Sheets

Cell biology

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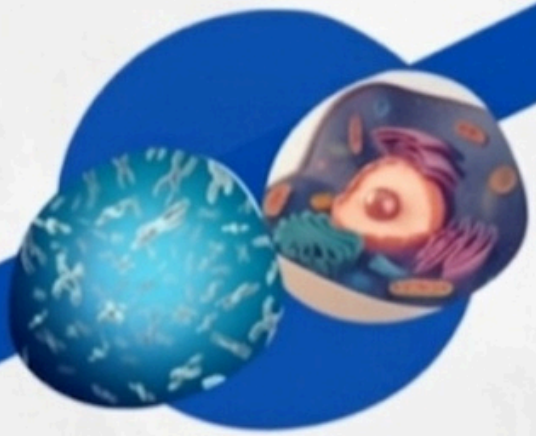

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FIGURE 7.20 Hemidesmosomes. Hemidesmosomes are differentiated sites at the basal surfaces of epithelial cells where the cells are attached to the underlying basement membrane. (a) Electron micrograph of several hemidesmosomes showing the dense plaque on the inner surface of the plasma membrane and the intermediate filaments projecting into the cytoplasm. (b) Schematic diagram showing the major components of a hemidesmosome connecting the epidermis to the underlying dermis. The $\alpha_6\beta_4$ integrin molecules of the epidermal cells are linked to cytoplasmic intermediate filaments by a protein called plectin that is present in the dark-staining plaque and to the basement membrane by anchoring filaments of a particular type of laminin. A second transmembrane protein (BP180) is also present in hemidesmosomes. The collagen fibers are part of the underlying dermis.

The importance of hemidesmosomes is revealed by a rare disease, *bullous pemphigoid*, in which individuals produce antibodies that recognize proteins present in these adhesive structures. Autoimmune disorders, diseases caused by production of antibodies directed against one's own tissues (i.e., autoantibodies), are responsible for a wide variety of conditions. In this case, the presence of autoantibodies causes the lower layer of the epidermis to lose attachment to the underlying basement membrane (and thus to the underlying connective tissue layer of the dermis). The leakage of fluid into the space beneath the epidermis results in severe blistering of the skin. A similar inherited blistering disease, *epidermolysis bullosa*, can occur in patients with genetic alterations in any one of a number of hemidesmosomal proteins, including the α_6 or β_4 integrin subunit, collagen VII, or laminin-5.

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1. How are integrins able to link the cell surface with materials that make up the ECM? How do the inactive and active structures of integrins differ from one another structurally and functionally? What is the significance of the presence of an RGD motif in an integrin ligand?
2. How can a cell-surface protein be involved in both cell adhesion and transmembrane signal transduction?
3. What is the difference between inside-out and outside-in signaling? What is the importance of each to cell activities?
4. List two characteristics that distinguish hemidesmosomes from focal adhesions.

7.4 Interactions of Cells with Other Cells

Examination of a thin section through a major organ of an animal reveals a complex architecture involving a variety of different types of cells. Little is known about the precise mechanisms responsible for generating the complex three-dimensional cellular arrangements found within developing organs, but scientists are learning more through studies of in vitro grown structures such as organoids (see Section 7.2). It is thought that this process depends

heavily on *selective* interactions between cells of the same type, as well as those of different types. It is evident that cells can recognize the surfaces of other cells, interacting with some and ignoring others.

Early attempts to learn about cell–cell recognition and adhesion were carried out by removing a developing organ from a chick or amphibian embryo, dissociating the organ's tissues to form a suspension of single cells, and determining the ability of the cells to reaggregate in culture (**Figure 7.21a**). In experiments in which cells from two different developing organs were dissociated and mixed together, the cells would initially aggregate to form a mixed clump. Over time, however, the cells would move around within the aggregate and eventually "sort themselves out" so that each cell adhered only to cells of the same type (Figure 7.21*b*). Once separated into a homogeneous cluster, these embryonic cells would often differentiate into many of the structures they would have formed within an intact embryo.

Summary

1. Scientists study organ developmentusingorganoids grown in vitro.

2. Cell organization depends on selective cell–cell interactions.

3. Cells can recognize and selectively adhere to specific other cells.

4.When cells from different organs are mixed, they first form a mixed aggregate.

Over time, cells rearrange(redistribute) and sort themselves out.

5. Each cell eventually adheres only to cells of the same type.

6. After sorting, embryonic cells can differentiate into structures similar to those formed in a normal embryo.

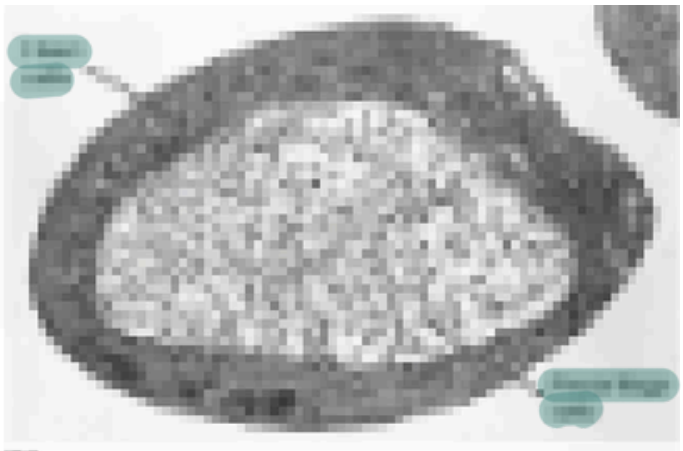

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FIGURE 7.21 Experimental demonstration of cell–cell recognition. When cells from different parts of an embryo are dissociated and then intermixed, the cells initially aggregate and then sort out by associating with other cells of the same type. The results of two such classic experiments are shown here. (a) In this experiment, two regions of an early amphibian embryo (the ectoderm and mesoderm) were dissociated into single cells and combined. At first the cells form a mixed aggregate, but eventually they sort out. The ectodermal cells (shown in red) move to the outer surface of the aggregate, which is where they would be located in the embryo, and the mesodermal cells (shown in purple) move to the interior, the position they would occupy in the embryo. Both types of cells then differentiate into the types of structures they would normally give rise to. (b) Light micrograph showing the results of an experiment in which precartilage cells from a chick limb are mixed with chick heart ventricle cells. The two types of cells have sorted themselves out of the mixed aggregate with the heart cells forming a layer on the outside of the precartilage cells. It is proposed that the precartilage cells collect in the center of the aggregate because the cells adhere to one another more strongly than do the cells from the heart. (This and other models are discussed in *Nat. Cell Biol.* 10:375, 2008.)

Researchers now have identified dozens of different proteins involved in cell adhesion. Different arrays of these molecules on the surfaces of different types of cells are thought to be responsible for the specific interactions between cells within complex tissues.

Four distinct families of integral membrane proteins play a major role in mediating cell–cell adhesion: (1) selectins, (2) certain members of the immunoglobulin superfamily (IgSF), (3) certain members of the integrin family, and (4) cadherins.

