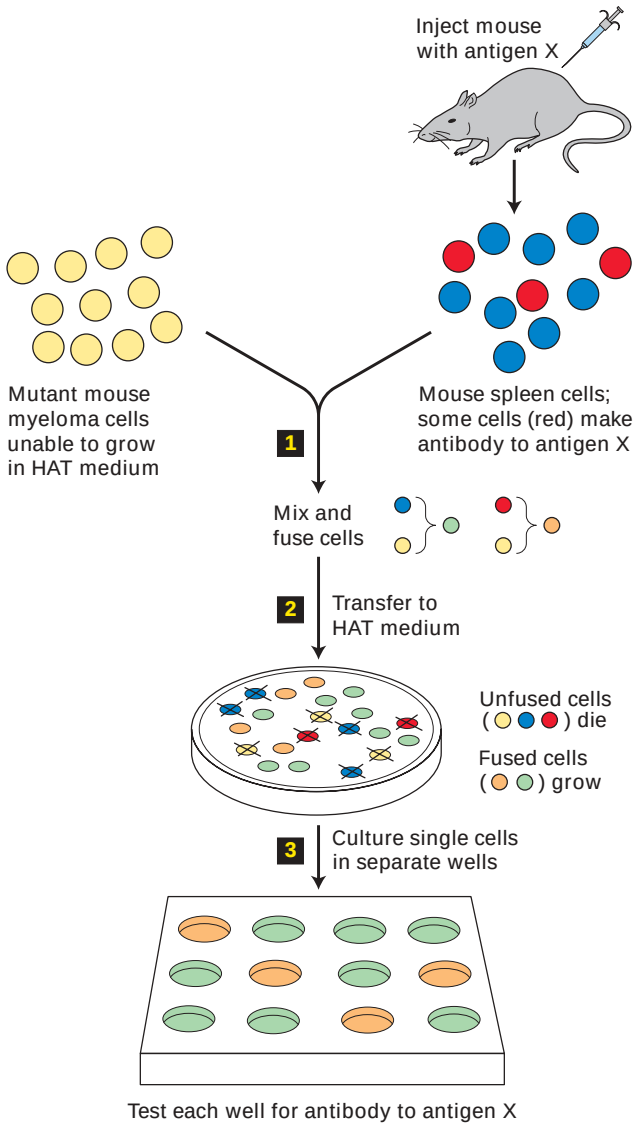
▲ FIGURE 6-37 **Stages in the establishment of a cell culture.** (a) When cells isolated from human tissue are initially cultured, some cells die and others (mainly fibroblasts) start to grow; overall, the growth rate increases (phase I). If the remaining cells are harvested, diluted, and replated into dishes again and again, the cell strain continues to divide at a constant rate for about 50 cell generations (phase II), after which the growth rate falls rapidly. In the ensuing period (phase III), all the cells in the culture stop growing (senescence). (b) In a culture prepared from mouse or other rodent cells, initial cell death (not shown) is coupled with the emergence of healthy growing cells. As these dividing cells are diluted and allowed to continue growth, they soon begin to lose growth potential, and most stop growing (i.e., the culture goes into senescence). Very rare cells survive and continue dividing until their progeny overgrow the culture. These cells constitute a cell line, which will grow indefinitely if it is appropriately diluted and fed with nutrients: such cells are said to be immortal.

Most cell lines have lost some or many of the functions characteristic of the differentiated cells from which they were derived. Such relatively undifferentiated cells are poor models for investigating the normal functions of specific cell types. Better in this regard are several more-differentiated cell lines that exhibit many properties of normal nontransformed cells. These lines include the liver tumor (hepatoma) HepG2 line, which synthesizes most of the serum proteins made by normal liver cells (hepatocytes). Another example consists of cells from a certain cultured fibroblast line, which under certain experimental conditions behave as muscle precursor cells, or myoblasts. These cells can be induced to fuse to form myotubes, which resemble differentiated multinucleated muscle cells and synthesize many of the specialized proteins associated with contraction. The results of studies with this cell line have provided valuable information about the differentiation of muscle (Chapter 22). Finally, as discussed previously, the MDCK cell line retains many properties of highly differentiated epithelial cells and forms well-defined epithelial sheets in culture (see Figure 6-6).

