Cell Junctions, Cell Adhesion, and the Extracellular Matrix

19

Of all the social interactions between cells in a multicellular organism, the most fundamental are those that hold the cells together. Cells may cling to one another through direct cell–cell junctions, or they may be bound together by extracellular materials that they secrete; but by one means or another, they must cohere if they are to form an organized multicellular structure.

The mechanisms of cohesion govern the architecture of the body—its shape, its strength, and the arrangement of its different cell types. The junctions between cells create pathways for communication, allowing the cells to exchange the signals that coordinate their behavior and regulate their patterns of gene expression. Attachments to other cells and to extracellular matrix control the orientation of each cell's internal structure. The making and breaking of the attachments and the modeling of the matrix govern the way cells move within the organism, guiding them as the body grows, develops, and repairs itself. Thus, the apparatus of cell junctions, cell adhesion mechanisms, and extracellular matrix is critical for every aspect of the organization, function, and dynamics of multicellular structures. Defects in this apparatus underlie an enormous variety of diseases.

As examples of structural engineering, large multicellular organisms represent a most surprising feat. Cells are small, squishy, and often motile objects, filled with an aqueous medium and enclosed in a flimsy plasma membrane; yet they can combine in their millions to form a structure as massive, as strong, and as stable as a horse or a tree. How is this possible?

The answer lies in two basic building strategies, corresponding to two ways in which stresses can be transmitted across a multicellular structure. One strategy depends on the strength of the *extracellular matrix*, a complex network of proteins and polysaccharide chains that the cells secrete. The other strategy depends on the strength of the cytoskeleton inside the cells and on *cell–cell adhesions* that tie the cytoskeletons of neighboring cells together. In plants, the extracellular matrix is all-important: plant tissues owe their strength to the cell walls that surround each cell. In animals, both architectural strategies are used, but to different extents in different tissues.

Animal tissues are extraordinarily varied, as we shall see in Chapter 23, but most fall into one or other of two broad categories, representing two architectural extremes (**Figure 19–1**). In **connective tissues**, such as bone or tendon, the extracellular matrix is plentiful, and cells are sparsely distributed within it. The matrix is rich in fibrous polymers, especially *collagen*, and it is the matrix—rather than the cells—that bears most of the mechanical stress to which the tissue is subjected. Direct attachments between one cell and another are relatively rare, but the cells have important attachments to the matrix, allowing them to pull on it and to be pulled by it.

By contrast, in **epithelial tissues**, such as the lining of the gut or the epidermal covering of the skin, cells are closely bound together into sheets called **epithelia**. The extracellular matrix is scanty, consisting mainly of a thin mat called the *basal lamina* (or *basement membrane*), underlying one face of the sheet. Within the epithelium, the cells are attached to each other directly by cell–cell adhesions, where cytoskeletal filaments are anchored, transmitting stresses across the interior of each cell, from adhesion site to adhesion site.

In This Chapter

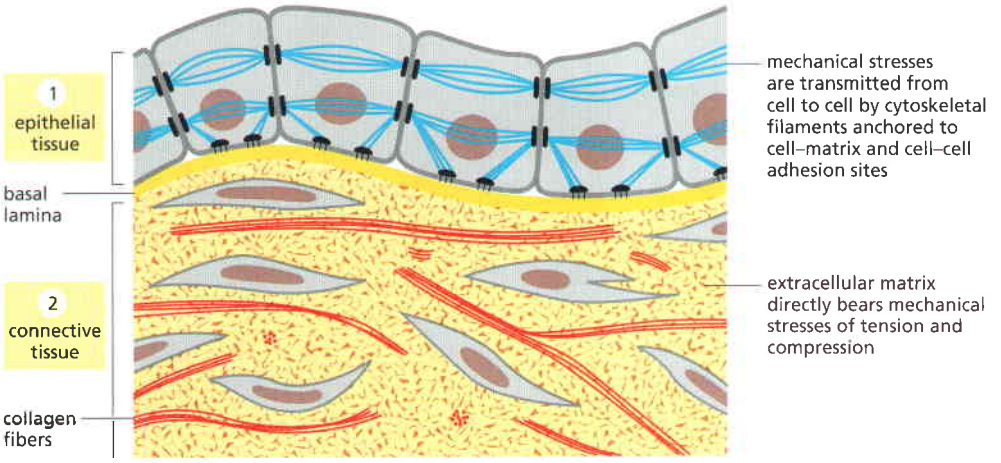


Figure 19–1 Two main ways in which animal cells are bound together. In connective tissue, the main stress-bearing component is the extracellular matrix. In epithelial tissue, it is the cytoskeletons of the cells themselves, linked from cell to cell by anchoring junctions. Cell–matrix attachments bond epithelial tissue to the connective tissue beneath it.

Physical attachment is critical, both in epithelia and in nonepithelial tissues, but junctions between cell and cell or between cells and matrix are diverse in structure and do more than just transmit physical forces. Four main functions can be distinguished, each with a different molecular basis (**Figure 19–2** and **Table 19–1**):

1. **Anchoring junctions**, including both *cell–cell adhesions* and *cell–matrix adhesions*, transmit stresses and are tethered to cytoskeletal filaments inside the cell.
2. **Occluding junctions** seal the gaps between cells in epithelia so as to make the cell sheet into an impermeable (or selectively permeable) barrier.
3. **Channel-forming junctions** create passageways linking the cytoplasms of adjacent cells.
4. **Signal-relaying junctions** allow signals to be relayed from cell to cell across their plasma membranes at sites of cell-to-cell contact.

Chemical synapses in the nervous system (discussed in Chapter 11) and immunological synapses, where T lymphocytes interact with antigen-presenting cells (discussed in Chapter 25), are the most obvious examples of signal-relaying junctions, but they are not the only ones. Sites of cell–cell communication via transmembrane ligand–receptor pairs such as Delta and Notch, or ephrins and Eph receptors, as discussed in Chapter 15, fall under this heading: the cell membranes must be held in contact with one another for the ligands to activate the receptors. Moreover, we shall see that anchoring junctions, occluding junctions, and channel-forming junctions, in different ways, all can have important roles in signal transmission.

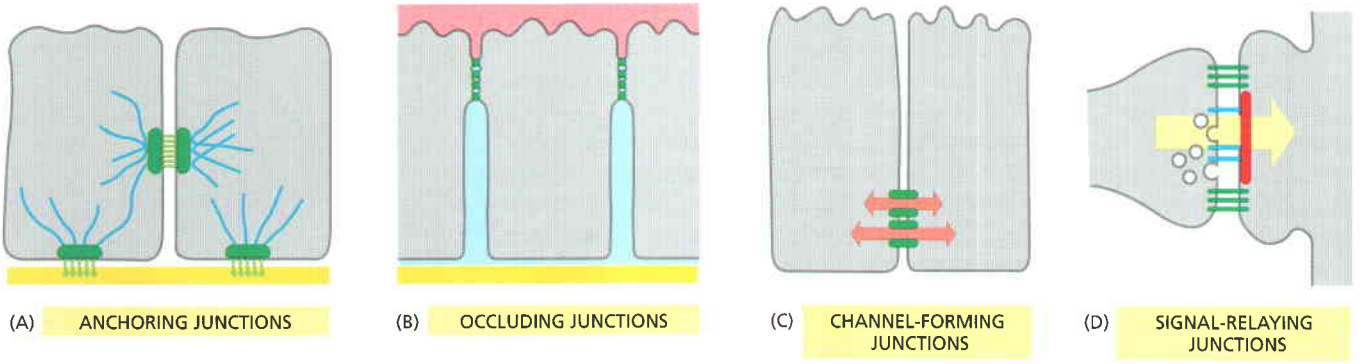


Figure 19–2 Four functional classes of cell junctions in animal tissues. (A) Anchoring junctions link cell to cell (typically via transmembrane *cadherin* proteins) or cell to matrix (typically via transmembrane *integrin* proteins). (B) Occluding junctions (involving *claudin* proteins) seal gaps between epithelial cells. (C) Channel-forming junctions (composed of *connexin* or *innexin* proteins) form passageways for small molecules and ions to pass from cell to cell. (D) Signal-relaying junctions are complex structures, typically involving anchorage proteins alongside proteins mediating signal transduction.

Table 19–1 A Functional Classification of Cell Junctions

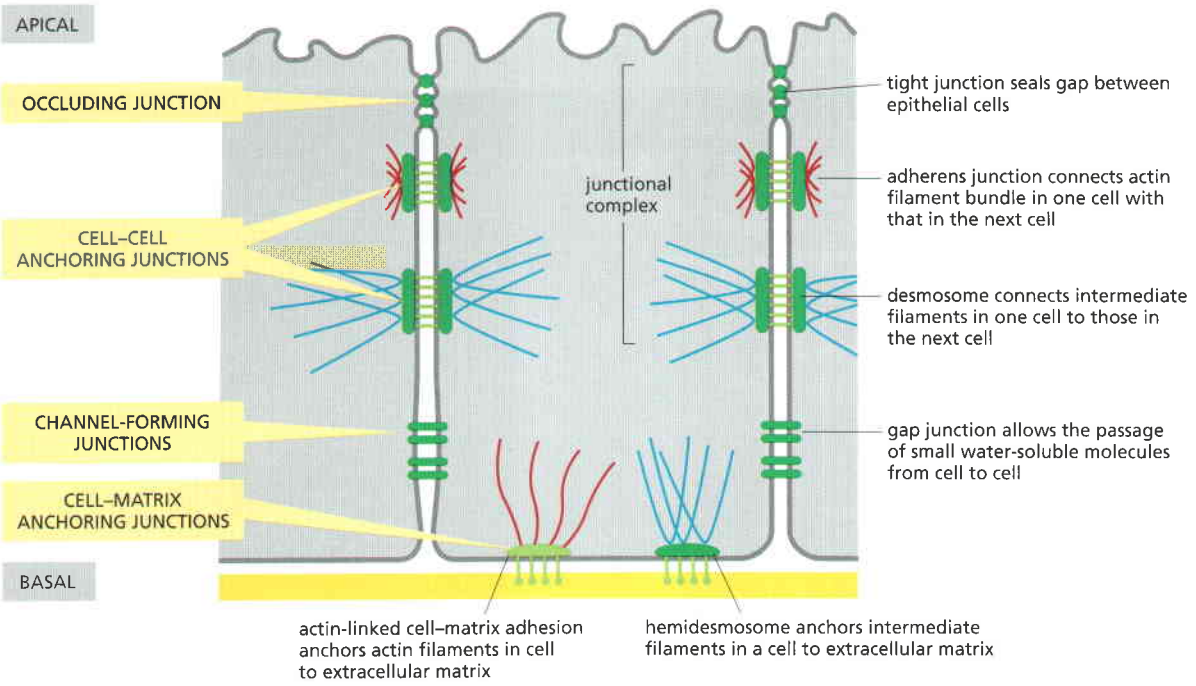
ANCHORING JUNCTIONS
Actin filament attachment sites
1. cell–cell junctions (adherens junctions)
2. cell–matrix junctions (actin-linked cell–matrix adhesions)
Intermediate filament attachment sites
1. cell–cell junctions (desmosomes)
2. cell–matrix junctions (hemidesmosomes)
OCCLUDING JUNCTIONS
1. tight junctions (in vertebrates)
2. septate junctions (in invertebrates)
CHANNEL-FORMING JUNCTIONS
1. gap junctions (in animals)
2. plasmodesmata (in plants)
SIGNAL-RELAYING JUNCTIONS
1. chemical synapses (in the nervous system)
2. immunological synapses (in the immune system)
3. transmembrane ligand–receptor cell–cell signaling contacts (Delta-Notch, ephrin-Eph, etc.). Anchoring, occluding, and channel-forming junctions can all have signaling functions in addition to their structural roles

The first part of this chapter will focus on animal cells and tissues, beginning with the cell–cell adhesions, occluding junctions, and channel-forming junctions that link cell to cell directly. As examples of signal-relaying junctions, we shall briefly examine neuronal synapses from the point of view of their adhesion mechanisms and assembly. We shall see how the different kinds of junctions together organize cells into polarized epithelial sheets. We shall then discuss the extracellular matrix in animals and the ways in which the cells interact with it through cell–matrix adhesions. Last, we shall turn to plants and the central role of the plant cell wall in their construction.

CADHERINS AND CELL–CELL ADHESION

The structures of **cell–cell adhesions** are most clearly seen in mature epithelia and in some other tissues, such as heart muscle, that are held together by strong direct anchorage of cell to cell. Study of these tissues by electron microscopy provided the first general classification of cell junctions. Biochemistry and molecular biology have since shown that the different structures seen in the electron microscope relate to distinct systems of molecules, important not only in adult epithelia but also in other tissues where the junctional specializations are not always so plainly visible.

Figure 19–3 illustrates schematically the types of junctions that the electron microscope reveals in a section of mature epithelium and shows how the cell–cell adhesions (anchoring junctions) that will concern us in this section are distributed in relation to other types of junctions to be discussed later. The diagram shows the typical arrangement in a *simple columnar* epithelium such as the lining of the small intestine of a vertebrate. Here, a single layer of tall cells all stand on a basal lamina, with their uppermost surface, or *apex*, free and exposed to the extracellular medium. On their sides, or *lateral* surfaces, the cells make junctions with one another. Closest to the apex lie occluding junctions (known as *tight junctions* in vertebrates), preventing molecules from leaking across the epithelium through gaps between the cells. Below these are two types of cell–cell



adhesions. **Adherens junctions** are anchorage sites for actin filaments; **desmosome junctions** are anchorage sites for intermediate filaments. Still lower, often mingled with additional desmosome junctions, lie channel-forming junctions, called *gap junctions.*

Additional sets of adhesions attach the epithelial cells to the basal lamina and will be discussed in a later section. We classify these cell–matrix adhesions, like the cell–cell adhesions, according to their cytoskeletal connections: *actin-linked cell–matrix adhesions* (indistinct in the small intestine, but prominent elsewhere) anchor actin filaments to the matrix, while *hemidesmosomes* anchor intermediate filaments to it.

At each of the four types of anchoring junctions, the central role is played by **transmembrane adhesion proteins** that span the membrane, with one end linking to the cytoskeleton inside the cell and the other end linking to other structures outside it (**Figure 19–4**). These cytoskeleton-linked transmembrane molecules fall neatly into two superfamilies, corresponding to the two basic kinds of external attachment (**Table 19–2**). Proteins of the **cadherin** superfamily chiefly mediate attachment of cell to cell. Proteins of the **integrin** superfamily chiefly mediate attachment of cells to matrix. Within each family, there is specialization: some cadherins link to actin and form adherens junctions, while others link to intermediate filaments and form desmosome junctions; likewise,

Figure 19–3 A summary of the various cell junctions found in a vertebrate epithelial cell, classified according to their primary functions. In the most apical portion of the cell, the relative positions of the junctions are the same in nearly all vertebrate epithelia. The tight junction occupies the most apical position, followed by the adherens junction (adhesion belt) and then by a special parallel row of desmosomes; together these form a structure called a junctional complex. Gap junctions and additional desmosomes are less regularly organized.The drawing is based on epithelial cells of the small intestine. Specialized signal-relaying junctions are discussed later in the chapter.

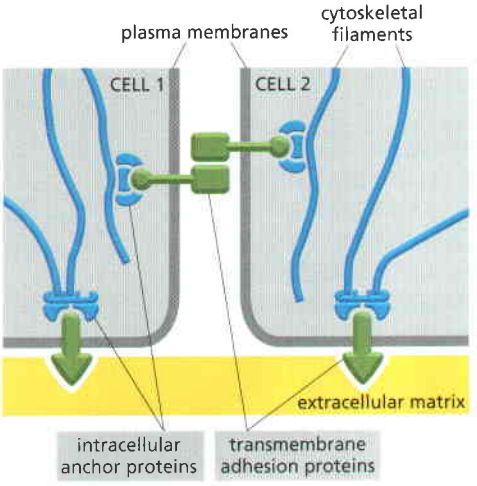


Figure 19–4 Transmembrane adhesion proteins link the cytoskeleton to extracellular structures. The external linkage may be either to parts of other cells (cell–cell anchorage, mediated typically by cadherins) or to extracellular matrix (cell–matrix anchorage, mediated typically by integrins). The internal linkage to the cytoskeleton is generally indirect, via intracellular anchor proteins, to be discussed later.