Like the integrins discussed in the previous section, these proteins play a dual role. One major function is their structural role in adhesion. Another major role is to transfer information across the plasma membrane, a process known as **transmembrane signaling** (which will be discussed further in Chapter 15). For example, integrins and cadherins can transmit signals from the extracellular environment to the cytoplasm by means of linkages with the cytoskeleton and with cytosolic regulatory molecules, such as protein kinases and G proteins. Protein kinases activate (or inhibit) their target proteins through phosphorylation, whereas G proteins activate (or inhibit) their protein targets through physical interaction (see Figure 15.21*b*). A variety of responses within the cell can result, including changes in gene expression which can, in turn, alter a cell's growth potential, migratory activity, state of differentiation, or survival.

Selectins

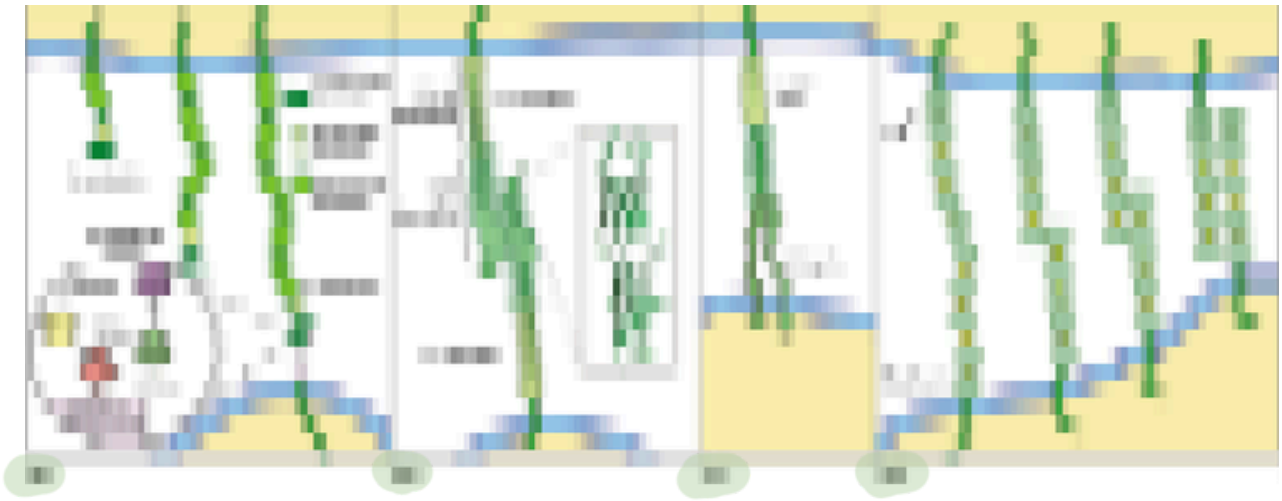
During the 1960s, it was discovered that lymphocytes that had been removed from peripheral lymph nodes, radioactively labeled, and injected back into the body would return to the sites from which they were originally derived—a phenomenon called *lymphocyte homing*. It was subsequently found that homing could be studied in vitro by allowing lymphocytes to adhere to frozen sections of lymphoid organs. Under these experimental conditions, the lymphocytes

would selectively adhere to the endothelial lining of the venules (the smallest veins) of peripheral lymph nodes. Binding of lymphocytes to venules could be blocked by antibodies directed against a specific glycoprotein, called L-selectin, on the lymphocyte surface.

Selectins comprise a family of integral membrane glycoproteins that recognize and bind to a particular arrangement of sugars in the oligosaccharides that project from the surfaces of other cells (Section 4.3). The name of this class of cell-surface receptors is derived from the word "lectin," a general term for a compound that binds to specific carbohydrate groups. Selectins possess a small cytoplasmic segment, a single membrane-spanning domain, and a large extracellular portion that consists of a number of separate domains, including an outermost domain that acts as the lectin (**Figure 7.22a**). There are three known selectins: E-selectin, present on endothelial cells; P-selectin, present on platelets and endothelial cells; and L-selectin, present on leukocytes (white blood cells). All three selectins recognize a particular grouping of sugars (Figure 7.22*a*) that is found at the ends of the carbohydrate chains of certain complex glycoproteins. Binding of selectins to their carbohydrate ligands requires calcium. As a group, selectins mediate transient interactions between circulating leukocytes and vessel walls at sites of inflammation and clotting. Capturing a leukocyte is a challenging task because these cells are flowing through the bloodstream at a considerable rate of speed. Selectins are well suited for this function because the bonds that they form with their ligands become stronger when the interaction is placed under mechanical stress, as occurs when the leukocyte is being pulled away from a given site on the vessel wall. The role of selectins in inflammation is discussed further in the Human Perspective feature.

Summary

- What are Selectins?
  - A family of integral membrane glycoproteins.
  - Bind to specific carbohydrate (sugar) groups on other cells.
  - Binding is calcium-dependent.
- Structure
  - Small cytoplasmic tail
  - Single membrane-spanning domain
  - Large extracellular region
  - The outermost domain functions as a lectin (binds carbohydrates)
- Types of Selectins
  - E-selectin → found on endothelial cells
  - P-selectin → found on platelets and endothelial cells
  - L-selectin → found on leukocytes (white blood cells)
- Main Function
  - Mediate transient (temporary) adhesion between circulating leukocytes and blood vessel walls.
  - Essential in inflammation and clotting.
  - Help capture leukocytes from fast-moving blood flow.



Source: Inset of (*b*) From E. Yvonne Jones et al., *Nature* 373:540, 1995. ©1995. Reprinted by permission from Springer Nature.

FIGURE 7.22 Overview ofcell adhesion molecules. (a) Selectins. All three types of known selectins recognize and bind to a similar carbohydrate ligand at the ends of oligosaccharide chains on glycoproteins. In the inset, the detailed structure of the carbohydrate ligand is shown. The terminal fucose and sialic acid moieties are particularly important in selectin recognition, and the *N*-acetylglucosamine moiety is often sulfated. (b) L1 molecules. L1 is a member of the immunoglobulin (Ig) superfamily. Cell–cell adhesion results from the specific interactions of the immunoglobulin (Ig) domains of two L1 molecules projecting from the surfaces of neighboring cells. Each L1 molecule contains a small cytoplasmic domain, a transmembrane segment, several segments that resemble one type of module found in fibronectin, and six Ig domains situated at the N-terminal portion of the molecule. The inset shows the structure of the two N-terminal Ig domains of VCAM, an IgSF molecule on the surface of endothelial cells. The Ig domains of VCAM and L1 have a similar three-dimensional structure consisting of two sheets packed face-to-face. (c) Integrin-IgSF interactions. IgSF family members can also bind to integrins of neighboring cells (d) Cadherins. Cell–cell adhesion results from the interactions between similar types of cadherins projecting from the plasma membrane of each cell. Calcium ions (shown as small yellow spheres) are situated between the successive domains of the cadherin molecule where they play a critical role in maintaining the rigidity of the extracellular portion of the protein. This illustration shows several alternate models by which cadherins from opposing cells might interact. Different types of studies have suggested different degrees of overlap (interdigitation) between the extracellular domains of molecules from opposing cells. For consistency, subsequent figures will depict cadherins with a single domain overlap, which is likely the predominant configuration.