Hybrid Cells Called Hybridomas Produce Abundant Monoclonal Antibodies

In addition to serving as research models for studies on cell function, cultured cells can be converted into "factories" for producing specific proteins. In Chapter 9, we describe how it is done by introducing genes encoding insulin, growth factors, and other therapeutically useful proteins into bacterial or eukaryotic cells. Here we consider the use of special cultured cells to generate **monoclonal antibodies,** which are widely used experimental tools and increasingly are being used for diagnostic and therapeutic purposes in medicine.

To understand the challenge of generating monoclonal antibodies, we need to briefly review how antibodies are produced by mammals. Each normal B lymphocyte in a mammal is capable of producing a single type of antibody directed against (can bind to) a specific chemical structure (called a determinant or epitope) on an antigen molecule. If an animal is injected with an antigen, B lymphocytes that make antibodies recognizing the antigen are stimulated to grow and secrete the antibodies. Each antigen-activated B lymphocyte forms a clone of cells in the spleen or lymph nodes, with each cell of the clone producing the identical antibody—that is, a monoclonal antibody. Because most natural antigens contain multiple epitopes, exposure of an animal to an antigen usually stimulates the formation of multiple different B-lymphocyte clones, each producing a different antibody. The resulting mixture of antibodies that recognize different epitopes on the same antigen is said to be *polyclonal*. Such polyclonal antibodies circulate in the blood and can be isolated as a group and used for a variety of experiments. However, monoclonal antibodies are required for many types of experiments or medical applications. Unfortunately, the biochemical purification of any



▲ EXPERIMENTAL FIGURE 6-38 **Use of cell fusion and selection to obtain hybridomas producing monoclonal antibody to a specific protein.** Step 1: Immortal myeloma cells that lack HGPRT, an enzyme required for growth on HAT selection medium, are fused with normal antibody-producing spleen cells from an animal that was immunized with antigen X. The spleen cells can make HGPRT. Step 2: When plated in HAT medium, the unfused cells do not grow; neither do the mutant myeloma cells, because they cannot make purines through an HGPRT-dependent metabolic "salvage" pathway (see Figure 6-41), and the spleen cells, because they have a limited life span in culture. Thus only fused cells formed from a myeloma cell and a spleen cell survive on HAT medium, proliferating into clones called hybridomas. Each hybridoma produces a single antibody. Step 3: Testing of individual clones identifies those that recognize antigen X. After a hybridoma that produces a desired antibody has been identified, the clone can be cultured to yield large amounts of that antibody.

MEDIA CONNECTIONS
Technique Animation: Preparing Monoclonal Antibodies

one type of monoclonal antibody from blood is not feasible, in part because the concentration of any given antibody is quite low.

Because of their limited life span, primary cultures of normal B lymphocytes are of limited usefulness for the production of monoclonal antibody. Thus the first step in producing a monoclonal antibody is to generate immortal, antibody-producing cells. This immortality is achieved by fusing normal B lymphocytes from an immunized animal with transformed, immortal lymphocytes called *myeloma cells.* During cell fusion, the plasma membranes of two cells fuse together, allowing their cytosols and organelles to intermingle. Treatment with certain viral glycoproteins or the chemical polyethylene glycol promotes cell fusion. Some of the fused cells can undergo division and their nuclei eventually coalesce, producing viable *hybrid cells* with a single nucleus that contains chromosomes from both "parents." The fusion of two cells that are genetically different can yield a hybrid cell with novel characteristics. For instance, the fusion of a myeloma cell with a normal antibody-producing cell from a rat or mouse spleen yields a hybrid that proliferates into a clone called a **hybridoma.** Like myeloma cells, hybridoma cells grow rapidly and are immortal. Each hybridoma produces the monoclonal antibody encoded by its B-lymphocyte parent.

The second step in this procedure for producing monoclonal antibody is to separate, or select, the hybridoma cells from the unfused parental cells and the self-fused cells generated by the fusion reaction. This selection is usually performed by incubating the mixture of cells in a special culture medium, called *selection medium*, that permits the growth of only the hybridoma cells because of their novel characteristics. Such a selection is readily performed if the myeloma cells used for the fusion carry a mutation that blocks a metabolic pathway and renders them, but not their lymphocyte fusion partners that do not have the mutation, sensitive to killing by the selection medium. In the immortal hybrid cells, the functional gene from the lymphocyte can supply the gene product missing because of the mutation in the myeloma cell, and thus the hybridoma cells but not the myeloma cells, will be able to grow in the selection medium. Because the lymphocytes used in the fusion are not immortalized and do not divide rapidly, only the hybridoma cells will proliferate rapidly in the selection medium and can thus be readily isolated from the initial mixture of cells.