Table 19–2 Anchoring Junctions

JUNCTION	TRANSMEMBRANE ADHESION PROTEIN	EXTRACELLULAR LIGAND	INTRACELLULAR CYTOSKELETAL ATTACHMENT	INTRACELLULAR ANCHOR PROTEINS
Cell–Cell				
adherens junction	cadherin (classical cadherin)	cadherin in neighboring cell	actin filaments	α-catenin, β-catenin, plakoglobin (γ-catenin), p120-catenin, vinculin, α-actinin
desmosome	cadherin (desmoglein, desmocollin)	desmoglein and desmocollin in neighboring cell	intermediate filaments	plakoglobin (γ-catenin), plakophilin, desmoplakin
Cell–Matrix				
actin-linked cell–matrix adhesion	integrin	extracellular matrix proteins	actin filaments	talin, vinculin, α-actinin, filamin, paxillin, focal adhesion kinase (FAK)
hemidesmosome	integrin α6β4, type XVII collagen (BP180)	extracellular matrix proteins	intermediate filaments	plectin, dystonin (BP230)

some integrins link to actin and form actin-linked cell–matrix adhesions, while others link to intermediate filaments and form hemidesmosomes.

There are some exceptions to these rules. Some integrins, for example, mediate cell–cell rather than cell–matrix attachment. Moreover, there are other types of cell adhesion molecules that can provide attachments more flimsy than anchoring junctions, but sufficient to stick cells together in special circumstances. Cell–cell adhesions based on cadherins, however, seem to be the most fundamentally important class, and we begin our account of cell–cell adhesion with them. <CGAA>

Cadherins Mediate Ca^{2+}-Dependent Cell–Cell Adhesion in All Animals

Cadherins are present in all multicellular animals whose genomes have been analyzed, and in one other known group, the choanoflagellates. These creatures can exist either as free-living unicellular organisms or as multicellular colonies and are thought to be representatives of the group of protists from which all animals evolved. Other eucaryotes, including fungi and plants, lack cadherins, and they are absent from bacteria and archaea also. Cadherins therefore seem to be part of the essence of what it is to be an animal.

The cadherins take their name from their dependence on Ca^{2+} ions: removing Ca^{2+} from the extracellular medium causes adhesions mediated by cadherins to come adrift. Sometimes, especially for embryonic tissues, this is enough to let the cells be easily separated. In other cases, a more severe treatment is required, combining Ca^{2+} removal with exposure to a protease such as trypsin. The protease loosens additional connections mediated by extracellular matrix and by other cell–cell adhesion molecules that do not depend on Ca^{2+}. In either case, when the dissociated cells are put back into a normal culture medium, they will generally stick together again by reconstructing their adhesions.

This type of cell–cell association provided one of the first assays that allowed cell–cell adhesion molecules to be identified. In these experiments, monoclonal antibodies were raised against the cells of interest, and each antibody was tested for its ability to prevent the cells from sticking together again after they had been dissociated. Rare antibodies that bound to the cell–cell adhesion molecules showed this blocking effect. These antibodies then were used to isolate the adhesion molecule that they recognized.

Virtually all cells in vertebrates, and probably in other animals too, seem to express one or more proteins of the cadherin family, according to the cell type.

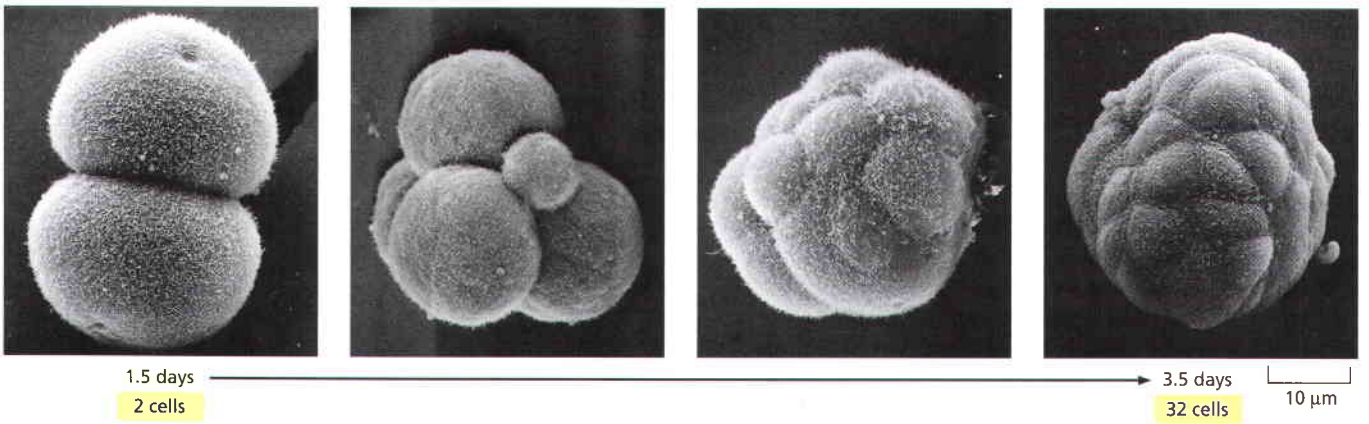


Figure 19–5 **Compaction of an early mouse embryo.** The cells of the early embryo at first stick together only weakly. At about the eight-cell stage, they begin to express E-cadherin and as a result become strongly and closely adherent to one another. (Scanning electron micrographs courtesy of Patricia Calarco; 16–32-cell stage is from P. Calarco and C.J. Epstein, *Dev. Biol.* 32:208–213, 1973. With permission from Academic Press.)

Several lines of evidence indicate that they are the main adhesion molecules holding cells together in early embryonic tissues. For example, embryonic tissues in culture disintegrate when treated with anti-cadherin antibodies, and if cadherin-mediated adhesion is left intact, antibodies against other adhesion molecules have little effect. Studies of the early mouse embryo illustrate the role of cadherins in development. Up to the eight-cell stage, the mouse embryo cells are only very loosely held together, remaining individually more or less spherical; then, rather suddenly, in a process called compaction, they become tightly packed together and joined by cell–cell junctions, so that the outer surface of the embryo becomes smoother (**Figure 19–5**). Antibodies against a specific cadherin, called *E-cadherin*, block compaction, whereas antibodies that react with various other cell-surface molecules on these cells do not. Mutations that inactivate E-cadherin cause the embryos to fall apart and die early in development.

The Cadherin Superfamily in Vertebrates Includes Hundreds of Different Proteins, Including Many with Signaling Functions

The first three cadherins that were discovered were named according to the main tissues in which they were found: *E-cadherin* is present on many types of epithelial cells; *N-cadherin* on nerve, muscle, and lens cells; and *P-cadherin* on cells in the placenta and epidermis. All are also found in various other tissues; N-cadherin, for example, is expressed in fibroblasts, and E-cadherin is expressed in parts of the brain (**Figure 19–6**). These and other **classical cadherins** are closely related in sequence throughout their extracellular and intracellular domains. While all of them have well-defined adhesive functions, they are also important in signaling. Through their intracellular domains, as we shall see later, they relay information into the cell interior, enabling the cell to adapt its behavior according to whether it is attached or detached from other cells.

There are also a large number of **nonclassical cadherins** more distantly related in sequence, with more than 50 expressed in the brain alone. The nonclassical cadherins include proteins with known adhesive function, such as the diverse *protocadherins* found in the brain, and the *desmocollins* and *desmogleins* that form desmosome junctions. They also include proteins that appear to be primarily involved in signaling, such as *T-cadherin*, which lacks a transmembrane domain and is attached to the plasma membrane of nerve and muscle

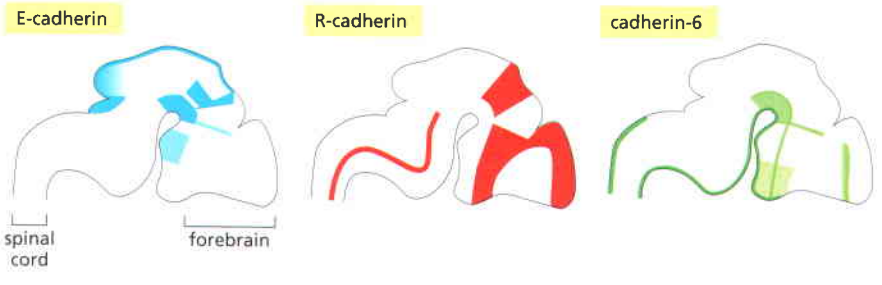


Figure 19–6 **Cadherin diversity in the central nervous system.** The diagram shows the expression patterns of three different classical cadherins in the embryonic mouse brain. More than 70 other cadherins, both classical and nonclassical, are also expressed in the brain, in complex patterns that are thought to reflect their roles in guiding and maintaining the organization of this intricate organ.

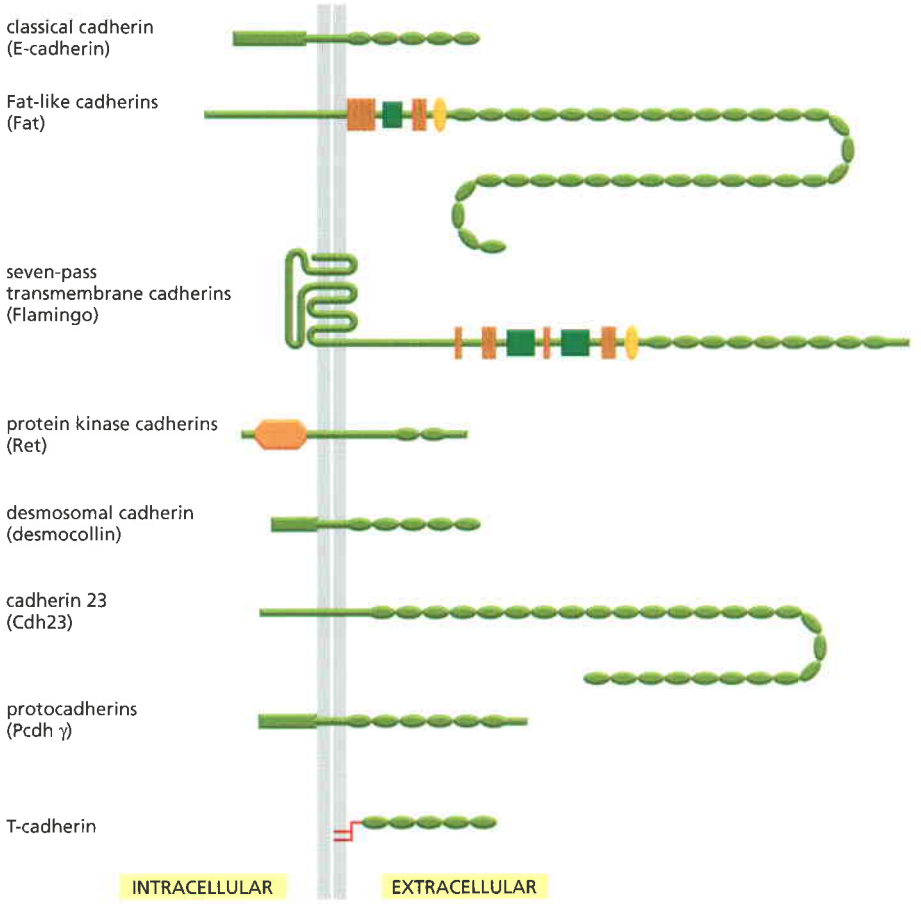


Figure 19–7 The cadherin superfamily. The diagram shows some of the diversity among cadherin superfamily members. These proteins all have extracellular portions containing multiple copies of the cadherin domain motif *(green ovals)*, but their intracellular portions are more varied, reflecting interactions with a wide variety of intracellular ligands, including signaling molecules as well as components that anchor the cadherin to the cytoskeleton. The differently colored motifs in Fat, Flamingo, and Ret represent conserved domains that are also found in other protein families.

cells by a glycosylphosphatidylinositol (GPI) anchor, and the *Fat* and *Flamingo* proteins, which were first identified as the products of genes in *Drosophila* that regulate, respectively, epithelial growth and cell polarity. Together, the classical and nonclassical cadherin proteins constitute the **cadherin superfamily** (**Figure 19–7** and **Table 19–3**), with more than 180 members in humans. How do the structures of these proteins relate to their functions, and why are there so many of them?

Cadherins Mediate Homophilic Adhesion

Anchoring junctions between cells are usually symmetrical: if the linkage is to actin, for example, in the cell on one side of the junction, it will be to actin in the cell on the other side also. In fact, the binding between cadherins is generally **homophilic** (like-to-like, **Figure 19–8**): cadherin molecules of a specific subtype on one cell bind to cadherin molecules of the same or closely related subtype on adjacent cells. According to a current model, the binding occurs at the N-terminal tips of the cadherin molecules—the ends that lie furthest from the membrane. The protein chain here forms a terminal knob and a nearby pocket, and the cadherin molecules protruding from opposite cell membranes bind by insertion of the knob of each one in the pocket of the other (**Figure 19–9**A).

The spacing between the cell membranes at an anchoring junction is precisely defined and depends on the structure of the participating cadherin molecules. All the members of the superfamily, by definition, have an extracellular portion consisting of several copies of a motif called the *cadherin domain.* In the classical cadherins of vertebrates there are 5 of these repeats, and in desmogleins and desmocollins there are 4 or 5, but some nonclassical cadherins have more than 30. Each cadherin domain forms a more or less rigid unit, joined to the next cadherin domain by a hinge (Figure 19–9B). Ca^{2+} ions bind to sites

Table 19–3 Some Members of the Cadherin Superfamily

NAME	MAIN LOCATION	JUNCTION ASSOCIATION	PHENOTYPE WHEN INACTIVATED IN MICE
Classical cadherins			
E-cadherin	many epithelia	adherens junctions	death at blastocyst stage; embryos fail to undergo compaction
N-cadherin	neurons, heart, skeletal muscle, lens, and fibroblasts	adherens junctions and chemical synapses	embryos die from heart defects
P-cadherin	placenta, epidermis, breast epithelium	adherens junctions	abnormal mammary gland development
VE-cadherin	endothelial cells	adherens junctions	abnormal vascular development (apoptosis of endothelial cells)
Nonclassical cadherins			
Desmocollin	skin	desmosomes	blistering of skin
Desmoglein	skin	desmosomes	blistering skin disease due to loss of keratinocyte cell–cell adhesion
T-cadherin	neurons, muscle, heart	none	unknown
Cadherin 23	inner ear, other epithelia	links between stereocilia in sensory hair cells	deafness
Fat (in *Drosophila*)	epithelia and central nervous system	signal-relaying junction (planar cell polarity)	enlarged imaginal discs and tumors; disrupted planar cell polarity
Fat1 (in mammals)	various epithelia and central nervous system	slit diaphragm in kidney glomerulus and other cell junctions	loss of slit diaphragm; malformation of forebrain and eye
α, β, and γ-Protocadherins	neurons	chemical synapses and nonsynaptic membranes	neuronal degeneration
Flamingo	sensory and some other epithelia	cell–cell junctions	disrupted planar cell polarity; neural tube defects

near each hinge and prevent it from flexing, so that the whole string of cadherin domains behaves as a rigid, slightly curved, rod. When Ca^{2+} is removed, the hinges can flex, and the structure becomes floppy. At the same time, the conformation at the N terminus is thought to change slightly, weakening the binding affinity for the matching cadherin molecule on the opposite cell. Cadherin molecules destabilized in this way by loss of Ca^{2+} are rapidly degraded by proteolytic enzymes.