The Human Perspective

TheRoleofCellAdhesioninInflammation and Metastasis

Inflammation is one of the primary responses to infection. If a part of the body becomes contaminated by bacteria, as might occur following a puncture wound to the skin, the site of injury becomes a magnet for a variety of white blood cells. White blood cells (leukocytes) that would normally remain in the bloodstream are stimulated to traverse the endothelial layer that lines the smallest veins (venules) in the region and enter the tissue. Once in the tissue, in response to chemical signals, the leukocytes move toward the invading microorganisms, which they ingest.* Although inflammation is a protective response, it also produces adverse side effects, such as fever, swelling due to fluid accumulation, redness, and pain.

Inflammation can also be triggered inappropriately. For example, damage to the tissues of the heart or brain can occur when blood flow to these organs is blocked during a heart attack or stroke. When blood flow to the organ is restored, circulating leukocytes may attack the damaged tissue, causing a condition known as *reperfusion damage*. An overzealous inflammatory response can also lead to asthma, toxic shock syndrome, and respiratory distress syndrome. A great deal of research has focused on questions related to these conditions: How are leukocytes recruited to sites of inflammation? How are they able to stop flowing through the bloodstream and adhere to vessel walls? How do they penetrate the walls of the vessels? How ca n s o m e of the negative side effects of inflammation be blocked without interfering with the beneficial aspects of the response? Answers to questions about inflammation have focused on three types of cell adhesion molecules: selectins, integrins, and IgSF proteins. → Recruitment.

A chain of events that has been proposed to occur during acu te inflammation is shown in **Figure 1**. The first step is activation of the walls of the venules in response to chemical signals from nearby damaged tissue. The endothelial cells that line these venules become more adhesive to circulating neutrophils, a type of phagocytic leukocyte that carries out a rapid, nonspecific attack on invading pathogens. This change in adhesion is mediated by a temporary display of P- and E-selectins on the surfaces of the activated endothelial cells in the damaged area (step 2, Figure 1). When neutrophils encounter the selectins, they form transient adhesions that dramatically slow their movement through the vessel. At this stage, the neutrophils can be seen to “roll” slowly along the wall of the vessel. A number of biotechnology companies are attempting to develop anti-inflammatory drugs that act by interfering with binding of ligands to E- and P-selectins. Anti-selectin antibodies block neutrophil rolling on selectin-coated surfaces in vitro and suppress inflammation and reperfusion damage in animals. A similar type of blocking effect has been attained using synthetic carbohydrates (e.g., efomycines) that bind to E- and P-selectin, thereby competing with carbohydrate ligands on the surfaces of the neutrophils.

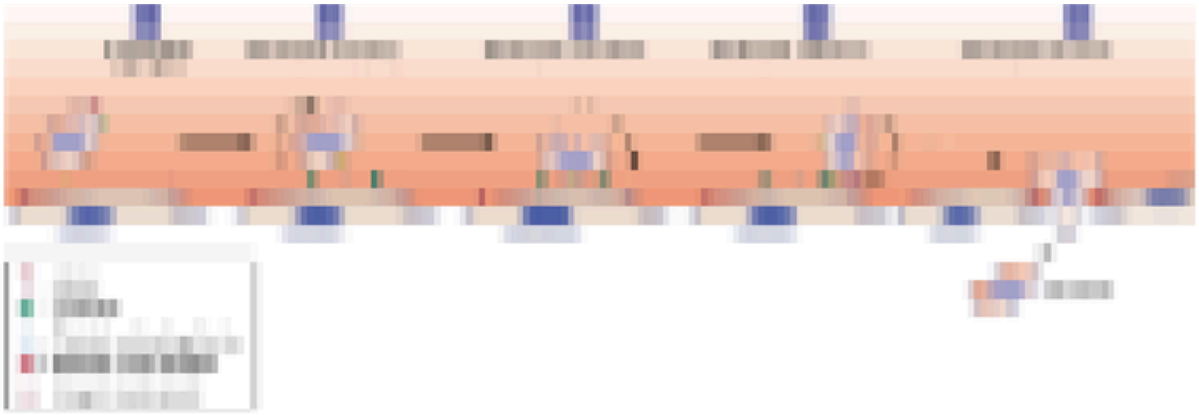


FIGURE 1 Steps in the movement of neutrophils from the bloodstream during inflammation. The steps are described in the text.

As the neutrophils interact with the inflamed venule endothelium, interactions take place between other molecules on the surfaces of the two types of cells. One of the molecules displayed on the surfaces of endothelial cells is a phospholipid called *platelet activating factor* (*PAF*). PAF binds to a receptor on the surface of the neutrophil, sending a signal into the neutrophil that leads to an increase in the binding activity of certain integrins (e.g., $\alpha_L\beta_2$ and $\alpha_4\beta_1$) already situated on the neutrophil surface (step 3, Figure 1). This is an example of the type of inside-out signal that is illustrated in Figure 7.14. The activated integrins then bind with high affinity to IgSF molecules (e.g., ICAM-1 and VCAM-1) on the surface of the endothelial cells, causing the neutrophils to stop their rolling and adhere firmly to the wall of the vessel (step 4). The bound neutrophils then change their shape and squeeze between adjacent endothelial cells into the damaged tissue (step 5). Invading neutrophils appear capable of disassembling the adherens junctions described later in this section, which form the major barrier between cells of the vessel wall. This cascade of events, which involves several different types of cell-adhesion molecules, ensures that attachment of blood cells to the walls of blood vessels and subsequent penetration will occur only at sites where leukocyte invasion is required.

The importance of integrins in the inflammatory response is demonstrated by a rare disease called *leukocyte adhesion deficiency* (*LAD*). Persons with the type I form of LAD are unable to produce the β_2 subunit as part of a number of leukocyte integrins. As a result, their leukocytes lack the ability to adhere to the endothelial layer of venules, a step required for exit from the bloodstream. These patients suffer from repeated life-threatening bacterial infections. The disease is best treated by bone marrow transplantation, which provides the patient with stem cells capable of forming normal leukocytes. Administration of antibodies against the β_2 subunit can mimic the effects of LAD, blocking the movement of neutrophils and other leukocytes out of blood vessels. Such antibodies might prove useful in preventing inflammatory responses associated with diseases such as asthma and rheumatoid arthritis or with reperfusion.