Figure 6-38 depicts the general procedure for generating and selecting hybridomas. In this case, normal B lymphocytes are fused with myeloma cells that cannot grow in *HAT medium*, the most common selection medium used in the production of hybridomas. Only the myeloma-lymphocyte hybrids can survive and grow for an extended period in HAT medium for reasons described shortly. Thus, this selection medium permits the separation of hybridoma cells from both types of parental cells and any

self-fused cells. Finally, each selected hybridoma is then tested for the production of the desired antibody; any clone producing that antibody is then grown in large cultures, from which a substantial quantity of pure monoclonal antibody can be obtained.

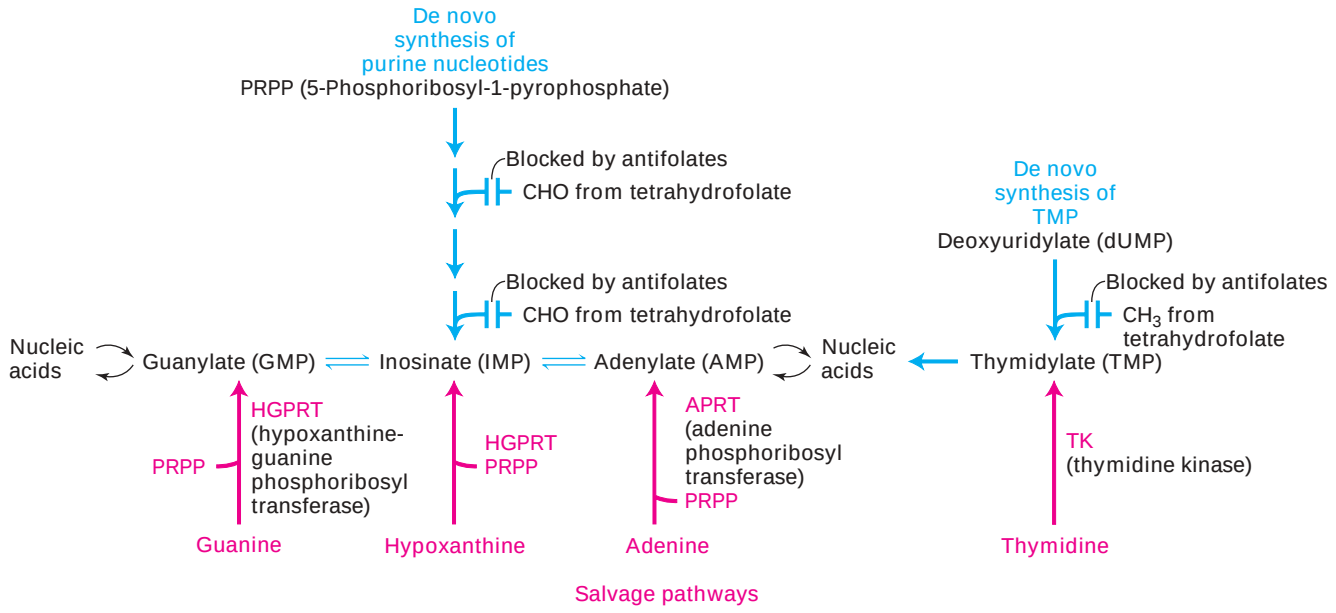
BIOTECH

Monoclonal antibodies are commonly employed in affinity chromatography to isolate and purify proteins from complex mixtures (see Figure 3-34c). They can also be used to label and thus locate a particular protein in specific cells of an organ and within cultured cells with the use of immunofluorescence microscopy techniques (see Figures 6-26a and 6-27) or in specific cell fractions with the use of immunoblotting (see Figure 3-35). Monoclonal antibodies also have become important diagnostic and therapeutic tools in medicine. For example, monoclonal antibodies that bind to and inactivate toxic proteins (toxins) secreted by bacterial pathogens are used to treat diseases caused by these pathogens. Other monoclonal antibodies are specific for cell-surface proteins expressed by certain types of tumor cells; chemical complexes of such monoclonal antibodies with toxic drugs or simply the antibodies themselves have been developed for cancer chemotherapy. ▮

HAT Medium Is Commonly Used to Isolate Hybrid Cells

The principles underlying HAT selection are important not only for understanding how hybridoma cells are isolated but also for understanding several other frequently used selection methods, including selection of the ES cells used in generating knockout mice (Chapter 9). HAT medium contains *h*ypoxanthine (a purine), *a*minopterin, and *t*hymidine. Most animal cells can synthesize the purine and pyrimidine nucleotides from simpler carbon and nitrogen compounds (Figure 6-39, *top*). The folic acid antagonists amethopterin and aminopterin interfere with the donation of methyl and formyl groups by tetrahydrofolic acid in the early stages of the synthesis of glycine, purine nucleoside monophosphates, and thymidine monophosphate. These drugs are called *antifolates* because they block reactions of tetrahydrofolate, an active form of folic acid.