Unlike receptors for soluble signal molecules, which bind their specific ligand with high affinity, cadherins (and most other cell–cell adhesion proteins) typically bind to their partners with relatively low affinity. Strong attachments result from the formation of many such weak bonds in parallel. When binding to oppositely oriented partners on another cell, cadherin molecules are often clustered side-to-side with many other cadherin molecules on the same cell. Many cadherin molecules packed side by side in this way collaborate to form an anchoring junction (Figure 19–9C). The strength of this junction is far greater than that of any individual intermolecular bond, and yet it can be easily disassembled by separating the molecules sequentially, just as two pieces of fabric can be strongly joined by Velcro and yet easily peeled apart. A similar "Velcro principle" also operates at cell–cell and cell–matrix adhesions formed by other

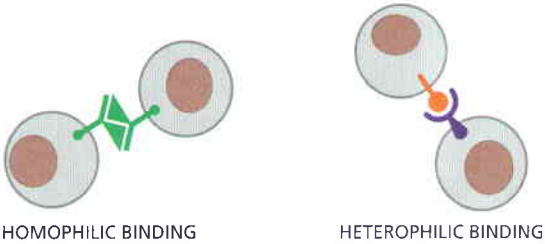


Figure 19–8 Homophilic versus heterophilic binding. Cadherins in general bind homophilically; some other cell adhesion molecules, discussed later, bind heterophilically.

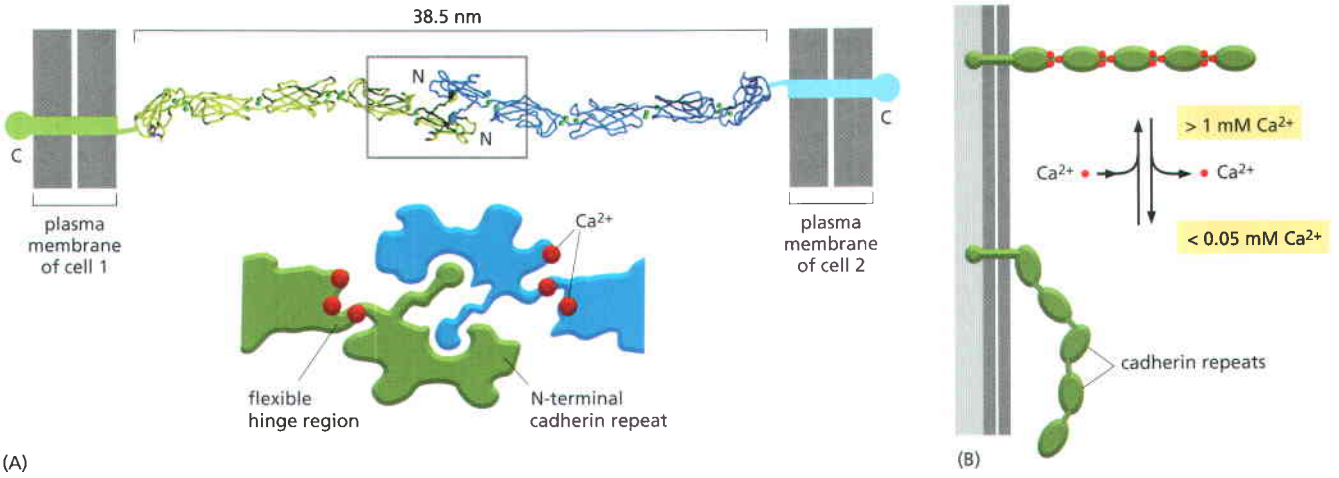


Figure 19–9 Cadherin structure and function. (A) The extracellular domain of a classical cadherin (C-cadherin) is shown here, illustrating how two such molecules on opposite cells are thought to bind homophilically, end-to-end. The structure was determined by x-ray diffraction of the crystallized C-cadherin extracellular domain. (B) The extracellular part of each polypeptide consists of a series of compact domains called cadherin repeats, joined by flexible hinge regions. Ca^{2+} binds in the neighborhood of each hinge, preventing it from flexing. In the absence of Ca^{2+}, the molecule becomes floppy and adhesion fails. (C) At a typical junction, many cadherin molecules are arrayed in parallel, functioning like Velcro to hold cells together. Cadherins on the same cell are thought to be coupled by side-to-side interactions between their N-terminal head regions, and via the attachments of their intracellular tails to a mat of other proteins (not shown here). (Based on T.J. Boggon et al., *Science* 296:1308–1313, 2002. With permission from AAAS.)

types of transmembrane adhesion proteins. The making and breaking of anchoring junctions plays a vital part in development and in the constant turnover of tissues in many parts of the mature body. <CGAA>

Selective Cell–Cell Adhesion Enables Dissociated Vertebrate Cells to Reassemble into Organized Tissues

Cadherins form specific homophilic attachments, and this explains why there are so many different family members. Cadherins are not like glue, making cell surfaces generally sticky. Rather, they mediate highly selective recognition, enabling cells of a similar type to stick together and to stay segregated from other types of cells.

This selectivity in the way that animal cells consort with one another was demonstrated more than 50 years ago, long before the discovery of cadherins, in experiments in which amphibian embryos were dissociated into single cells. These cells were then mixed up and allowed to reassociate. Remarkably, the dissociated cells often reassembled *in vitro* into structures resembling those of the original embryo (**Figure 19–10**). The same phenomenon occurs when dissociated cells from two embryonic vertebrate organs, such as the liver and the retina, are mixed together and artificially formed into a pellet: the mixed aggregates

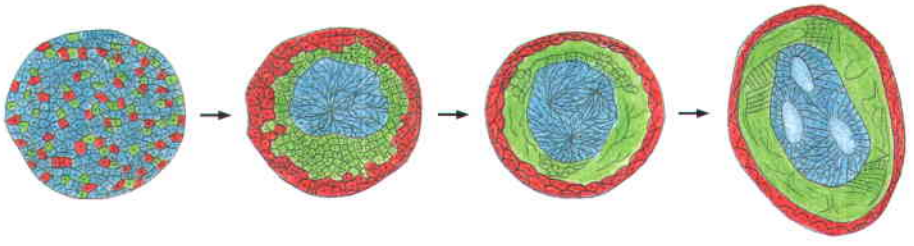


Figure 19–10 Sorting out. Cells from different parts of an early amphibian embryo will sort out according to their origins. In the classical experiment shown here, mesoderm cells (*green*), neural plate cells (*blue*), and epidermal cells (*red*) have been disaggregated and then reaggregated in a random mixture. They sort out into an arrangement reminiscent of a normal embryo, with a "neural tube" internally, epidermis externally, and mesoderm in between. (Modified from P.L. Townes and J. Holtfreter, *J. Exp. Zool.* 128:53–120, 1955. With permission from Wiley-Liss.)

Catenins Link Classical Cadherins to the Actin Cytoskeleton

The extracellular domains of cadherins mediate homophilic binding. The intracellular domains of typical cadherins, including all classical and some nonclassical ones, provide anchorage for filaments of the cytoskeleton: anchorage to actin at adherens junctions, and to intermediate filaments at desmosome junctions, as mentioned earlier (see Figure 19–3). The linkage to the cytoskeleton is indirect and depends on a cluster of accessory *intracellular anchor proteins* that assemble on the tail of the cadherin. This linkage, connecting the cadherin family member to actin or intermediate filaments, includes several different components (**Figure 19–14**). These components vary somewhat according to the type of anchorage—but in general a central part is played by *β-catenin* and/or its close relative *γ-catenin (plakoglobin).*

At adherens junctions, a remote relative of this pair of proteins, *p120-catenin,* is also present and helps to regulate assembly of the whole complex. When p120-catenin is artificially depleted, cadherin proteins are rapidly degraded, and cell–cell adhesion is lost. An artificial increase in the level of p120-catenin has an opposite effect. It is possible that cells use changes in the level of p120-catenin or in its phosphorylation state as one way to regulate their strength of adhesion. In any case, it seems that the linkage to actin is essential for efficient cell–cell adhesion, as classical cadherins that lack their cytoplasmic domain cannot hold cells strongly together.

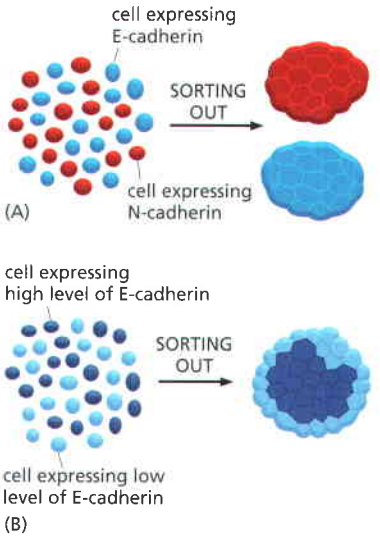


Figure 19–13 Cadherin-dependent cell sorting. Cells in culture can sort themselves out according to the type and level of cadherins they express. This can be visualized by labeling different populations of cells with dyes of different colors. (A) Cells expressing N-cadherin sort out from cells expressing E-cadherin. (B) Cells expressing high levels of E-cadherin sort out from cells expressing low levels of E-cadherin.

Adherens Junctions Coordinate the Actin-Based Motility of Adjacent Cells

Adherens junctions are an essential part of the machinery for modeling the shapes of multicellular structures in the animal body. By indirectly linking the actin filaments in one cell to those in its neighbors, they enable the cells in the tissue to use their actin cytoskeletons in a coordinated way.

Adherens junctions occur in various forms. In many nonepithelial tissues, they appear as small punctate or streaklike attachments that indirectly connect the cortical actin filaments beneath the plasma membranes of two interacting cells. In heart muscle (discussed in Chapter 23), they anchor the actin bundles of the contractile apparatus and act in parallel with desmosome junctions to link the contractile cells end-to-end. (The cell–cell interfaces in the muscle where these adhesions occur are so substantial that they show up clearly in stained light-microscope sections as so-called *intercalated discs.*) But the prototypical examples of adherens junctions occur in epithelia, where they often form a continuous **adhesion belt** (or *zonula adherens)* close beneath the apical face of the epithelium, encircling each of the interacting cells in the sheet (**Figure 19–15**). Within each cell, a contractile bundle of actin filaments lies adjacent to the adhesion belt, oriented parallel to the plasma membrane and tethered to it by the cadherins and their associated intracellular anchor proteins. The actin bundles are thus linked, via the cadherins and anchor proteins, into an extensive transcellular network. This network can contract with the help of myosin motor proteins (discussed in Chapter 16), providing the motile force for a fundamental process in animal morphogenesis—the folding of epithelial cell sheets into tubes, vesicles, and other related structures (**Figure 19–16**).

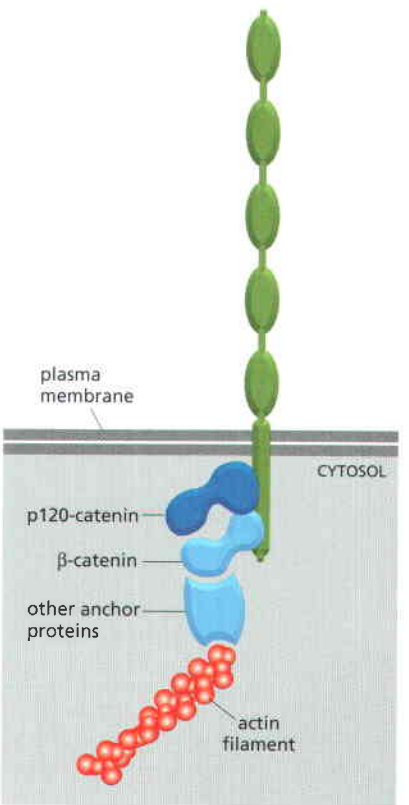


Figure 19–14 The linkage of classical cadherins to actin filaments. The cadherins are coupled indirectly to actin filaments via β-catenin and other anchor proteins. α-Catenin, vinculin, and plakoglobin (a relative of β-catenin, also called γ-catenin) are probably also present in the linkage or involved in control of its assembly, but the details of the anchorage are not well understood. Another intracellular protein, called p120-catenin, also binds to the cadherin cytoplasmic tail and regulates cadherin function. β-Catenin has a second, and very important, function in intracellular signaling, as we discuss in Chapter 15 (see Figure 15–77).

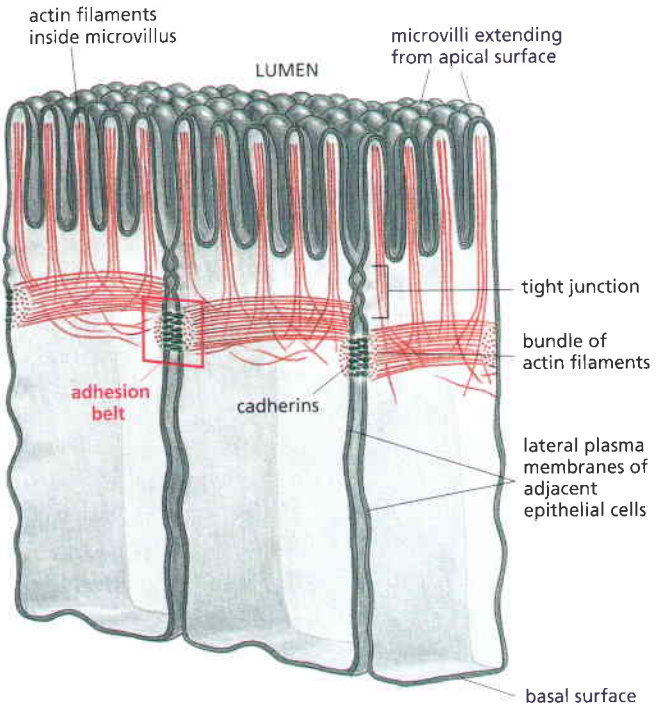


Figure 19–15 Adherens junctions between epithelial cells in the small intestine. These cells are specialized for absorption of nutrients; at their apex, facing the lumen of the gut, they have many microvilli (protrusions that serve to increase the absorptive surface area). The adherens junction takes the form of an *adhesion belt*, encircling each of the interacting cells. Its most obvious feature is a contractile bundle of actin filaments running along the cytoplasmic surface of the junctional plasma membrane. The actin filament bundles are tethered by intracellular anchor proteins to cadherins. The cadherins span the plasma membrane, and their extracellular domains bind homophilically to those of the cadherins on the adjacent cell. In this way, the actin filament bundles in adjacent cells are tied together.

Desmosome Junctions Give Epithelia Mechanical Strength

Desmosome junctions are structurally similar to adherens junctions but link to intermediate filaments instead of actin. Their main function is to provide mechanical strength. Desmosome junctions are important in vertebrates but are not found, for example, in *Drosophila*. They are present in most mature vertebrate epithelia, and are extremely plentiful in the epidermis, the epithelium that forms the outer layer of the skin; a favorite source for biochemical studies is the epidermis of the snout of cows, which has to withstand constant battering as the animal grazes.