Cancer is a disease in which cells escape from the body's normal growth control mechanisms and proliferate in an unregulated manner. Were the malignant cells to remain in a single mass, as often occurs in some types of skin cancer or thyroid cancer, most cancers would be readily cured by surgical removal of the diseased tissue. Most malignant tumors, however, spawn cells that are capable of leaving the primary tumor mass and entering the bloodstream or lymphatic channels, thereby initiating the growth

of secondary tumors in other parts of the body (**Figure 2a**). **Metastasis**, the spread of a tumor within the body, is the reason cancer is such a devastating disease. Metastatic cells (cancer cells that are able to initiate the formation of secondary tumors) are thought to have special cell-surface properties that are not shared by most other cells in the tumor. For example:

1. Metastatic cells must be less adhesive than other cells to break free of the tumor mass.
2. Metastatic cells must be able to penetrate numerous barriers, such as the extracellular matrices of surrounding connective tissue and the basement membranes that underlie the epithelium and line the blood vessels that carry them to distant sites (Figure 2*a*). Although the presence of cancer cells in the bloodstream is a potentially lethal circumstance, it does provide an opportunity for nonsurgical access to a patient's cancer. A number of devices have recently been developed to trap rare circulating tumor cells (CTCs) present in blood samples taken from cancer patients (Figure 2*b*). Once they have been captured, CTCs from a patient can be studied to determine the molecular characteristics of the tumor, how aggressive the tumor is likely to be, how it might be best treated, and how well a particular therapy is progressing as reflected in the decreasing number of CTCs over time. One day CTCs might be used as the basis for early detection of disease.
3. Metastatic cells must be able to invade normal tissues and survive to form secondary colonies. For some reason, this third step appears to pose the greatest obstacle in the process of metastasis for most types of tumors. Consequently, only a very small fraction of cancer cells that exit the bloodstream typically produce viable secondary tumors.

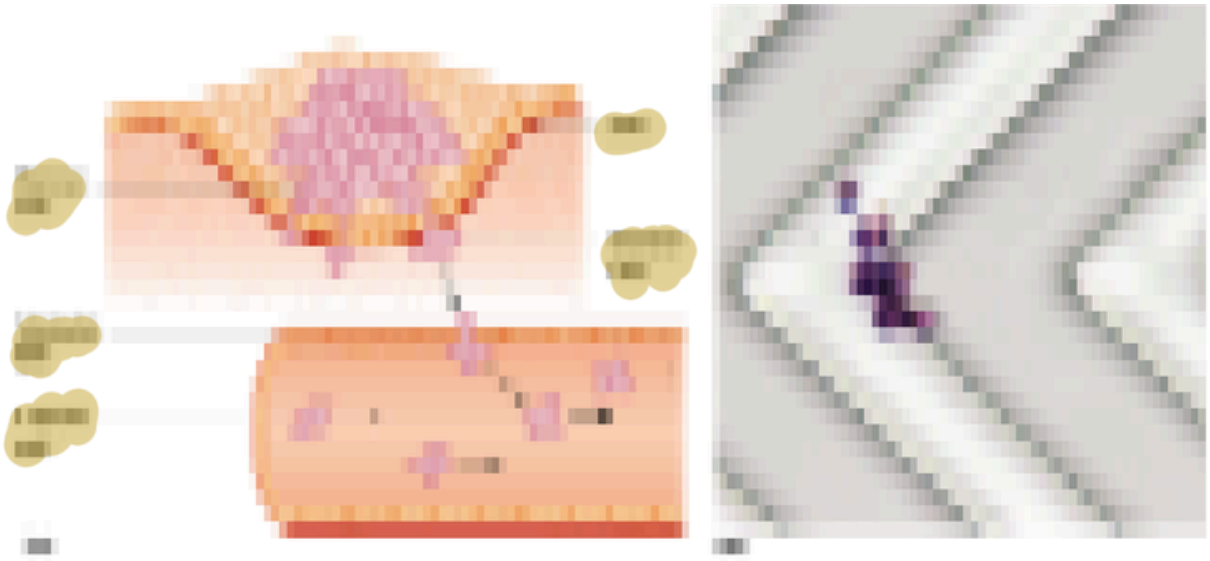


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FIGURE2Steps leading to the metastatic spread of an epithelial cancer(a carcinoma). (a) A fraction of the cells of the primary tumor lose their adhesiveness to other tumor cells and gain the capability to penetrate the basement membrane (BM) barrier that underlies the epithelial tissue. These cells, which have assumed a mesenchymal-like appearance, migrate through the surrounding stromal tissue and cross the BM of a blood or lymph vessel, thereby entering the general circulation. The cells are carried to other tissues, where they migrate back across the BM of the vessel and enter a tissue in which they possess the potential to form secondary tumors. Only a very small percentage of tumor cells that are released from a primary tumor manage to overcome these numerous hurdles, but those that do pose a threat to the life of the host. (b) These circulating tumor cells (CTCs) have been isolated from a blood sample of a patient with prostate cancer. Even though the blood of a cancer patient may contain less than one cancer cell for every billion normal cells, these rare cancer cells can be selectively trapped on a chip that has been coated with antibody molecules directed against a cell-surface protein (in this case, EpCAM) that is present on cancer cells but absent from normal blood cells.

The mechanisms used by cancer cells to penetrate extracellular matrices are poorly understood because such events have been virtually impossible to study within the tissues of a living animal. It is thought that movement through basement membranes is accomplished largely by ECM-digesting enzymes, most notably the matrix metalloproteinases (MMPs) discussed at the end of Section 7.1.These enzymes are presumed to degrade the proteins and proteoglycans that stand in the way of cancer cell migration. In some types of cancer cells, MMPs are directly associated with long cell projections, called *invadopodia*, that push their way through the ECM. Among other tumor-promoting functions of MMPs, cleavage of certain proteins of the ECM or cell

surface produces active protein fragments that stimulate the growth and invasive character of the cancer cells. Because of their apparent roles in the development of malignant tumors, MMPs became a prominent target of the pharmaceutical industry. Once it was demonstrated that synthetic MMP inhibitors were able to reduce metastasis in mice, a number of clinical trials of these drugs were conducted on patients with a variety of advanced, inoperable cancers. Unfortunately, these inhibitors have shown little promise in stopping late-stage tumor progression and, in some cases, have led to joint damage. To date, the only FDA-approved MMP inhibitor (Periostat) is used to treat periodontal disease.

Studies have shown that changes to the ECM itself accompany many diseases, including cancer. In breast cancer, for instance, the ECM surrounding tumor cells can be up to 10 times stiffer, resulting in the ability to feel a "lump" during a breast exam. This increased stiffness is thought to be due to increased crosslinks in collagen and other components of the ECM. In addition, breast tumors show an increase in linearized and bundled forms of collagen compared to normal breast tissue. This reorganization of the ECM by cancer tissue appears to play an important role in metastases, because stiffer, bundled collagen tends to promote cell migration.

Changes in the numbers and types of various cell-adhesion molecules—and thus the ability of cells to adhere to other cells or to extracellular matrices—have also been implicated in promoting metastasis. The greatest focus in this area has centered on E-cadherin, which is the predominant cell-adhesion molecule of the adherens junctions that hold epithelial cells in a cohesive sheet. Section 7.4 on cadherins describes how the loss of E-cadherin from epithelial cells during certain events of embryonic development is associated with conversion of the cells into a less adhesive, more motile, mesenchymal phenotype. A remarkably similar epithelial-mesenchymal transition occurs at the peripheral edges of a tumor as malignant cells separate from the primary tumor mass and invade the adjacent normal tissue (Figure 2*a*). This is an important step in the process of metastasis. Surveys of a variety of epithelial cell tumors (e.g., breast, prostate, and colon cancers) confirm that these malignant cells have greatly reduced levels of E-cadherin; the lower the level of expression of E-cadherin, the greater the cell's metastatic potential. Conversely, when malignant cells are forced to express extra copies of the E-cadherin gene, the cells become much less capable of causing tumors when injected into host animals. The presence of E-cadherin is thought to favor the adhesion of cells to one another and suppress the dispersal of tumor cells to distant sites. E-cadherin may also inhibit the signaling pathways within the cell that lead to tissue invasion and metastasis. The importance of E-cadherin is evident from a study of a family of native New Zealanders that had lost 25 members to stomach cancer over a 30-year period. Analysis of the DNA of family members has revealed that susceptible individuals carry mutations in the gene encoding E-cadherin.