Many cells, however, are resistant to antifolates because they contain enzymes that can synthesize the necessary nucleotides from purine bases and thymidine (Figure 6-39, *bottom*). Two key enzymes in these *nucleotide salvage pathways* are thymidine kinase (TK) and hypoxanthine-guanine phosphoribosyl transferase (HGPRT). Cells that produce these enzymes can grow on HAT medium, which supplies a



▲ FIGURE 6-39 **De novo and salvage pathways for nucleotide synthesis.** Animal cells can synthesize purine nucleotides (AMP, GMP, IMP) and thymidylate (TMP) from simpler compounds by de novo pathways (blue). They require the transfer of a methyl or formyl ("CHO") group from an activated form of tetrahydrofolate (e.g., N^5,N^{10}-methylenetetrahydrofolate), as shown in the upper part of the diagram. Antifolates, such as aminopterin and amethopterin, block the reactivation of tetrahydrofolate, preventing purine and thymidylate synthesis. Many animal cells can also use salvage pathways (red) to incorporate purine bases or nucleosides and thymidine. If these precursors are present in the medium, normal cells will grow even in the presence of antifolates. Cultured cells lacking one of the enzymes—HGPRT, APRT, or TK—of the salvage pathways will not survive in media containing antifolates.

salvageable purine and thymidine, whereas those lacking one of them cannot.

Cells with a TK mutation that prevents the production of the functional TK enzyme can be isolated because such cells are resistant to the otherwise toxic thymidine analog 5-bromodeoxyuridine. Cells containing TK convert this compound into 5-bromodeoxyuridine monophosphate, which is then converted into a nucleoside triphosphate by other enzymes. The triphosphate analog is incorporated by DNA polymerase into DNA, where it exerts its toxic effects. This pathway is blocked in TK^- mutants, and thus they are resistant to the toxic effects of 5-bromodeoxyuridine. Similarly, cells lacking the HGPRT enzyme, such as the $HGPRT^-$ myeloma cell lines used in producing hybridomas, can be isolated because they are resistant to the otherwise toxic guanine analog 6-thioguanine.

Normal cells can grow in HAT medium because even though the aminopterin in the medium blocks de novo synthesis of purines and TMP, the thymidine in the medium is transported into the cell and converted into TMP by TK and the hypoxanthine is transported and converted into usable purines by HGPRT. On the other hand, neither TK^- nor $HGPRT^-$ cells can grow in HAT medium because each lacks an enzyme of the salvage pathway. However, hybrids formed by the fusion of these two mutants will carry a normal *TK* gene from the $HGPRT^-$ parent and a normal *HGPRT* gene from the TK^- parent. The hybrids will thus produce both functional salvage-pathway enzymes and will grow on HAT medium.

KEY CONCEPTS OF SECTION 6.7

Growth and Use of Cultured Cells

- Growth of vertebrate cells in culture requires rich media containing essential amino acids, vitamins, fatty acids, and peptide or protein growth factors; the last are frequently provided by serum.
- Most cultured vertebrate cells will grow only when attached to a negatively charged substratum that is coated with components of the extracellular matrix.
- Primary cells, which are derived directly from animal tissue, have limited growth potential in culture and may give rise to a cell strain. Transformed cells, which are derived from animal tumors or arise spontaneously from primary cells, grow indefinitely in culture, forming cell lines (see Figure 6-37).
- The fusion of an immortal myeloma cell and a single B lymphocyte yields a hybrid cell that can proliferate indefinitely, forming a clone called a hybridoma (see Figure 6-38). Because each individual B lymphocyte produces antibodies specific for one antigenic determinant (epitope), a hybridoma produces only the monoclonal antibody synthesized by its original B-lymphocyte parental cell.
- HAT medium is commonly used to isolated hybridoma cells and other types of hybrid cells.