Figure 19–17A shows the general structure of a desmosome, and Figure 19–17B shows some of the proteins that form it. Desmosomes typically appear as buttonlike spots of intercellular adhesion, riveting the cells together (Figure 19–17C). Inside the cell, the bundles of ropelike intermediate filaments that are

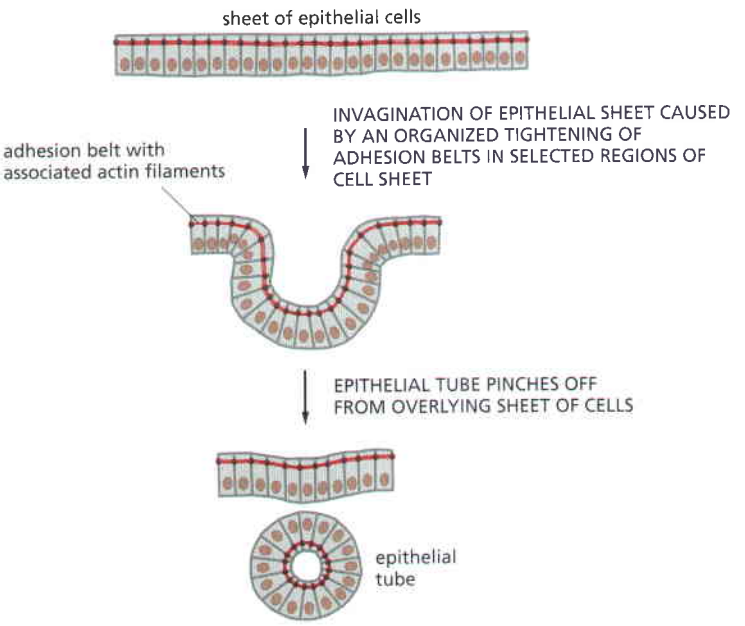


Figure 19–16 The folding of an epithelial sheet to form an epithelial tube. The oriented contraction of the bundles of actin filaments running along adhesion belts causes the epithelial cells to narrow at their apex and helps the epithelial sheet to roll up into a tube. An example is the formation of the neural tube in early vertebrate development (see Figure 19–12 and Chapter 22). Although not shown here, rearrangements of the cells within the epithelial sheet are also thought to have an important role in the process.

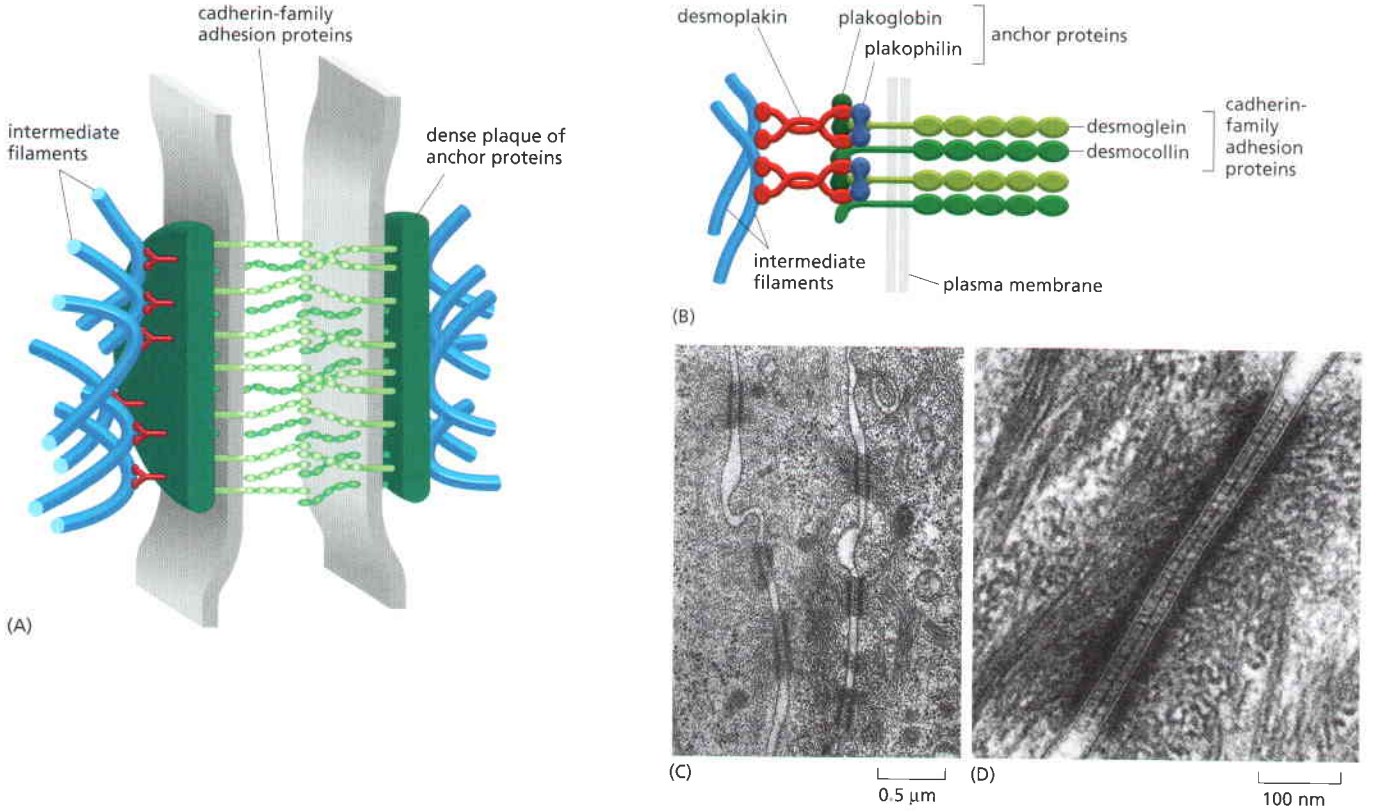


Figure 19–17 Desmosomes. (A) The structural components of a desmosome. On the cytoplasmic surface of each interacting plasma membrane is a dense plaque composed of a mixture of intracellular anchor proteins. A bundle of keratin intermediate filaments is attached to the surface of each plaque. Transmembrane adhesion proteins of the cadherin family bind to the plaques and interact through their extracellular domains to hold the adjacent membranes together by a Ca^{2+}-dependent mechanism. (B) Some of the molecular components of a desmosome. Desmoglein and desmocollin are members of the cadherin family of adhesion proteins. Their cytoplasmic tails bind *plakoglobin* (γ-catenin) and *plakophilin* (a distant relative of p120-catenin), which in turn bind to *desmoplakin*. Desmoplakin binds to the sides of intermediate filaments, thereby tying the desmosome to these filaments. (C) An electron micrograph of desmosome junctions between epidermal cells in the skin of a baby mouse. (D) Part of the same tissue at higher magnification, showing a single desmosome, with intermediate filaments attached to it. (C and D, from W. He, P. Cowin and D.L. Stokes, *Science* 302:109–113, 2003. With permission from AAAS.)

anchored to the desmosomes form a structural framework of great tensile strength (Figure 19–17D), with linkage to similar bundles in adjacent cells, creating a network that extends throughout the tissue (**Figure 19–18**). The particular type of intermediate filaments attached to the desmosomes depends on the cell type: they are *keratin filaments* in most epithelial cells, for example, and *desmin filaments* in heart muscle cells.

The importance of desmosome junctions is demonstrated by some forms of the potentially fatal skin disease *pemphigus*. Affected individuals make antibodies against one of their own desmosomal cadherin proteins. These antibodies bind to and disrupt the desmosomes that hold their epidermal cells (keratinocytes) together. This results in a severe blistering of the skin, with leakage of body fluids into the loosened epithelium.

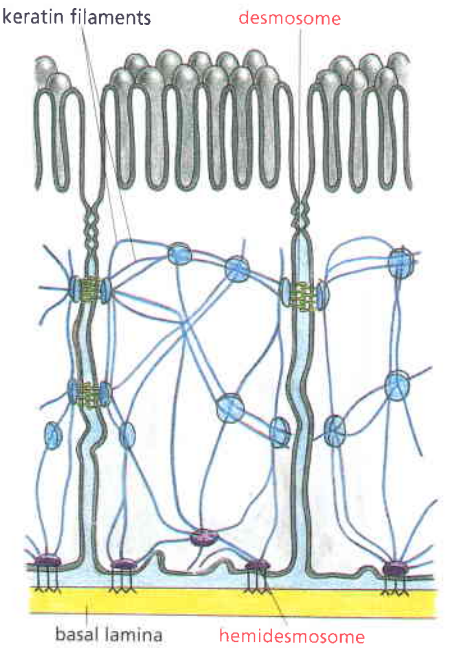


Figure 19–18 Desmosomes, hemidesmosomes, and the intermediate filament network. The keratin intermediate filament networks of adjacent cells—in this example, epithelial cells of the small intestine—are indirectly connected to one another through desmosomes, and to the basal lamina through hemidesmosomes.

cadherin molecule, and are involved not only in physical anchorage but also in the genesis of intracellular signals. Conversely, intracellular signals can regulate the formation of cadherin-mediated adhesions. β-Catenin, for example, is also a key component of the Wnt cell signaling pathway.

In addition to cadherins, at least three other classes of transmembrane molecules are also important mediators of cell–cell adhesion: selectins, immunoglobulin (Ig) superfamily members, and integrins. Selectins are expressed on white blood cells, blood platelets, and endothelial cells, and they bind heterophilically to carbohydrate groups on cell surfaces. They help to trap circulating white blood cells at sites of inflammation. Ig-superfamily proteins also play a part in this trapping, as well as in many other adhesive processes; some of them bind homophilically, some heterophilically. Integrins, though they mainly serve to attach cells to the extracellular matrix, can also mediate cell–cell adhesion by binding to the Ig-superfamily members.

Many different Ig-superfamily members, cadherins, and other cell–cell adhesion molecules guide the formation of nerve connections and hold neuronal membranes together at synapses. In these complicated structures, as well as at other types of cell–cell junctions, intracellular scaffold proteins containing multiple PDZ protein-binding domains have an important role in holding the many different adhesive and signaling molecules in their proper arrangements.

TIGHT JUNCTIONS AND THE ORGANIZATION OF EPITHELIA

An epithelial sheet, with its cells joined side by side and standing on a basal lamina, may seem a specialized type of structure, but it is central to the construction of multicellular animals. In fact, more than 60% of the cell types in the vertebrate body are epithelial. Just as cell membranes enclose and partition the interior of the eucaryotic cell, so epithelia enclose and partition the animal body, lining all its surfaces and cavities, and creating internal compartments where specialized processes occur. The epithelial sheet seems to be one of the inventions that lie at the origin of animal evolution, diversifying in a huge variety of ways (as we see in Chapter 23), but retaining an organization based on a set of conserved molecular mechanisms that practically all epithelia have in common.

Essentially all epithelia are anchored to other tissue on one side—the **basal** side—and free of such attachment on their opposite side—the **apical** side. A basal lamina lies at the interface with the underlying tissue, mediating the attachment, while the apical surface of the epithelium is generally bathed by extracellular fluid (but sometimes covered by material that the cells have secreted at their apices). Thus all epithelia are structurally **polarized**, and so are their individual cells: the basal end of a cell, adherent to the basal lamina below, differs from the apical end, exposed to the medium above.

Correspondingly, all epithelia have at least one function in common: they serve as selective permeability barriers, separating the fluid that permeates the tissue on their basal side from fluid with a different chemical composition on their apical side. This barrier function requires that the adjacent cells be sealed together by **occluding junctions**, so that molecules cannot leak freely across the cell sheet. In this section we consider how the occluding junctions are formed, and how the polarized architecture of the epithelium is maintained. These two fundamental aspects of epithelia are closely linked: the junctions play a key part in organizing and maintaining the polarity of the cells in the sheet.

Tight Junctions Form a Seal Between Cells and a Fence Between Membrane Domains

The occluding junctions found in vertebrate epithelia are called **tight junctions**. The epithelium of the small intestine provides a good illustration of their structure and function (see Figure 19–3). This epithelium has a *simple columnar* structure; that is, it consists of a single layer of tall (columnar) cells. These are of

several differentiated types, but the majority are absorptive cells, specialized for uptake of nutrients from the internal cavity, or *lumen*, of the gut.

The absorptive cells have to transport selected nutrients across the epithelium from the lumen into the extracellular fluid that permeates the connective tissue on the other side. From there, these nutrients diffuse into small blood vessels to provide nourishment to the organism. This *transcellular transport* depends on two sets of transport proteins in the plasma membrane of the absorptive cell. One set is confined to the apical surface of the cell (facing the lumen) and actively transports selected molecules into the cell from the gut. The other set is confined to the *basolateral* (basal and lateral) surfaces of the cell, and it allows the same molecules to leave the cell by facilitated diffusion into the extracellular fluid on the other side of the epithelium. For this transport activity to be effective, the spaces between the epithelial cells must be tightly sealed, so that the transported molecules cannot leak back into the gut lumen through these spaces (**Figure 19–23**). Moreover, the proteins that form the pumps and channels must be correctly distributed in the cell membranes: the apical set of active transport proteins must be delivered to the cell apex (as discussed in Chapter 13) and must not be allowed to drift to the basolateral surface, and the basolateral set of channel proteins must be delivered to the basolateral surface and must not be allowed to drift to the apical surface. The tight junctions between epithelial cells, besides sealing the gaps between the cells, may also function as "fences" helping to separate domains within the plasma membrane of each cell, so as to hinder apical proteins (and lipids) from diffusing into the basal region, and vice versa (see Figure 19–23).

The sealing function of tight junctions is easy to demonstrate experimentally: a low-molecular-weight tracer added to one side of an epithelium will generally not pass beyond the tight junction (**Figure 19–24**). This seal is not absolute, however. Although all tight junctions are impermeable to macromolecules, their permeability to small molecules varies. Tight junctions in the epithelium lining the small intestine, for example, are 10,000 times more permeable to inorganic ions,

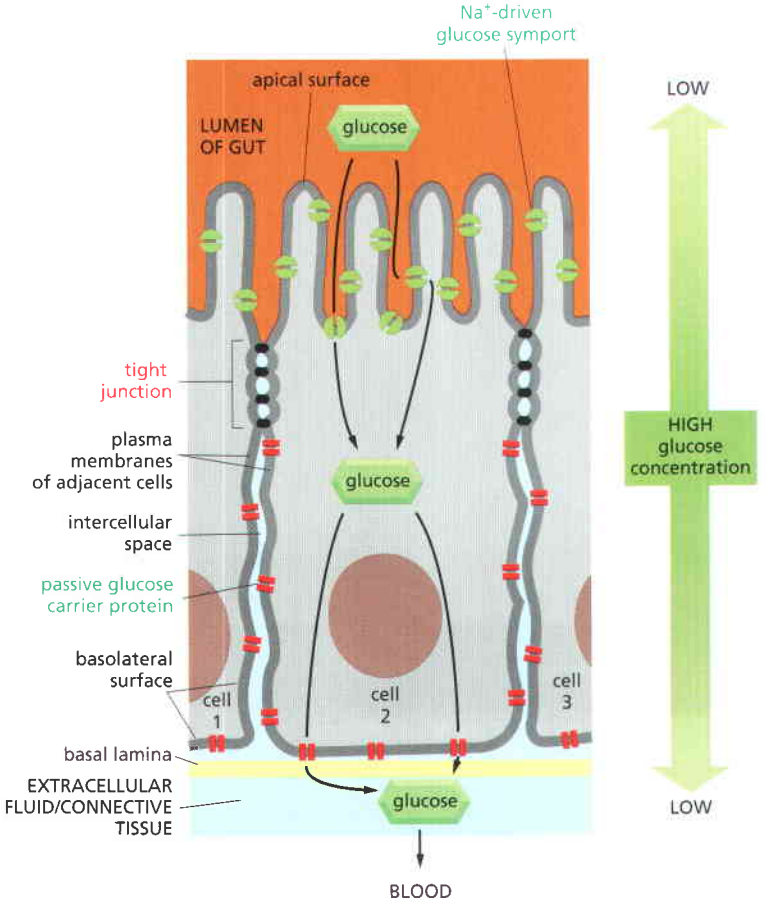


Figure 19–23 The role of tight junctions in transcellular transport. Transport proteins are confined to different regions of the plasma membrane in epithelial cells of the small intestine. This segregation permits a vectorial transfer of nutrients across the epithelium from the gut lumen to the blood. In the example shown, glucose is actively transported into the cell by Na^+-driven glucose symports at its apical surface, and it diffuses out of the cell by facilitated diffusion mediated by glucose carriers in its basolateral membrane. Tight junctions are thought to confine the transport proteins to their appropriate membrane domains by acting as diffusion barriers or "fences" within the lipid bilayer of the plasma membrane; these junctions also block the backflow of glucose from the basal side of the epithelium into the gut lumen.