* A remarkable film of a leukocyte "chasing" a bacterium can be found on the Web by searching the keywords "neutrophil crawling."

The Immunoglobulin Superfamily

Elucidation of the structure of bloodborne antibody molecules in the 1960s was one of the milestones in our understanding of the immune response. Antibodies, which are a type of protein called an immunoglobulin (or Ig), were found to consist of polypeptide

chains composed of a number of similar domains. Each of these Ig domains, as they are called, is composed of 70 to 110 amino acids organized into a tightly folded structure, as shown in the inset of Figure 7.22*b*. The human genome encodes 765 distinct Ig domains, making it the most abundant domain in human proteins. Taken as a group, these proteins are members of the **immunoglobulin superfamily**, or **IgSF**. Most members of the IgSF are involved in various aspects of immune function, but some of these proteins mediate calcium-independent cell–cell adhesion. In fact, the discovery of Ig-like domains in cell-adhesion receptors in invertebrates—animals that lack a classic immune system—suggests that Ig-like proteins originally evolved as cell-adhesion mediators and only secondarily took on their functions as effectors of the vertebrate immune system.

Most IgSF cell-adhesion molecules mediate the specific interactions of lymphocytes with cells required for an immune response (e.g., macrophages, other lymphocytes, and target cells). However, some IgSF members, such as VCAM (vascular cell-adhesion molecule), NCAM (neural cell-adhesion molecule), and L1 (also called L1CAM), mediate adhesion between nonimmune cells. NCAM and L1, for example, play important roles in nerve outgrowth, synapse formation, and other events during the development of the nervous system. Like fibronectin and many other proteins involved in cell adhesion, the IgSF cell adhesion molecules have a modular construction (Figure 7.22*b,c*) and are composed of individual domains of similar structure to those in other proteins.

The importance of L1 in neural development has been revealed in several ways. In humans, mutations in the L1 gene can have devastating consequences. In extreme cases, babies are born with a fatal condition of hydrocephalus ("water on the brain"). Children with less severe mutations typically exhibit mental retardation and difficulty in controlling limb movements (spasticity). Autopsies on patients that have died of an L1-deficiency disease reveal a remarkable condition: They are often missing two large nerve tracts, one that runs between the two halves of the brain and the other that runs between the brain and the spinal cord. The absence of such nerve tracts suggests that L1 is involved in the directed growth of axons within the embryonic nervous system.

Various types of proteins serve as ligands for IgSF cell surface molecules. As described earlier, most integrins facilitate adhesion of cells to their substratum, but a few integrins mediate cell–cell adhesion by binding to proteins on other cells (Figure 7.22*c*). For example, the integrin $\alpha_4\beta_1$ on the surface of leukocytes binds to VCAM, an IgSF protein on the endothelial lining of certain blood vessels (see the Human Perspective feature).

Cell–cell adhesion from homotypic interactions of two L1 molecules through Ig domains at the N-termin. i.

Ca dhe ri ns

The **cadherins** are a large family of glycoproteins that mediate Ca^{2+}-dependent cell–cell adhesion and transmit signals from the ECM to the cytoplasm. Cadherins typically join cells of similar type to one another and do so predominantly by binding to the same cadherin present on the surface of the neighboring cell. This property of cadherins was first demonstrated by genetically engineering cells that were normally nonadhesive to express one of a variety of different cadherins. The cells were then mixed in various combinations and their interactions monitored. It was found that cells expressing one species of cadherin preferentially adhered to other cells expressing the same cadherin. These types of findings have led researchers to believe that cadherins are largely responsible for the ability of like cells to "sort out" of mixed aggregates, as was illustrated in Figure 7.21. In fact, cadherins may be the single most important factor in molding

cells into cohesive tissues in the embryo and holding them together in the adult. As discussed in the Human Perspective feature, the loss of cadherin function may play a key role in the spread of malignant tumors.

Like many other proteins involved in adhesion, cadherins have a modular construction. The best-studied cadherins are E-cadherin (epithelial), N-cadherin (neural), and P-cadherin (placental). These "classical" cadherins, as they are called, contain a relatively large extracellular segment consisting of five tandem domains of similar size and structure, a single transmembrane segment, and a small cytoplasmic domain (Figure 7.22*d*). The cytoplasmic domain is often associated with members of the *catenin* family of cytosolic proteins, which have a dual role: They tether the cadherins to the cytoskeleton (see Figure 7.26), and they transmit signals to the cytoplasm and nucleus. A number of models of cadherin adhesion are depicted in Figure 7.22*d*. Structural studies indicate that cadherins from the same cell-surface associate laterally to form parallel dimers. These studies also shed light on the role of calcium, which has been known for decades to be essential for cell–cell adhesion. As indicated in Figure 7.22*d*, calcium ions form bridges between successive domains of a given molecule. These calcium ions maintain the extracellular portion of each cadherin in a rigid conformation required for cell adhesion. Adhesion between cells results from the interaction between the extracellular domains of cadherins from opposing cells to form a "cell-adhesion zipper." Different types of cells bearing different cadherins likely engage in different types of interactions, so that many different configurations (as depicted in Figure 7.22*d*) may occur within an organism. Just as cadherin interdigitation can be compared to a zipper, cadherin clusters can be compared to Velcro; the greater the number of interacting cadherins in a cluster, the greater the strength of adhesion between apposing cells.

Embryonic development is characterized by change: change in gene expression, change in cell shape, change in cell motility, change in cell adhesion, and so forth. Cadherins are thought to mediate many of the dynamic changes in adhesive contacts that are required to construct the tissues and organs of an embryo, which is known as the process of *morphogenesis*. For example, a number of morphogenetic events during embryonic development involve a group of cells changing from an epithelium (tightly adherent, apical-basal polarized, stationary cell layer) to a mesenchyme (solitary, nonadhesive, nonpolarized, migratory cells), or vice versa. The **epithelial-mesenchymal transition** (or **EMT**) is illustrated by the formation of the mesoderm during gastrulation in a chick or mammalian embryo. Typically, these cells break away from a cohesive epithelial layer (called the epiblast) at the dorsal surface of the early embryo and wander into the interior regions as mesenchymal cells (**Figure 7.23a,b**). These mesenchymal cells will eventually give rise to mesodermal tissues such as blood, muscle, and bone. The cells of the epiblast display E-cadherins on their surface, which is presumed to promote their close association with one another. Prior to their separation from the epiblast, the future mesodermal cells stop expressing E-cadherin, which is thought to promote their release from the epithelium and transformation into mesenchymal cells (Figure 7.23*a*). At a later stage of development, another major event, the formation of the primitive nervous system, is also characterized by changes in cadherin expression. Following gastrulation, the dorsal surface of the embryo is covered by a single-celled epithelial layer, which will become the ectodermal tissues of the animal (including the skin and nervous system). At this stage, the cells in the central region of this layer stop expressing E-cadherin and begin expressing N-cadherin (Figure 7.23*c*). In

subsequent stages, the epithelial cells expressing N-cadherin separate from their neighbors on either side and roll into the neural tube, which will go on to become the animal's brain and spinal cord. It is thought that cadherins (and other cell-adhesion molecules) play a key role in these events by changing the adhesive properties of cells.