PERSPECTIVES FOR THE FUTURE

A deeper understanding of the integration of cells into tissues in complex organisms will draw on insights and techniques from virtually all subdisciplines of molecular cell biology: biochemistry, biophysics, microscopy, genetics, genomics, proteomics, and developmental biology. An important set of questions for the future deals with the mechanisms by which cells detect mechanical forces on them and the extracellular matrix, as well as the influence of their three-dimensional arrangements and interactions. A related question is how this information is used to control cell and tissue structure and function. Shear stresses can induce distinct patterns of gene expression and cell growth and can greatly alter cell metabolism and responses to extracellular stimuli. Future research should give us a far more sophisticated understanding of the roles of the three-dimensional organization of cells and ECM components in controlling the structures and activities of tissues.

Numerous questions relate to intracellular signaling from CAMs and adhesion receptors. Such signaling must be integrated with other cellular signaling pathways that are activated by various external signals (e.g., growth factors) so that the cell responds appropriately and in a single coordinated fashion to many different simultaneous internal and external stimuli. How are the logic circuits constructed that allow cross-talk between diverse signaling pathways? How do these circuits integrate the information from these pathways? How is the combination of outside-in and inside-out signaling mediated by CAMs and adhesion receptors merged into such circuits?

The importance of specialized GAG sequences in controlling cellular activities, especially interactions between some growth factors and their receptors, is now clear. With the identification of the biosynthetic mechanisms by which these complex structures are generated and the development of tools to manipulate GAG structures and test their functions in cultured systems and intact animals, we can expect a dramatic increase in our understanding of the cell biology of GAGs in the next several years.

A structural hallmark of CAMs, adhesion receptors, and ECM proteins is the presence of multiple domains that impart diverse functions to a single polypeptide chain. It is generally agreed that such multidomain proteins arose evolutionarily by the assembly of distinct DNA sequences encoding the distinct domains. Genes encoding multiple domains provide opportunities to generate enormous sequence and functional diversity by alternative splicing and the use of alternate promoters within a gene. Thus, even

though the number of independent genes in the human genome seems surprisingly small in comparison with other organisms, far more distinct protein molecules can be produced than predicted from the number of genes. Such diversity seems especially well suited to the generation of proteins that take part in specifying adhesive connections in the nervous system, especially the brain. Indeed, several groups of proteins expressed by neurons appear to have just such combinatorial diversity of structure. They include the protocadherins, a family of cadherins with as many as 70 proteins encoded per gene; the neurexins, which comprise more than 1000 proteins encoded by three genes; and the Dscams, members of the IgCAM superfamily encoded by a *Drosophila* gene that has the potential to express 38,016 distinct proteins owing to alternative splicing. A continuing goal for future work will be to describe and understand the molecular basis of functional cell–cell and cell–matrix attachments—the "wiring"—in the nervous system and how that wiring ultimately permits complex neuronal control and, indeed, the intellect required to understand molecular cell biology.

KEY TERMS

adhesion receptor *199*
anchoring junction *202*
basal lamina *202*
cadherin *199*
cell-adhesion molecule (CAM) *198*
cell line *236*
cell strain *236*
cell wall *231*
connexin *231*
dystrophin glycoprotein complex (DGC) *227*
epithelium *201*
extracellular matrix (ECM) *199*
fibril-associated collagen *217*
fibrillar collagen *217*
fibronectin *220*
glycosaminoglycan (GAG) *213*
HAT medium *238*
hyaluronan *217*
hybridoma *238*
immunoglobulin cell-adhesion molecule (IgCAM) *227*
integrin *199*
laminin *211*
monoclonal antibody *237*
multiadhesive matrix protein *209*
paracellular pathway *208*
plasmodesma *233*
proteoglycan *209*
RGD sequence *221*
selectin *199*
syndecan *214*
tight junction *202*

REVIEW THE CONCEPTS

1. Using specific examples, describe the two phenomena that give rise to the diversity of adhesive molecules.

2. Cadherins are known to mediate homophilic interactions between cells. What is a homophilic interaction, and how can it be demonstrated experimentally for E-cadherins?

3. What is the normal function of tight junctions? What can happen to tissues when tight junctions do not function properly?

4. What is collagen, and how is it synthesized? How do we know that collagen is required for tissue integrity?

5. You have synthesized an oligopeptide containing an RGD sequence surrounded by other amino acids. What is the effect of this peptide when added to a fibroblast cell culture grown on a layer of fibronectin absorbed to the tissue culture dish? Why does this happen?

6. Blood clotting is a crucial function for mammalian survival. How do the multiadhesive properties of fibronectin lead to the recruitment of platelets to blood clots?