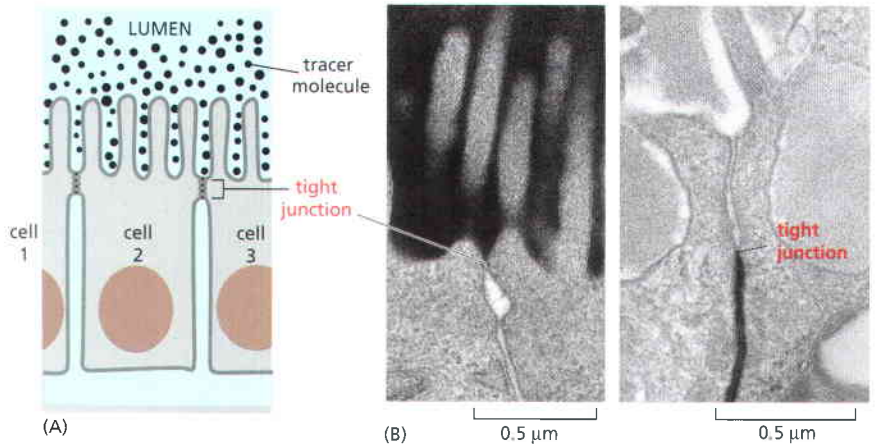


Figure 19–24 The role of tight junctions in allowing epithelia to serve as barriers to solute diffusion. (A) The drawing shows how a small extracellular tracer molecule added on one side of an epithelium is prevented from crossing the epithelium by the tight junctions that seal adjacent cells together. (B) Electron micrographs of cells in an epithelium in which a small, extracellular, electron-dense tracer molecule has been added to either the apical side (on the *left*) or the basolateral side (on the *right*). In both cases, the tight junction blocks passage of the tracer. (B, courtesy of Daniel Friend.)

such as Na^+, than the tight junctions in the epithelium lining the urinary bladder. These differences reflect differences in the proteins that form the junctions.

Epithelial cells can also alter their tight junctions transiently to permit an increased flow of solutes and water through breaches in the junctional barriers. Such *paracellular transport* is especially important in the absorption of amino acids and monosaccharides from the lumen of the intestine, where the concentration of these nutrients can increase enough after a meal to drive passive transport in the proper direction.

When tight junctions are visualized by freeze-fracture electron microscopy, they seem to consist of a branching network of *sealing strands* that completely encircles the apical end of each cell in the epithelial sheet (**Figure 19–25**A and B). In conventional electron micrographs, the outer leaflets of the two interacting

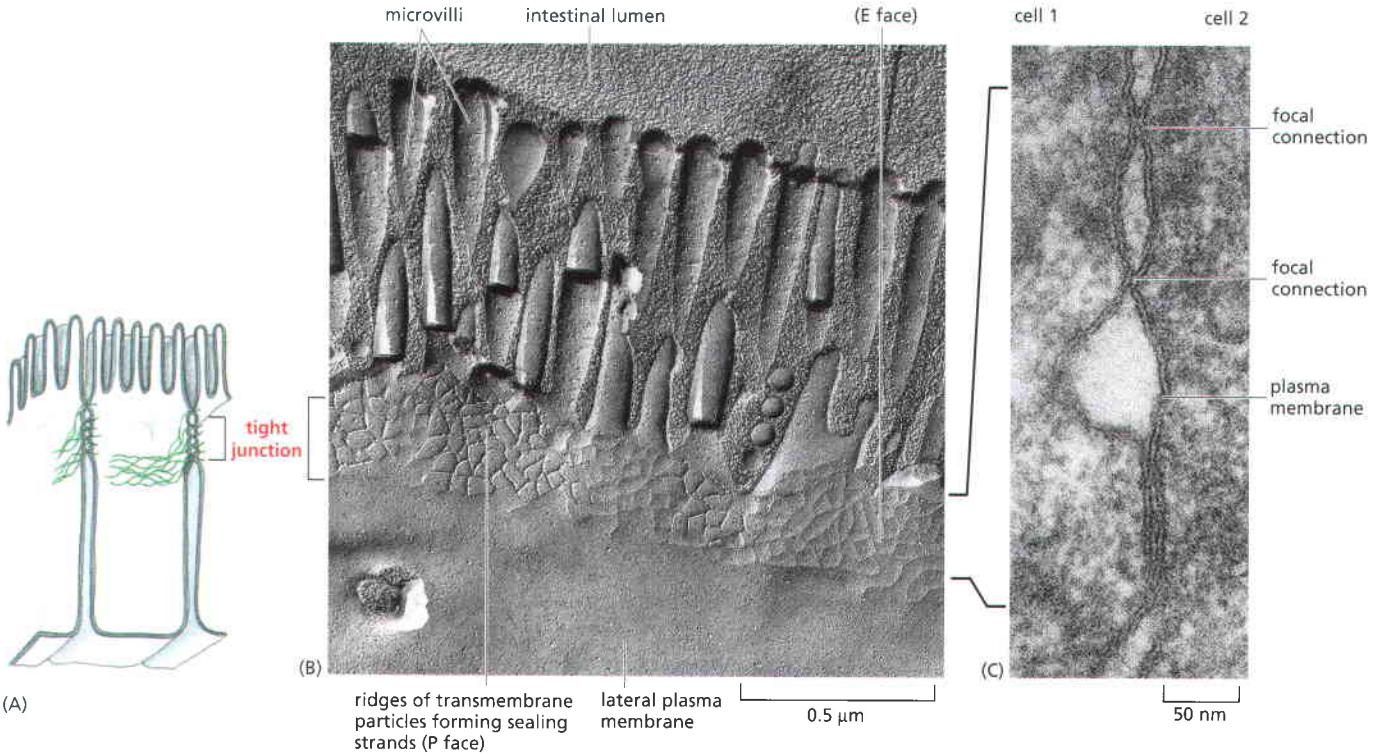


Figure 19–25 The structure of a tight junction between epithelial cells of the small intestine. The junctions are shown (A) schematically, (B) in a freeze-fracture electron micrograph, and (C) in a conventional electron micrograph. In (B), the plane of the micrograph is parallel to the plane of the membrane, and the tight junction appears as a band of branching sealing strands that encircle each cell in the epithelium. The sealing strands are seen as ridges of intramembrane particles on the cytoplasmic fracture face of the membrane (the P face) or as complementary grooves on the external face of the membrane (the E face) (see Figure 19–26A). In (C), the junction is seen in cross section as a series of focal connections between the outer leaflets of the two interacting plasma membranes, each connection corresponding to a sealing strand in cross section. (B and C, from N.B. Gilula, in Cell Communication [R.P. Cox, ed.], pp. 1–29. New York: Wiley, 1974.)

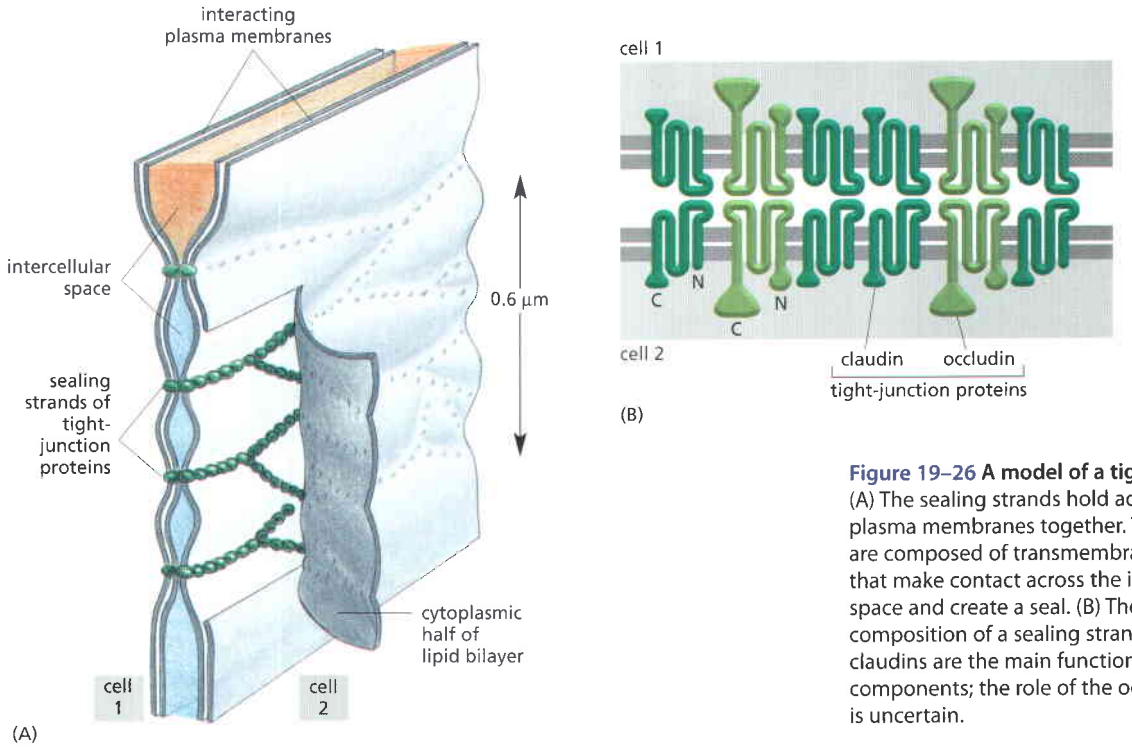


Figure 19–26 A model of a tight junction. (A) The sealing strands hold adjacent plasma membranes together. The strands are composed of transmembrane proteins that make contact across the intercellular space and create a seal. (B) The molecular composition of a sealing strand. The claudins are the main functional components; the role of the occludins is uncertain.

plasma membranes are seen to be tightly apposed where sealing strands are present (Figure 19–25C). Each tight junction sealing strand is composed of a long row of transmembrane adhesion proteins embedded in each of the two interacting plasma membranes. The extracellular domains of these proteins adhere directly to one another to occlude the intercellular space (**Figure 19–26**).

The main transmembrane proteins forming these strands are the *claudins,* which are essential for tight junction formation and function. Mice that lack the *claudin-1* gene, for example, fail to make tight junctions between the cells in the epidermal layer of the skin; as a result, the baby mice lose water rapidly by evaporation through the skin and die within a day after birth. Conversely, if nonepithelial cells such as fibroblasts are artificially caused to express claudin genes, they will form tight-junctional connections with one another. Normal tight junctions also contain a second major transmembrane protein called *occludin,* but the function of this protein is uncertain, and it does not seem to be as essential as the claudins. A third transmembrane protein, *tricellulin* (related to occludin), is required to seal cell membranes together and prevent transepithelial leakage at the points where three cells meet.

The claudin protein family has many members (24 in humans), and these are expressed in different combinations in different epithelia to confer particular permeability properties on the epithelial sheet. They are thought to form *paracellular pores*—selective channels allowing specific ions to cross the tight-junctional barrier, from one extracellular space to another. A specific claudin found in kidney epithelial cells, for example, is needed to let Mg^{2+} pass between the cells of the sheet so that this ion can be resorbed from the urine into the blood. A mutation in the gene encoding this claudin results in excessive loss of Mg^{2+} in the urine.

Scaffold Proteins in Junctional Complexes Play a Key Part in the Control of Cell Proliferation

The claudins and occludins have to be held in the right position in the cell, so as to form the tight-junctional network of sealing strands. This network usually lies just apical to the adherens and desmosome junctions that bond the cells together mechanically, and the whole assembly is called a *junctional complex* (**Figure 19–27**). The parts of this junctional complex depend on each other for

such as *Frizzled*, for example, and *Dishevelled*, code for proteins that have since been shown to be components of the Wnt signaling pathway (discussed in Chapter 15). Two others, *Flamingo* (see Figure 19–32C) and *Dachsous*, code for members of the cadherin superfamily. Still others are less easily classified functionally, but it is clear that planar cell polarity is organized by machinery formed from these components and assembled at cell–cell junctions in such a way that a polarizing influence can propagate from cell to cell. Essentially the same system of proteins controls planar cell polarity in vertebrates. Mice with mutations in a *Flamingo* homolog, for example, have incorrectly oriented hair cells in their ears (among other defects) and thus are deaf (see Figure 19–32D).

Summary

Occluding junctions—tight junctions in vertebrates, septate junctions in insects and molluscs—seal the gaps between cells in epithelia, creating a barrier to the diffusion of molecules across the cell sheet. They also form a bar to the diffusion of proteins in the plane of the membrane, and so help to maintain a difference between the populations of proteins in the apical and basolateral membrane domains of the epithelial cell. The major transmembrane proteins forming occluding junctions are called claudins; different members of the family are expressed in different tissues, conferring different permeability properties on the various epithelial sheets.

Intracellular scaffold proteins bind to the transmembrane components at occluding junctions and coordinate these junctions with cadherin-based anchoring junctions, so as to create junctional complexes. The junctional scaffold proteins have at least two other crucial functions. They play a part in the control of epithelial cell proliferation; and, in conjunction with other regulatory molecules such as Rac and Cdc42, they govern cell polarity. Epithelial cells have an intrinsic tendency to develop a polarized apico-basal axis. The orientation of this axis in relation to the cell's neighbors in an epithelial sheet depends on protein complexes involving scaffold proteins that assemble at cell–cell junctions, as well as on cytoskeletal polarization controlled by Rac/Cdc42 and on influences from the basal lamina.

The cells of some epithelia have an additional polarity in the plane of the epithelium, at right angles to the apico-basal axis. A separate set of conserved proteins, operating in a similar way in vertebrates and in insects, governs this planar cell polarity through poorly understood signaling processes that are likewise based on cell–cell junctions.

PASSAGEWAYS FROM CELL TO CELL: GAP JUNCTIONS AND PLASMODESMATA

Tight junctions block the passageways through the gaps between cells, preventing extracellular molecules from leaking from one side of an epithelium to the other. Another type of junctional structure has a radically different function: it bridges gaps between adjacent cells so as to create direct passageways from the cytoplasm of one into that of the other. These passageways take quite different forms in animal tissues, where they are called *gap junctions*, and in plants, where they are called *plasmodesmata* (singular *plasmodesma*). In both cases, however, the function is similar: the connections allow neighboring cells to exchange small molecules but not macromolecules (with some exceptions for plasmodesmata). Many of the implications of this cell coupling are only beginning to be understood.

Gap Junctions Couple Cells Both Electrically and Metabolically

Gap junctions are present in most animal tissues, including connective tissues as well as epithelia, allowing the cells to communicate with their neighbors. Each gap junction appears in conventional electron micrographs as a patch where the membranes of two adjacent cells are separated by a uniform narrow

gap of about 2–4 nm. The gap is spanned by channel-forming proteins, of which there are two distinct families, called the *connexins* and the *innexins*. These are unrelated in sequence but similar in shape and function: in vertebrates, both families are present, but connexins predominate, with 21 members in humans. In *Drosophila* and *C. elegans*, only innexins are present, with 15 family members in the fly and 25 in the worm.

The channels formed by the gap-junction proteins allow inorganic ions and other small water-soluble molecules to pass directly from the cytoplasm of one cell to the cytoplasm of the other, thereby coupling the cells both electrically and metabolically. Thus, when a suitable dye is injected into one cell, it diffuses readily into the other, without escaping into the extracellular space. Similarly, an electric current injected into one cell through a microelectrode causes an almost instantaneous electrical disturbance in the neighboring cell, due to the flow of ions carrying electric charge through gap junctions. With microelectrodes inserted into both cells, one can easily monitor this effect and measure properties of the gap junctions, such as their electrical resistance and the ways in which the coupling changes as conditions change. In fact, some of the earliest evidence of gap-junctional communication came from electrophysiological studies that demonstrated this type of rapid, direct electrical coupling between some types of neurons. Similar methods were used to identify connexins as the proteins that mediate the gap-junctional communication: when connexin mRNA is injected into either frog oocytes or gap-junction-deficient cultured cells, channels with the properties expected of gap-junction channels can be demonstrated electrophysiologically where pairs of injected cells make contact.