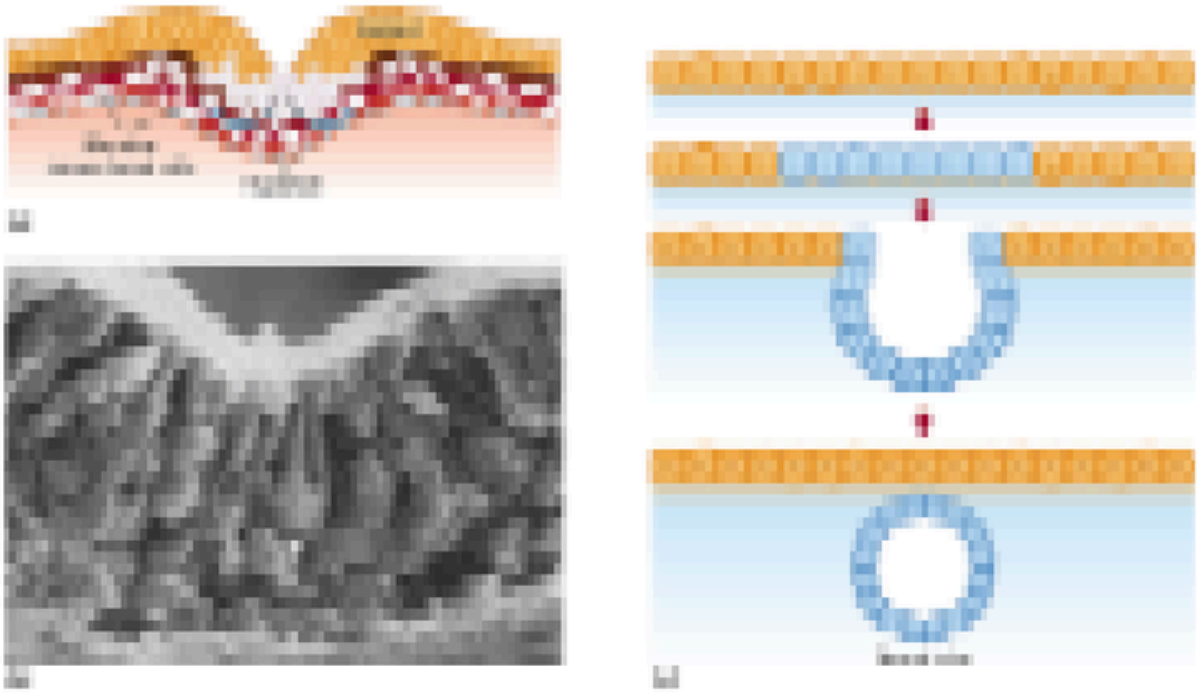

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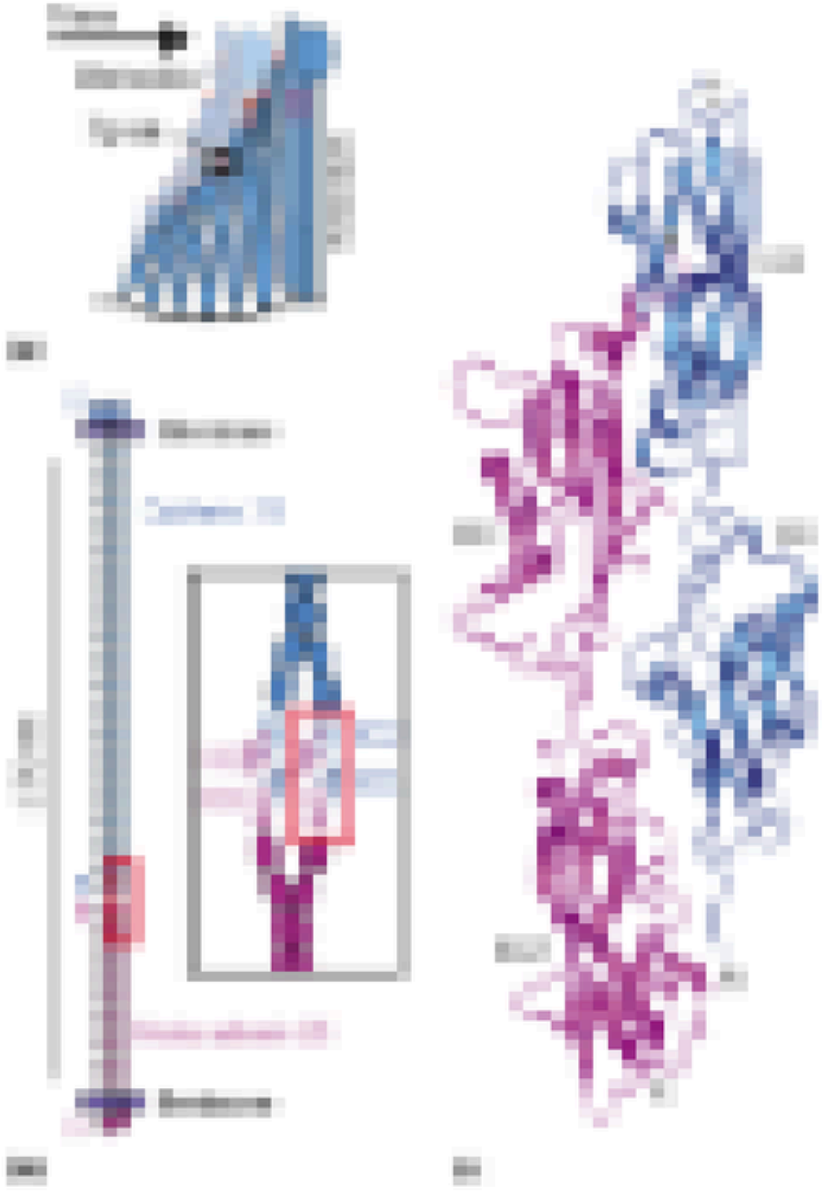
FIGURE 7.23Cadherins and morphogenesis. (a) During gastrulation, cells in the upper layer of the embryo (the epiblast) move toward a groove in the center of the embryo, sink into the groove, and then migrate laterally as mesenchymal cells in the space beneath the epiblast. This epithelial-mesenchymal transition is marked by a loss of expression of E-cadherin that is characteristic of epithelial cells. Cells expressing E-cadherin are depicted in orange. (b) Scanning electron micrograph of a chick embryo during gastrulation that has been fractured to reveal the cells undergoing the epithelial-mesenchymal transition (arrow) depicted in part *a*. (c) This sequence of drawings depicts the development of the neural tube, which is an epithelial layer that forms by separation from the overlying layer of dorsal ectoderm. In the top drawing, the epithelial cells are expressing E-cadherin. In the lower drawings, the cells of the neural tube stop expressing E-cadherin (orange) and instead express N-cadherin (blue).