7. Using structural models, explain how integrins mediate outside-in and inside-out signaling.

8. How do changes in molecular connections between the extracellular matrix (ECM) and cytoskeleton give rise to Duchenne muscular dystrophy?

9. What is the difference between a cell strain, a cell line, and a clone?

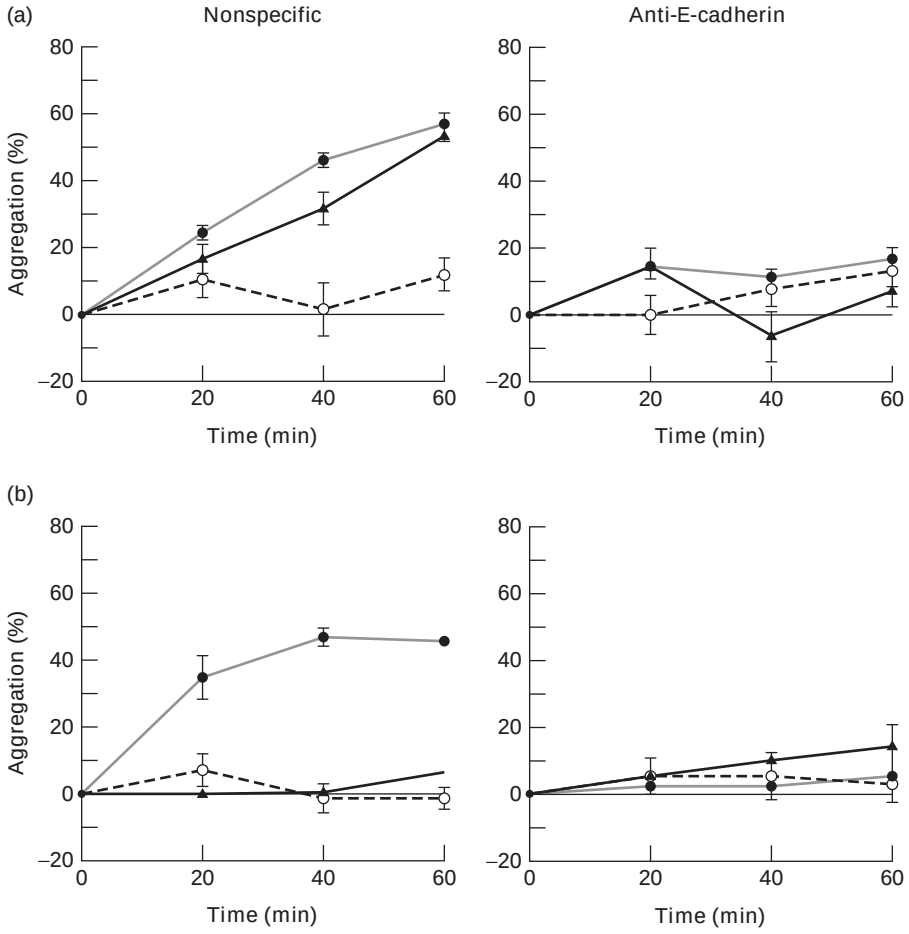
10. Explain why the process of cell fusion is necessary to produce monoclonal antibodies used for research.

ANALYZE THE DATA

Researchers have isolated two E-cadherin mutant isoforms that are hypothesized to function differently from that of the wild-type E-cadherin. An E-cadherin negative mammary carcinoma cell line was transfected with the mutant E-cadherin genes A (part a in the figure) and B (part b) (diamonds) and the wild-type E-cadherin gene (black circles) and compared to untransfected cells (open circles) in an aggregation assay. In this assay, cells are first dissociated by trypsin treatment and then allowed to aggregate in solution over a period of minutes. Aggregating cells from mutants A and B are presented in panels a and b respectively. To demonstrate that the observed adhesion was cadherin-mediated, the cells were pretreated with a nonspecific antibody (left panel) or a function-blocking anti-E-cadherin monoclonal antibody (right panel).

a. Why do cells transfected with the wild-type E-cadherin gene have greater aggregation than control, nontransfected cells?

b. From these data, what can be said about the function of mutants A and B?



c. Why does the addition of the anti-E-cadherin monoclonal antibody, but not the nonspecific antibody, block aggregation?

d. What would happen to the aggregation ability of the cells transfected with the wild-type E-cadherin gene if the assay were performed in media low in Ca^{2+}?

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