From experiments with injected dye molecules of different sizes, it seems that the largest functional pore size for gap-junctional channels is about 1.5 nm. Thus, the coupled cells share their small molecules (such as inorganic ions, sugars, amino acids, nucleotides, vitamins, and the intracellular mediators cyclic AMP and inositol trisphosphate) but not their macromolecules (proteins, nucleic acids, and polysaccharides) (**Figure 19–33**).

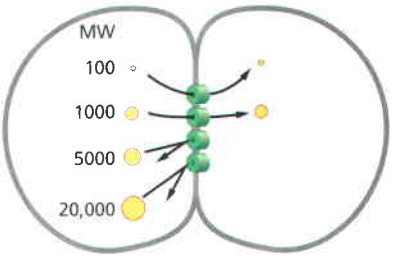


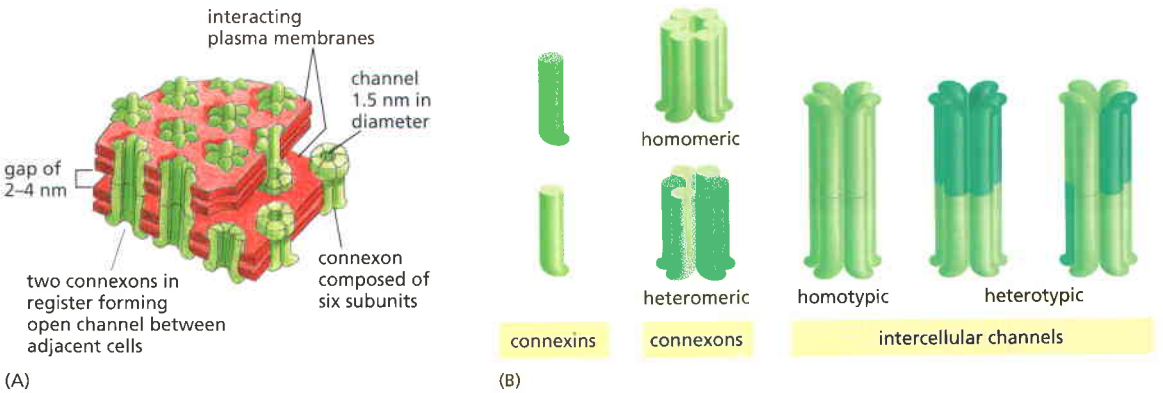
Figure 19–33 Determining the size of a gap-junction channel. When fluorescent molecules of various sizes are injected into one of two cells coupled by gap junctions, molecules with a mass of less than about 1000 daltons can pass into the other cell, but larger molecules cannot.

A Gap-Junction Connexon Is Made Up of Six Transmembrane Connexin Subunits

Connexins are four-pass transmembrane proteins, six of which assemble to form a *hemichannel*, or **connexon**. When the connexons in the plasma membranes of two cells in contact are aligned, they form a continuous aqueous channel that connects the two cell interiors (**Figure 19–34**A and **Figure 19–35**). A gap junction consists of many such connexon pairs in parallel, forming a sort of molecular sieve. The connexons hold the interacting plasma membranes a fixed distance apart—hence the gap.

Gap junctions in different tissues can have different properties because they are formed from different combinations of connexins, creating channels that differ in permeability. Most cell types express more than one type of connexin, and two different connexin proteins can assemble into a heteromeric connexon, with its own distinct properties. Moreover, adjacent cells expressing different

Figure 19–34 Gap junctions. (A) A three-dimensional drawing showing the interacting plasma membranes of two adjacent cells connected by gap junctions. Each lipid bilayer is shown as a pair of *red* sheets. Protein assemblies called connexons (*green*), each of which is formed by six connexin subunits, penetrate the apposed lipid bilayers (*red*). Two connexons join across the intercellular gap to form a continuous aqueous channel connecting the two cells. (B) The organization of connexins into connexons and connexons into intercellular channels. The connexons can be homomeric or heteromeric, and the intercellular channels can be homotypic or heterotypic.



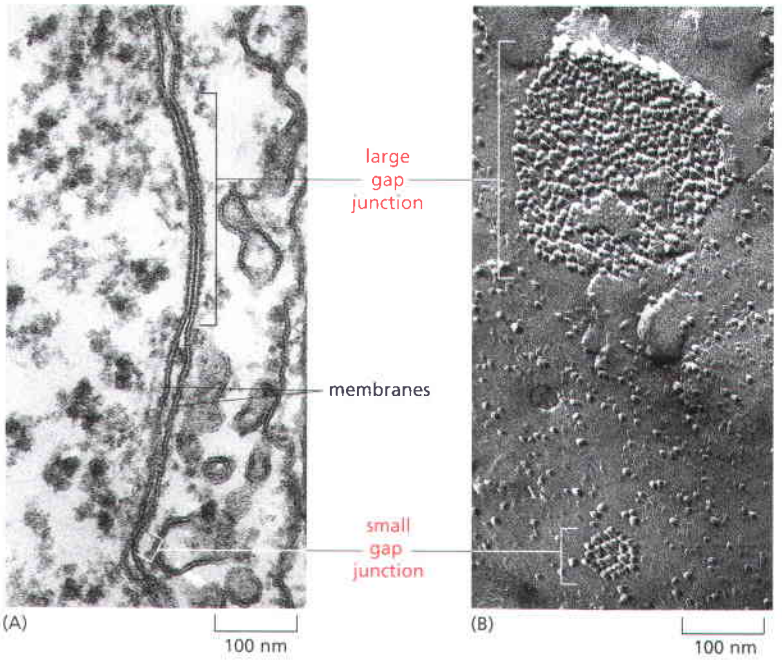


Figure 19–35 Gap junctions as seen in the electron microscope. (A) Thin-section and (B) freeze-fracture electron micrographs of a large and a small gap junction between fibroblasts in culture. In (B), each gap junction is seen as a cluster of homogeneous intramembrane particles. Each intramembrane particle corresponds to a connexon. (From N.B. Gilula, in Cell Communication [R.P. Cox, ed.], pp. 1–29. New York: Wiley, 1974.)

connexins can form intercellular channels in which the two aligned half-channels are different (Figure 19–34B).

Each gap-junctional plaque is a dynamic structure that can readily assemble, disassemble, or be remodelled, and it can contain a cluster of a few to many thousands of connexons (see Figure 19–35B). Studies with fluorescently labeled connexins in living cells show that new connexons are continually added around the periphery of an existing junctional plaque, while old connexons are removed from the middle of it and destroyed (**Figure 19–36**). This turnover is rapid: the connexin molecules have a half-life of a few hours.

The mechanism of removal of old connexons from the middle of the plaque is not known, but the route of delivery of new connexons to its periphery seems clear: they are inserted into the plasma membrane by exocytosis, like other integral membrane proteins, and then diffuse in the plane of the membrane until they bump into the periphery of a plaque and become trapped. This has a corollary: the plasma membrane away from the gap junction should contain connexons—hemichannels—that have not yet paired with their counterparts on another cell. It is thought that these unpaired hemichannels are normally held

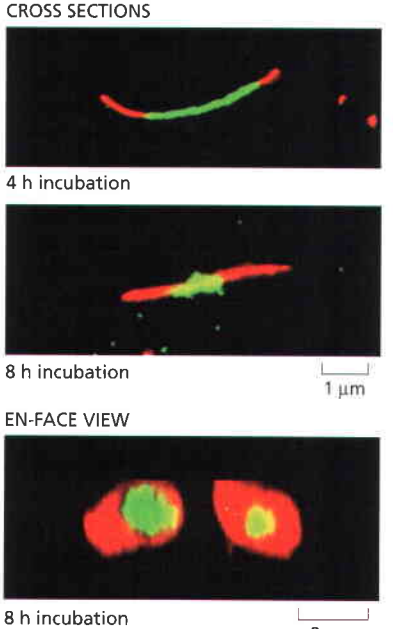


Figure 19–36 Connexin turnover at a gap junction. Cells were transfected with a slightly modified connexin gene, coding for a connexin with a short amino-acid tag containing four cysteines in the sequence ...Cys-Cys-X-X-Cys-Cys (where X denotes an arbitrary amino acid). This *tetracysteine tag* can bind strongly, and in effect irreversibly, to certain small fluorescent dye molecules that can be added to the culture medium and will readily enter cells by diffusing across the plasma membrane. In the experiment shown, a green dye was added first, and the cells were then washed and incubated for 4 or 8 hours. At the end of this time, a red dye was added to the medium and the cells were washed again and fixed. Connexin molecules already present at the beginning of the experiment are labeled green (and take up no red dye because their tetracysteine tags are already saturated with green dye), while connexins synthesized subsequently, during the 4- or 8-hour incubation, are labeled red. The fluorescence images show optical sections of gap junctions between pairs of cells prepared in this way. The central part of the gap-junction plaque is *green*, indicating that it consists of old connexin molecules, while the periphery is *red*, indicating that it consists of connexins synthesized during the past 4 or 8 hours. The longer the time of incubation, the smaller the green central patch of old molecules, and the larger the peripheral ring of new molecules that have been recruited to replace them. (From G. Gaietta et al., *Science* 296:503–507, 2002. With permission from AAAS.)

in a closed conformation, preventing the cell from losing its small molecules by leakage through them. But there is also evidence that in some physiological circumstances they can open and serve as channels for the release of small molecules, such as the neurotransmitter glutamate, to the exterior, or for the entry of small molecules into the cell.

Gap Junctions Have Diverse Functions

In tissues containing electrically excitable cells, cell–cell coupling via gap junctions serves an obvious purpose. Some nerve cells, for example, are electrically coupled, allowing action potentials to spread rapidly from cell to cell, without the delay that occurs at chemical synapses. This is advantageous when speed and reliability are crucial, as in certain escape responses in fish and insects, or where a set of neurons need to act in synchrony. Similarly, in vertebrates, electrical coupling through gap junctions synchronizes the contractions of heart muscle cells as well as those of the smooth muscle cells responsible for the peristaltic movements of the intestine.

Gap junctions also occur in many tissues whose cells are not electrically excitable. In principle, the sharing of small metabolites and ions provides a mechanism for coordinating the activities of individual cells in such tissues and for smoothing out random fluctuations in small-molecule concentrations in different cells. Gap junctions are required in the liver, for example, to coordinate the response of the liver cells to signals from nerve terminals that contact only a part of the cell population (see Figure 15–7). The normal development of ovarian follicles also depends on gap-junction-mediated communication—in this case, between the oocyte and the surrounding granulosa cells. A mutation in the gene that encodes the connexin that normally couples these two cell types causes infertility.

Mutations in connexins, especially connexin-26, are the commonest of all genetic causes of congenital deafness: they result in the death of cells in the organ of Corti, probably because they disrupt functionally important pathways for the flow of ions from cell to cell in this electrically active sensory epithelium. Connexin mutations are responsible for many other disorders besides deafness, ranging from cataracts in the lens of the eye to a form of demyelinating disease in peripheral nerves.

Cell coupling via gap junctions also seems to play a part in embryogenesis. In early vertebrate embryos (beginning with the late eight-cell stage in mouse embryos), most cells are electrically coupled to one another. As specific groups of cells in the embryo develop their distinct identities and begin to differentiate, they commonly uncouple from surrounding tissue. As the neural plate folds up and pinches off to form the neural tube, for instance (see Figure 19–16), its cells uncouple from the overlying ectoderm. Meanwhile, the cells within each group remain coupled with one another and therefore tend to behave as a cooperative assembly, all following a similar developmental pathway in a coordinated fashion.

Cells Can Regulate the Permeability of Their Gap Junctions

Like conventional ion channels (discussed in Chapter 11), individual gap-junction channels do not remain continuously open; instead, they flip between open and closed states. Moreover, the permeability of gap junctions is rapidly (within seconds) and reversibly reduced by experimental manipulations that decrease the cytosolic pH or increase the cytosolic concentration of free Ca^{2+} to very high levels.

The purpose of the pH regulation of gap-junction permeability is unknown. In one case, however, the purpose of Ca^{2+} control seems clear. When a cell is damaged, its plasma membrane can become leaky. Ions present at high concentration in the extracellular fluid, such as Ca^{2+} and Na^{+}, then move into the cell, and valuable metabolites leak out. If the cell were to remain coupled to its

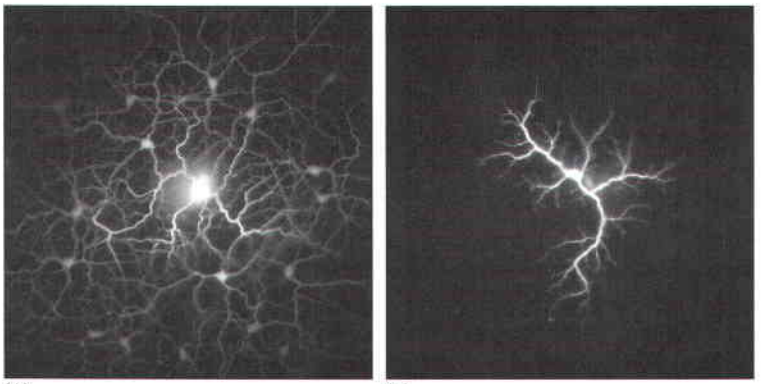


Figure 19–37 The regulation of gap-junction coupling by a neurotransmitter. (A) A neuron in a rabbit retina was injected with the dye Lucifer yellow, which passes readily through gap junctions and labels other neurons of the same type that are connected to the injected cell by gap junctions. (B) The retina was first treated with the neurotransmitter dopamine, before the neuron was injected with dye. As can be seen, the dopamine treatment greatly decreased the permeability of the gap junctions. Dopamine acts by increasing intracellular cyclic AMP levels. (Courtesy of David Vaney.)

healthy neighbors, these too would suffer a dangerous disturbance of their internal chemistry. But the large influx of Ca^{2+} into the damaged cell causes its gap-junction channels to close immediately, effectively isolating the cell and preventing the damage from spreading to other cells.

Gap-junction communication can also be regulated by extracellular signals. The neurotransmitter *dopamine*, for example, reduces gap-junction communication between a class of neurons in the retina in response to an increase in light intensity (**Figure 19–37**). This reduction in gap-junction permeability helps the retina switch from using rod photoreceptors, which are good detectors of low light, to cone photoreceptors, which detect color and fine detail in bright light.

In Plants, Plasmodesmata Perform Many of the Same Functions as Gap Junctions

The tissues of a plant are organized on different principles from those of an animal. This is because plant cells are imprisoned within tough *cell walls* composed of an extracellular matrix rich in cellulose and other polysacharides, as we discuss later. The cell walls of adjacent cells are firmly cemented to those of their neighbors, which eliminates the need for anchoring junctions to hold the cells in place. But a need for direct cell–cell communication remains. Thus, plant cells have only one class of intercellular junctions, **plasmodesmata**. Like gap junctions, they directly connect the cytoplasms of adjacent cells.

In plants, the cell wall between a typical pair of adjacent cells is at least 0.1 μm thick, and so a structure very different from a gap junction is required to mediate communication across it. Plasmodesmata solve the problem. With a few specialized exceptions, every living cell in a higher plant is connected to its living neighbors by these structures, which form fine cytoplasmic channels through the intervening cell walls. As shown in **Figure 19–38**A, the plasma membrane of one cell is continuous with that of its neighbor at each plasmodesma, which connects the cytoplasms of the two cells by a roughly cylindrical channel with a diameter of 20–40 nm.