To date, over 100 cadherins have been identified in humans, many of which are thought to be functionally redundant. Single mutations in a number of cadherins, however, have been linked to genetic diseases. One of the most notable is **Usher syndrome**, which is characterized by deafness and gradual loss of vision. Usher syndrome can be caused by mutations in a number of genes, but some of the most severe forms are caused by mutations in either cadherin 23 or protocadherin 15. These nonclassical cadherins form a fine tether, called a tip link, between adjacent stereocilia on the surface of hair cells in the inner ear, as shown in **Figure 7.24**. Tip links are essential for converting the mechanical movement of the stereocilia (caused by soundwaves) into an electrical

current, and also play a critical structural role in the maintenance of stereociliary organization. Mutations in these tip links can result in severe defects in the organization and development of stereocilia, resulting in profound deafness.



Source: From M. Sotomayor et al, Reprinted by permission from Macmillan Publishers Ltd: *Nature* 492:128–32,2012.

FIGURE 7.24Cadherins form tip links of stereocilia. Cadherin 23 and protocadherin 15 form the tip link on stereocilia that play a key role in converting mechanical force, in the form of sound waves, into electrical signals that are carried to the brain. (a) A schematic drawing of stereocilia, which are located on cells known as hair cells in the inner ear, is shown here. The tip links are colored in red. The structure of the tip link is shown in (b), with cadherin 23 shown in blue and protocadherin shown in purple. The crystal structure of the interacting domains is revealed in (c).

Adherens Junctions and Desmosomes

Whereas cadherins are typically distributed diffusely all along the surfaces of two adherent cells, they also participate in the formation of specialized intercellular junctions called *adherens junctions* and *desmosomes*. In addition to these adhesive junctions, epithelial cells often contain other types of cell junctions, called *tight junctions* and *gap junctions*, that will be discussed later in the chapter (see Sections 7.5 and 7.6).

Adherens junctions are found in a variety of sites within the body. They are particularly common in epithelia, such as the lining of the intestine, where they occur as "belts" (or *zonulae adherens*) that encircle each cell near its apical surface, binding that cell to its surrounding neighbors (**Figure 7.25**). In an adherens junction, cells are held together by calcium-dependent linkages formed between the extracellular domains of cadherin molecules that bridge the 30–nm gap between neighboring cells (**Figure 7.26**). As Figure 7.26*a,b* illustrates, the cytoplasmic domains of these cadherins are linked by α- and β-catenins to a variety of cytoplasmic proteins, including actin filaments of the cytoskeleton. Thus, like the integrins of a focal adhesion, the cadherin clusters of an adherens junction (1) connect the external environment to the actin cytoskeleton and (2) provide a pathway for signals to be transmitted from the cell exterior to the cytoplasm. To give one example, the adherens junctions situated between endothelial cells that line the walls of blood vessels transmit signals that ensure the survival of the cells. Mice that are lacking an endothelial cell cadherin are unable to transmit these survival signals, and these animals die during embryonic development as a result of the death of the cells lining the vessel walls.

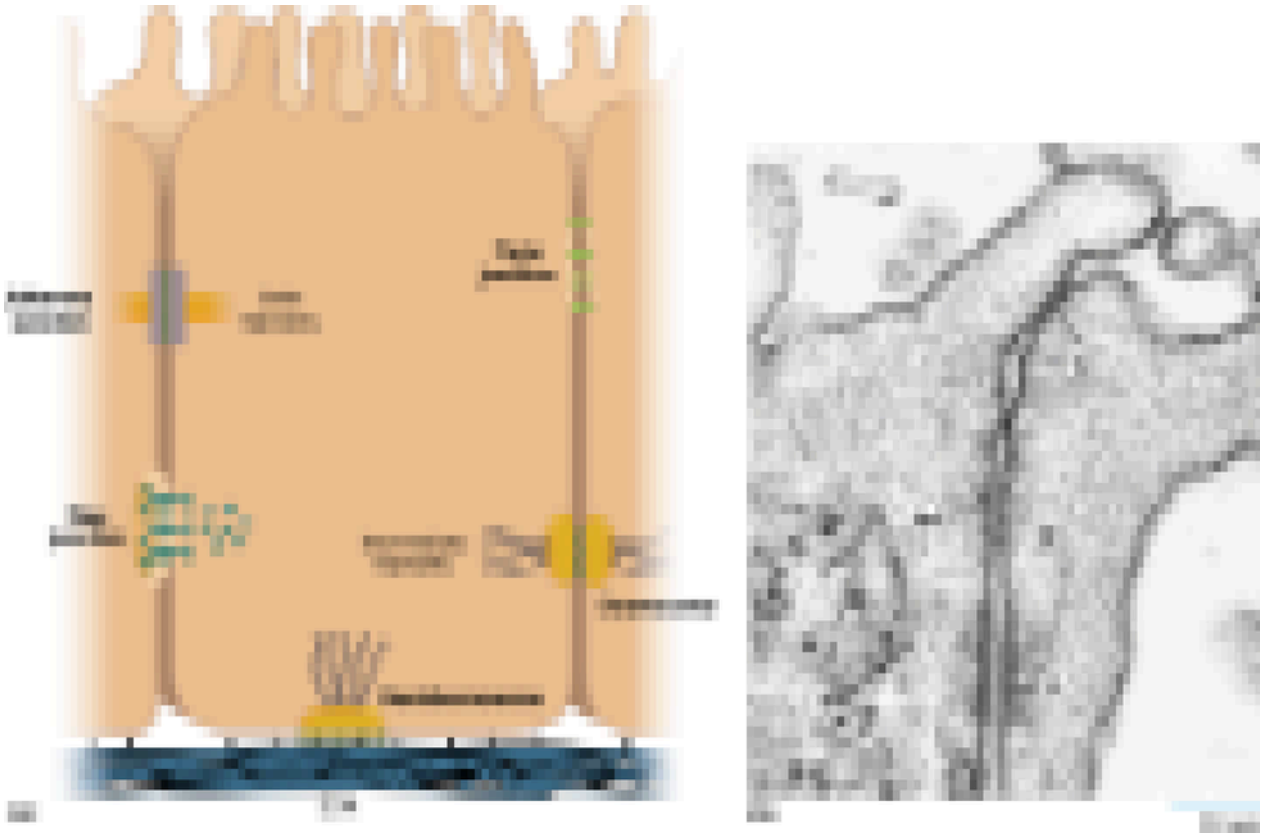
1. Cadherins are distributed on cell surfaces but also form specialized junctions: adherens junctions and desmosomes.

2. Epithelial cells also have tight junctions and gap junctions.

3. Adherens junctions are common in epithelia (e.g., intestine).
4. They form belt-like structures (zonula adherens) near the apical surface.
5. Cells are connected by calcium-dependent cadherin interactions across a 30. nm gap.
6. Cadherins bind to α- and β-catenins, linking them to actin filaments.
7. Functions of adherens junctions:

a) Connect extracellular space to actin cytoskeleton. .

b) Transmit signals from outside to inside the cell.