Running through the center of the channel in most plasmodesmata is a narrower cylindrical structure, the *desmotubule*, which is continuous with elements of the smooth endoplasmic reticulum in each of the connected cells (Figure 19–38B–D). Between the outside of the desmotubule and the inner face of the cylindrical channel formed by plasma membrane is an annulus of cytosol through which small molecules can pass from cell to cell. As each new cell wall is assembled during the cytokinesis phase of cell division, plasmodesmata are created within it. They form around elements of smooth ER that become trapped across the developing cell plate (discussed in Chapter 17). They can also be inserted *de novo* through preexisting cell walls, where they are commonly found in dense clusters called *pit fields*. When no longer required, plasmodesmata can be readily removed.

choline receptors and other proteins in the junctional plasma membrane of the muscle cell. Reciprocally, muscle cells deposit a particular isoform of laminin in the junctional basal lamina, and some evidence suggests that this binds directly to the extracellular domain of voltage-gated Ca^{2+} channels in the presynaptic membrane of the nerve cell, helping to hold them at the synapse where they are needed. Both agrin and the synaptic isoform of laminin are essential for the formation of normal neuromuscular junctions. Defects in components of the basal lamina or in proteins that tether muscle cell components to it at the synapse are responsible for many of the forms of muscular dystrophy, in which muscles at first develop normally but then degenerate in later years of life.

Summary

The basal lamina is a thin tough sheet of extracellular matrix that closely underlies epithelia in all multicellular animals. It also wraps around certain other cell types, such as muscle cells. All basal laminae are organized on a framework of laminin molecules, linked together by their side-arms and held close beneath the basal ends of the epithelial cells by attachment to integrins and other receptors in the basal plasma membrane. Type IV collagen molecules are recruited into this structure, assembling into a sheetlike mesh that is an essential component of all mature basal laminae. The collagen and laminin networks in mature basal laminae are bridged by the protein nidogen and the large heparan sulfate proteoglycan perlecan.

Basal laminae provide mechanical support for epithelia; they form the interface and the attachment between epithelia and connective tissue; they serve as filters in the kidney; they act as barriers to keep cells in their proper compartments; they influence cell polarity and cell differentiation; they guide cell migrations; and molecules embedded in them help to organize elaborate structures such as neuromuscular synapses. When cells are damaged or killed, basal laminae often survive and can help guide tissue regeneration.

INTEGRINS AND CELL–MATRIX ADHESION

Cells make extracellular matrix, organize it, and degrade it. The matrix in its turn exerts powerful influences on the cells. The influences are exerted chiefly through transmembrane cell adhesion proteins that act as *matrix receptors*. These tie the matrix outside the cell to the cytoskeleton inside it, but their role goes far beyond simple passive mechanical attachment. Through them, components of the matrix can affect almost any aspect of a cell's behavior. The matrix receptors have a crucial role in epithelial cells, mediating their interactions with the basal lamina beneath them; and they are no less important in connective-tissue cells, for their interactions with the matrix that surrounds them.

Several types of molecules can function as matrix receptors or co-receptors, including the transmembrane proteoglycans. But the principal receptors on animal cells for binding most extracellular matrix proteins are the **integrins**. Like the cadherins and the key components of the basal lamina, integrins are part of the fundamental architectural toolkit that is characteristic of multicellular animals. The members of this large family of homologous transmembrane adhesion molecules have a remarkable ability to transmit signals in both directions across the cell membrane. The binding of a matrix component to an integrin can send a message into the interior of the cell, and conditions in the cell interior can send a signal outward to control binding of the integrin to matrix (or, in some cases, to a cell-surface molecule on another cell, as we saw in the case of white blood cells binding to endothelial cells). Tension applied to an integrin can cause it to tighten its grip on intracellular and extracellular structures, and loss of tension can loosen its hold, so that molecular signaling complexes fall apart on either side of the membrane. In this way, integrins can also serve not only to transmit mechanical and molecular signals, but also to convert the one type of signal into the other. Studies of the structure of integrin molecules have begun to reveal how they perform these tasks.

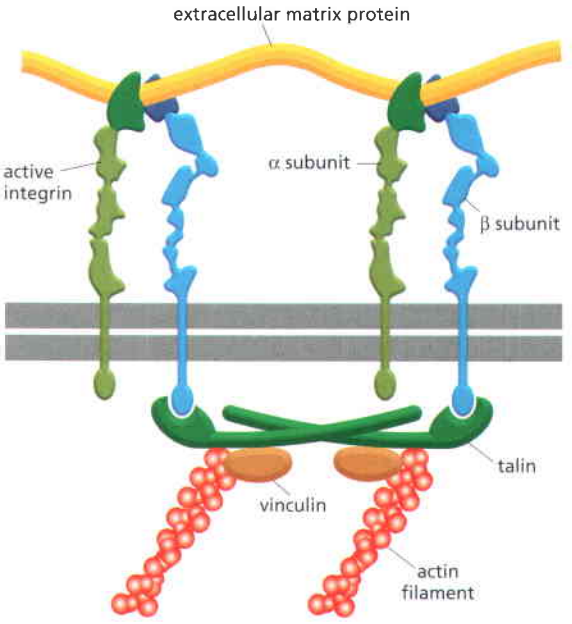


Figure 19–45 The subunit structure of an active integrin molecule, linking extracellular matrix to the actin cytoskeleton. The head of the integrin molecule attaches directly to an extracellular protein such as fibronectin; the intracellular tail of the integrin binds to talin, which in turn binds to filamentous actin. A set of other intracellular anchor proteins, including α-actinin, filamin, and vinculin, help to reinforce the linkage.

Integrins Are Transmembrane Heterodimers That Link to the Cytoskeleton

There are many varieties of integrins—at least 24 in humans—but they all conform to a common plan. An integrin molecule is composed of two noncovalently associated glycoprotein subunits called α and β. Both subunits span the cell membrane, with short intracellular C-terminal tails and large N-terminal extracellular domains. The extracellular portion of the integrin dimer binds to specific amino acid sequences in extracellular matrix proteins such as laminin or fibronectin or, in some cases, to ligands on the surfaces of other cells. The intracellular portion binds to a complex of proteins that form a linkage to the cytoskeleton.

For all but one of the 24 varieties of human integrins, this intracellular linkage is to actin filaments, via *talin* and a set of other intracellular anchorage proteins (**Figure 19–45**); talin, as we shall see later, seems to be the key component of the linkage. Like the actin-linked cell–cell junctions formed by cadherins, the actin-linked cell–matrix junctions formed by integrins may be small, inconspicuous and transient, or large, prominent, and durable. Examples of the latter are the *focal adhesions* that form when fibroblasts have sufficient time to form strong attachments to the rigid surface of a culture dish, and the *myotendinous junctions* that attach muscle cells to their tendons.

In epithelia, the most prominent cell–matrix attachment sites are the hemidesmosomes, where a specific type of integrin (α6β4) anchors the cells to laminin in the basal lamina. Here, uniquely, the intracellular attachment is to keratin filaments, via the intracellular anchor proteins plectin and dystonin (**Figure 19–46**).

Integrins Can Switch Between an Active and an Inactive Conformation

A cell crawling through a tissue—a fibroblast or a macrophage, for example, or an epithelial cell migrating along a basal lamina—has to be able both to make and to break attachments to the matrix, and to do so rapidly if it is to travel quickly. <TGAT> Similarly, a circulating white blood cell has to be able to switch on or off its tendency to bind to endothelial cells in order to crawl out of a blood vessel at a site of inflammation under the appropriate circumstances. Furthermore, if

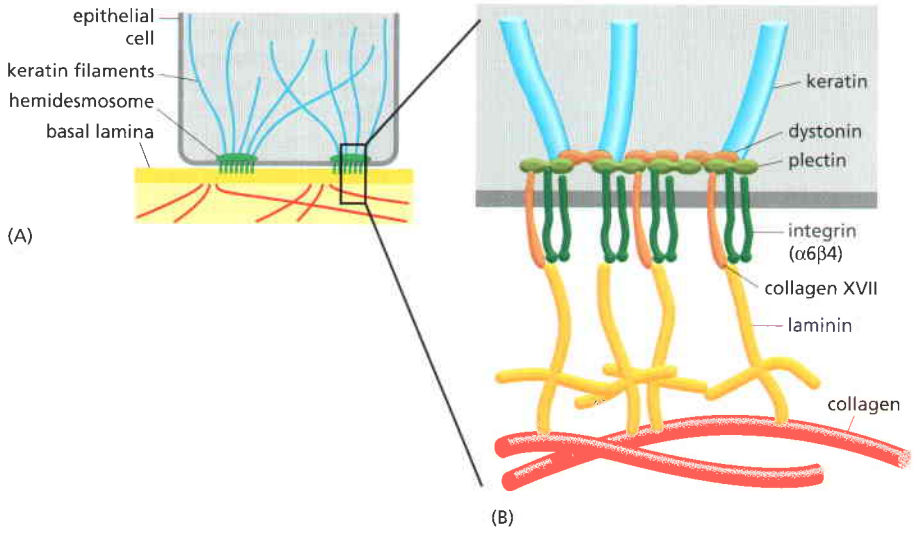


Figure 19–46 Hemidesmosomes. (A) Hemidesmosomes spot-weld epithelial cells to the basal lamina, linking laminin outside the cell to keratin filaments inside it. (B) Molecular components of a hemisdesmosome. A specialized integrin (α6β4 integrin) spans the membrane, attaching to keratin filaments intracellularly via anchor proteins called plectin and dystonin, and to laminin extracellularly. The adhesive complex also contains, in parallel with the integrin, an unusual collagen family member, collagen type XVII; this has a membrane-spanning domain attached to its extracellular collagenous portion. Defects in any of these components can give rise to a blistering disease of the skin.

force is to be applied where it is needed, the making and breaking of the extracellular attachments in all these cases has to be coupled to the prompt assembly and disassembly of cytoskeletal attachments inside the cell. The integrin molecules that span the membrane and mediate the attachments cannot simply be passive, rigid objects with sticky patches at their two ends. They must be able to switch between an active state, where they readily form attachments, and an inactive state, where they do not; and the binding of their ligands on one side of the membrane must alter their propensity to bind to a different set of ligands on the opposite side.

The basis for these dynamic phenomena is allosteric regulation: as an integrin binds to or detaches from its ligands, it undergoes conformational changes that affect both the intracellular and the extracellular ends of the molecule. Structural change at one end is coupled to structural change at the other, so that influences can be transmitted in either direction across the cell membrane. The timber tongs that lumberjacks use to grab hold of logs of wood provide a simple mechanical analogy (**Figure 19–47**).

The structural changes in integrins can be demonstrated by taking a purified preparation of integrin molecules and examining them at high resolution by electron microscopy. If the integrins are kept in a calcium-rich medium similar to normal extracellular fluid, but without any extracellular ligand, and then rapidly prepared for microscopy, they appear as tightly folded V-shaped objects. But if a small synthetic peptide containing a sequence that mimics the integrin-binding domain of a natural extracellular matrix protein is added to the medium, the integrins bind this molecule and extend into a different shape, with two legs that are no longer tightly bent, but are now straightened and separated from each other, supporting a head region high above them (**Figure 19–48**A). This pair of structures can be compared with more detailed data from x-ray crystallography, which reveals that the two legs correspond to the integrin α and β chains. The head region, where they meet, contains the binding site for the extracellular ligand. Binding of the ligand distorts this region so as to favor adoption of the extended, "active" conformation; conversely, adoption of the extended conformation creates a more favorable binding site, with a higher affinity for ligand (Figure 19–48B).

But how do these changes in the extracellular region of the integrin relate to events at the intracellular end of the integrin molecule? In its folded, inactive

Figure 19–47 Timber tongs. Holding the handles together causes the claws to grip the log; and closing the claws on the log causes the handles to come together. Moreover, the greater the pull on the tongs, the tighter the grip at both ends. In an integrin molecule, the details of the linkage are different, but the mechanical principles are similar: conformational changes at opposite ends of the molecule are coupled, and pulling tightens the grip.

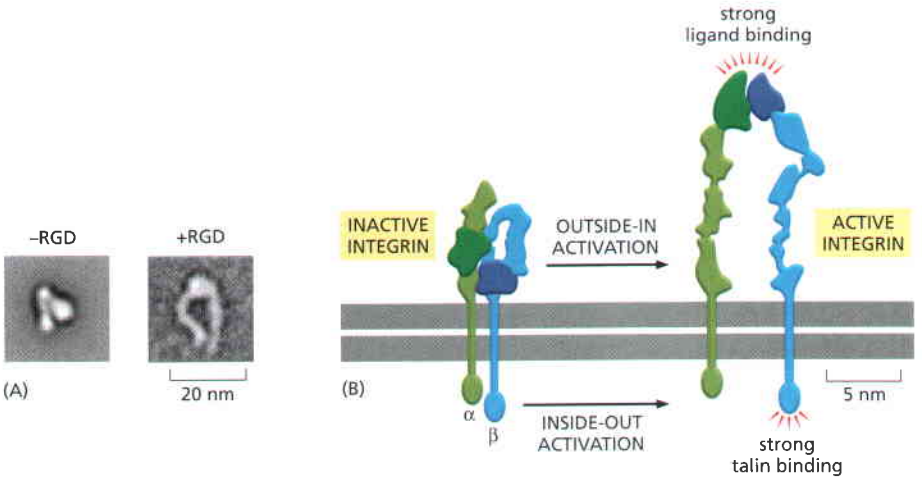


Figure 19–48 Change in conformation of an integrin molecule when it binds its ligand. (A) Images were produced by averaging many similarly aligned electron micrographs of individual integrin molecules. In the absence of extracellular ligand, the integrin molecules appear small and tightly folded. When incubated with an RGD peptide, the integrins unfold into an extended structure with two distinct legs. (B) Active (extended) and inactive (folded) structures of an integrin molecule, based on data from x-ray crystallography. Although it is difficult to crystallize the intact molecule in its natural conformations, with and without ligand bound, the complete structure can be inferred with reasonable confidence from x-ray crystallography of defined molecular fragments. (A, From J. Takagi et al., *Cell* 110:599–611, 2002. With permission from Elsevier; B, based on T. Xiao at al., *Nature* 432:59–67, 2004. With permission from Macmillan Publishers Ltd.)

state, the intracellular portions of its α and β chains lie close together and adhere to one another. When the extracellular domain unfolds, this contact is broken and the intracellular (and transmembrane) portions of these chains move apart. As a result, a binding site for talin on the tail of the β chain is exposed. The binding of talin then leads to assembly of actin filaments anchored to the intracellular end of the integrin molecule (see Figure 19–45). In this way, when an integrin catches hold of its ligand outside the cell, the cell reacts by tying its cytoskeleton to the integrin molecule, so that force can be applied at the point of attachment. This is referred to as "outside-in activation".

The chain of cause and effect can also operate in reverse, from inside to outside instead of outside to inside. Talin competes with the integrin α chain for its binding site on the tail of the β chain. Thus when talin binds to the β chain it undoes the intracellular α–β linkage, allowing the two legs of the integrin molecule to spring apart. This drives the extracellular portion of the integrin into its extended, active conformation.

This "inside-out activation" is triggered by intracellular regulatory molecules. These include the phosphoinositide PIP_2 (discussed in Chapter 15), which is thought to be capable of activating talin so that it binds to the integrin β chain strongly. In this way, a signal generated inside the cell can trigger its integrin molecules to reach out and grab hold of their extracellular ligands.

Intracellular signal molecules such as PIP_2 are themselves produced in response to signals received from outside the cell via other types of cell-surface receptors, such as G-protein-coupled receptors and receptor tyrosine kinases, which can thus control integrin activation (**Figure 19–49**). Conversely, the activation of integrins by attachment to matrix can influence the reception of signals by other pathways. The cross-talk between all these communication pathways, transmitting signals in both directions across the cell membrane, allows for some complex interactions between the cell and its physical and chemical environment.