Source:(*b*) From Eveline E. Schneeberger and Robert D. Lynch, *Am. J. Physiol.* 262:l648, 1992. © The American Physiological Society (APS). All rights reserved.

FIGURE 7.25An intercellular junctional complex.(a) Schematic diagram showing the junctional complex on the lateral surfaces of a simple columnar epithelial cell. The complex consists of a tight junction (zonula occludens), an adherens junction (zonula adherens), and a desmosome (macula adherens). Other desmosomes and gap junctions are located deeper along the lateral surfaces of the cells. Adherens junctions and tight junctions encircle the cell, whereas desmosomes and gap junctions are restricted to a particular site between adjacent cells. Hemidesmosomes are shown at the basal cell surface. (b) Electron micrograph of a junctional complex between two rat airway epithelial cells (TJ, tight junction; AJ, adherens junction; D, desmosome).

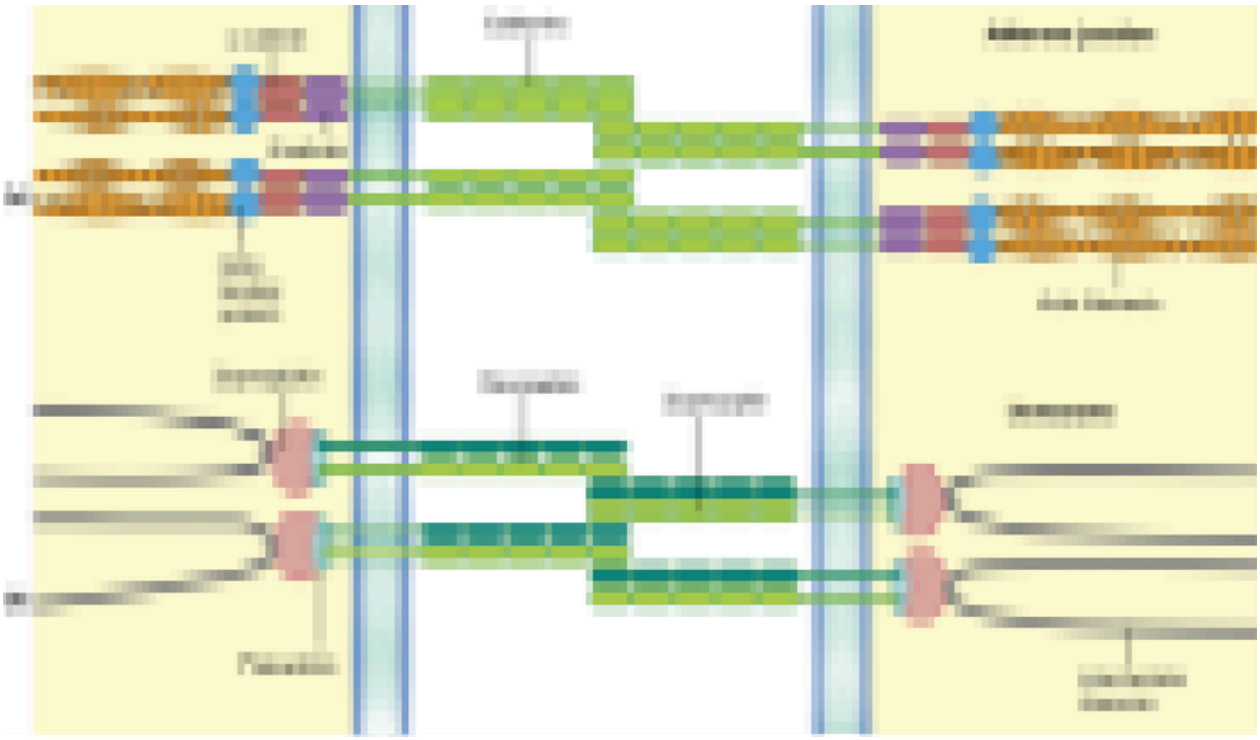


FIGURE 7.26Schematic model of the molecular architecture of an adherens junction and desomosome. (a) The cytoplasmic domain of each cadherin molecule is connected to the actin filaments of the cytoskeleton by linking proteins, including β-catenin, α-catenin, and various actin-binding proteins. One of these actin-binding proteins is formin, which participates in the polymerization of the actin filaments. (b) Schematic model of the molecular architecture of a desmosome. Specialized cadherins, called desmoglein and desmocollin, bind to one another in the extracellular space. In the cytoplasm, the desmosomal cadherins bind indirectly to intermediate filaments.

Desmosomes (or *maculae adherens*) are disk-shaped adhesive junctions approximately 1 μm in diameter (see Figure 7.27) that are found in a variety of tissues. Desmosomes are particularly numerous in tissues that are subjected to mechanical stress, such as cardiac muscle and the epithelial layers of the skin and uterine cervix. Like adherens junctions, desmosomes contain cadherins that link the two cells across a narrow extracellular gap. The cadherins of desmosomes have a different domain structure from the classical cadherins found in adherens junctions and are referred to as *desmogleins* and *desmocollins* (Figure 7.26*b*). Dense cytoplasmic plaques on the inner surfaces of the plasma membranes serve as sites of anchorage for looping intermediate filaments similar to those of hemidesmosomes (Figure 7.20). The three-dimensional network of ropelike intermediate filaments provides structural continuity and tensile strength to the entire sheet of cells. Intermediate filaments are linked to the cytoplasmic domains of the desmosomal cadherins by additional proteins, as depicted in **Figure 7.27**. The importance of cadherins in maintaining the structural integrity of an epithelium is illustrated by an autoimmune disease (*pemphigus vulgaris*) in which antibodies are produced against one of the desmogleins. The disease is characterized by a loss of epidermal cell–cell adhesion and severe blistering of the skin.

Source:©1996 Douglas E. Kelly et al. Originally published in *The Journal of Cell Biology* http://doi.org/10.1083/jcb.28.1.51.

FIGURE7.27Specialized cadherins, called desmogleinanddesmocollin, bind to one another in the extracellular space. In the cytoplasm, the desmosomal cadherins bind indirectly to intermediate filaments. Electron micrograph of a desmosome from newt epidermis.

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1. Which type(s) of cell junctions contain actin filaments? Which contain(s) intermediate filaments? Which contain(s) integrins? Which contain(s) cadherins?
2. How do cadherins, IgSF proteins, and selectins differ at the molecular level in the way they mediate cell–cell adhesion?
3. Distinguish between a hemidesmosome and a desmosome; between a desmosome and an adherens junction.

7.5 Tight Junctions: Sealing the Extracellular Space

A simple epithelium, like the lining of the intestine or lungs, is composed of a layer of cells that adhere tightly to one another to form a cellular sheet. Biologists have known for decades that when certain types of epithelia, such as frog skin or the wall of the urinary bladder, are mounted between two compartments containing different solute concentrations, very little diffusion of ions or solutes is observed across the wall of the epithelium from one