Integrin Defects Are Responsible for Many Different Genetic Diseases

The 24 types of integrins found in a human are formed from the products of 8 different β-chain genes and 18 different α-chain genes, dimerized in different combinations. Each integrin has distinctive properties and functions. Moreover, because the same integrin molecule in different cell types can have different ligand-binding specificities, it seems that additional cell-type-specific factors can interact with integrins to modulate their binding activity. The binding of integrins to their ligands is also affected by the concentration of Ca^{2+} and Mg^{2+} in the extracellular medium, reflecting the presence of divalent-cation-binding domains in the α and β subunits. The divalent cations can influence both the affinity and the specificity of the binding of an integrin to its ligands.

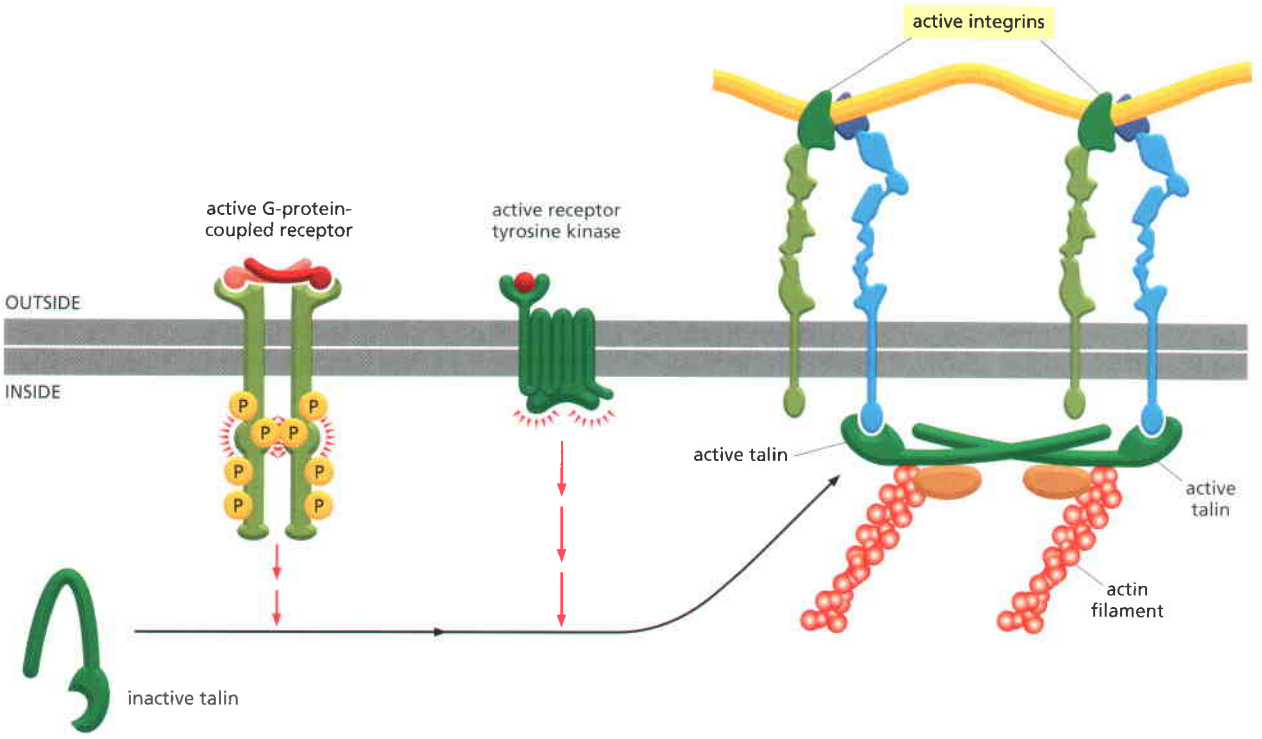


Figure 19–49 Activation of integrins by cross-talk from other signaling pathways. Signals received from outside the cell via other types of cell surface receptors, such as G-protein-coupled receptors and receptor tyrosine kinases, can alter the conformation of talin and thereby activate the cell's integrins.

Although there is some overlap in the activities of the different integrins—at least five bind laminin, for example—it is the diversity of integrin functions that is more remarkable. **Table 19–4** lists some of varieties of integrins and the problems that result when individual integrin α or β chains are defective.

The β1 subunits form dimers with at least 12 distinct α subunits and are found on almost all vertebrate cells: α5β1 is a fibronectin receptor and α6β1 a laminin receptor on many types of cells. Mutant mice that cannot make any β1 integrins die at implantation (very early in embryonic development). Mice that are only unable to make the α7 subunit (the partner for β1 in muscle) survive but develop muscular dystrophy (as do mice that cannot make the laminin ligand for the α7β1 integrin).

Table 19–4 Some Types of Integrins

INTEGRIN	LIGAND*	DISTRIBUTION	PHENOTYPE WHEN α SUBINUT IS MUTATED	PHENOTYPE WHEN β SUBUNIT IS MUTATED
α5β1	fibronectin	ubiquitous	death of embryo; defects in blood vessels, somites, neural crest	early death of embryo (at implantation)
α6β1	laminin	ubiquitous	severe skin blistering; defects in other epithelia also	early death of embryo (at implantation)
α7β1	laminin	muscle	muscular dystrophy; defective myotendinous junctions	early death of embryo (at implantation)
αLβ2 (LFA1)	Ig superfamily counterreceptors (ICAM)	white blood cells	impaired recruitment of leucocytes	leucocyte adhesion deficiency (LAD) impaired inflammatory responses; recurrent life-threatening infections
αIIbβ3	fibrinogen	platelets	bleeding; no platelet aggregation (Glanzmann's disease)	bleeding; no platelet aggregation (Glanzmann's disease); mild osteopetrosis
α6β4	laminin	hemidesmosomes in epithelia	severe skin blistering; defects in other epithelia also	severe skin blistering; defects in other epithelia also

*Not all ligands are listed.

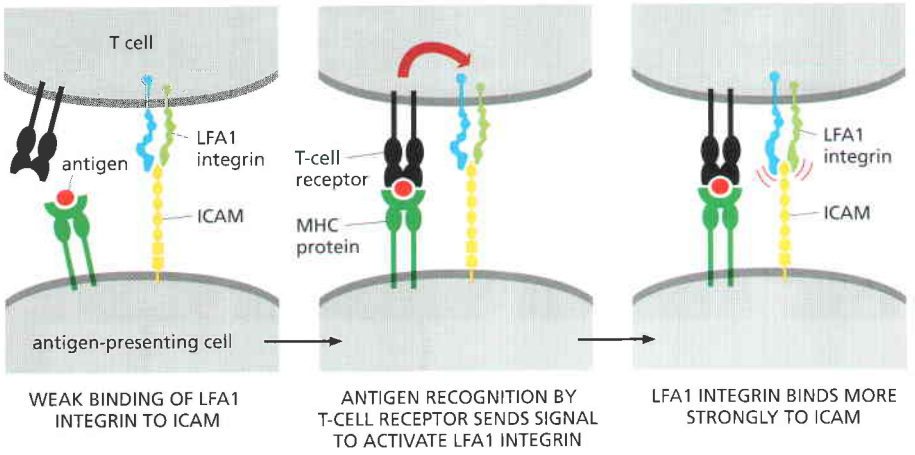


Figure 19–50 Integrin activation in the encounter of a T lymphocyte with an antigen-presenting cell. The two cells at first adhere weakly through binding of the LFA1 integrin in the T cell to the Ig-superfamily molecule ICAM in the membrane of the antigen-presenting cell. If the T-cell receptor at the same time recognizes its specific antigen, presented to it by the MHC molecule on the antigen-presenting cell, an intracellular signal is generated from the T cell receptor to activate the LFA1 integrin. As a result, LFA1 binds more strongly and persistently to ICAM. This gives the antigen-presenting cell time to activate the T cell and thereby elicit a specific immune response. (Adapted from K. Murphy et al., Janeway's Immunobiology, 7th ed. New York: Garland Science, 2008.)

The β2 subunits form dimers with at least four types of α subunit and are expressed exclusively on the surface of white blood cells, where they have an essential role in enabling these cells to fight infection. The β2 integrins mainly mediate cell–cell rather than cell–matrix interactions, binding to specific ligands on another cell, such as an endothelial cell. The ligands, sometimes referred to as *counterreceptors*, are members of the Ig superfamily of cell–cell adhesion molecules. We have already described an example earlier in the chapter: an integrin of this class (αLβ2, also known as LFA1) on white blood cells enables them to attach firmly to the Ig-family protein ICAM on endothelial cells at sites of infection and, through this attachment, to migrate out of the bloodstream into the infected tissue (see Figure 19–19B). People with the genetic disease called *leucocyte adhesion deficiency* fail to synthesize functional β2 subunits. As a consequence, their white blood cells lack the entire family of β2 receptors, and they suffer repeated bacterial infections.

The β3 integrins are found on blood platelets (as well as various other cells), and they bind several matrix proteins, including the blood clotting factor *fibrinogen*. Platelets have to interact with fibrinogen to mediate normal blood clotting, and humans with *Glanzmann's disease*, who are genetically deficient in β3 integrins, suffer from defective clotting and bleed excessively.

In both white blood cells and platelets, the ability to regulate integrin activity via inside-out signaling is particularly important. Regulated adhesion allows the cells to circulate unimpeded until they are activated by an appropriate stimulus. Because the integrins do not need to be synthesized *de novo*, the signaled adhesion response can be rapid. Platelets, for example, respond to contact with the wall of a damaged blood vessel and to various soluble signaling molecules, triggering activation of the β3 integrin in the platelet membrane. The resulting interaction of platelets with fibrinogen leads to formation of a platelet plug, which helps to stop the bleeding at just the site where it is needed. Similarly, the binding of a T lymphocyte to its specific antigen on the surface of an antigen-presenting cell (discussed in Chapter 25) switches on intracellular signaling pathways in the T cell that activate its β2 integrins (**Figure 19–50**). The activated integrins then enable the T cell to adhere strongly to the antigen-presenting cell so that it remains in contact long enough to become stimulated fully. The integrins may then return to an inactive state, allowing the T cell to disengage.

Integrins Cluster to Form Strong Adhesions

Integrins, like other cell adhesion molecules, differ from cell-surface receptors for hormones and for other extracellular soluble signal molecules in that they usually bind their ligand with lower affinity and are usually present at a 10- to 100-fold higher concentration on the cell surface. The Velcro principle, mentioned earlier, operates here too. Strong adhesion depends on clustering of

integrins, creating a plaque in which many cytoskeletal filaments are anchored, as at a hemidesmosome in the epidermis or at a focal adhesion made by a fibroblast on a culture dish. At focal adhesions, and probably also in the less prominent actin-linked cell–matrix adhesions that cells mainly make in normal tissues, activation of the small GTPase Rho plays a part in the maturation of the adhesive complex, by promoting recruitment of actin filaments and integrins to the contact site. Artificially mutated integrins that lack an intracellular tail fail to connect with cytoskeletal filaments, fail to cluster, and are unable to form strong adhesions.

Extracellular Matrix Attachments Act Through Integrins to Control Cell Proliferation and Survival

Like other transmembrane cell adhesion proteins, integrins do more than just create attachments. They also activate intracellular signaling pathways and thereby allow control of almost any aspect of the cell's behavior according to the nature of the surrounding matrix and the state of the cell's attachments to it.

Studies in culture show that many cells will not grow or proliferate unless they are attached to extracellular matrix; nutrients and soluble growth factors in the culture medium are not enough. For some cell types, including epithelial, endothelial, and muscle cells, even cell survival depends on such attachments. When these cells lose contact with the extracellular matrix, they undergo programmed cell death, or apoptosis. This dependence of cell growth, proliferation, and survival on attachment to a substratum is known as **anchorage dependence**, and it is mediated mainly by integrins and the intracellular signals they generate. Anchorage dependence is thought to help ensure that each type of cell survives and proliferates only when it is in an appropriate situation. Mutations that disrupt or override this form of control, allowing cells to escape from anchorage dependence, occur in cancer cells and play a major part in their invasive behavior.

The physical spreading of a cell on the matrix also has a strong influence on intracellular events. Cells that are forced to spread over a large surface area by the formation of multiple adhesions at widely separate sites survive better and proliferate faster than cells that are not so spread out (**Figure 19–51**). The stimulatory effect of cell spreading presumably helps tissues to regenerate after injury. If cells are lost from an epithelium, for example, the spreading of the remaining cells into the vacated space will help stimulate these survivors to proliferate until they fill the gap. It is uncertain how a cell senses its extent of spreading so as to adjust its behavior accordingly, but the ability to spread depends on integrins, and signals generated by integrins at the sites of adhesion must play a part in providing the spread cells with stimulation.

Our understanding of anchorage dependence and of the effects of cell spreading has come mainly from studies of cells living on the surface of matrix-coated culture dishes. For connective-tissue cells that are normally surrounded

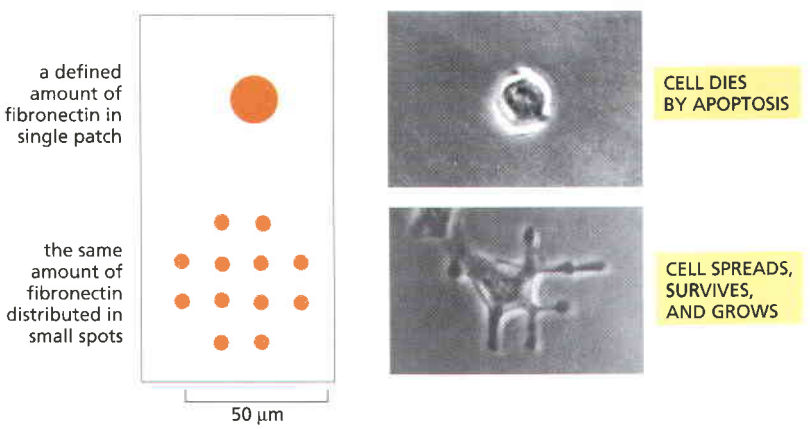


Figure 19–51 The importance of cell spreading. In this experiment, cell growth and survival are shown to depend on the extent of cell spreading on a substratum, rather than the mere fact of attachment or the number of matrix molecules the cell contacts. (Based on C.S. Chen et al., *Science* 276:1425–1428, 1997. With permission from AAAS.)

by matrix on all sides, this is a far cry from the natural environment. Walking over a plain is very different from clambering through a jungle. The types of contacts that cells make with a rigid substratum are not the same as those, much less well studied, that they make with the deformable web of fibers of the extracellular matrix, and there are substantial differences of cell behavior between the two contexts. Nevertheless, it is likely that the same basic principles apply. Both *in vitro* and *in vivo*, intracellular signals generated at cell–matrix adhesion sites, by molecular complexes organized around integrins, are crucial for cell proliferation and survival.

Integrins Recruit Intracellular Signaling Proteins at Sites of Cell–Substratum Adhesion

The mechanisms by which integrins signal into the cell interior are complex, involving several different pathways, and integrins and conventional signaling receptors often influence one another and work together to regulate cell behavior, as we have already emphasized. The Ras/MAP kinase pathway (see Figure 15–61), for example, can be activated both by conventional signaling receptors and by integrins, but cells often need both kinds of stimulation of this pathway at the same time to give sufficient activation to induce cell proliferation. Integrins and conventional signaling receptors also cooperate in activating similar pathways to promote cell survival (discussed in Chapters 15 and 17).

One of the best-studied modes of integrin signaling depends on a cytoplasmic protein tyrosine kinase called **focal adhesion kinase** (**FAK**). In studies of cells cultured in the normal way on rigid substrata, focal adhesions are often prominent sites of tyrosine phosphorylation (**Figure 19–52**A), and FAK is one of the major tyrosine-phosphorylated proteins found at these sites. When integrins cluster at cell–matrix contacts, FAK is recruited by intracellular anchor proteins such as talin (binding to the integrin β subunit) or *paxillin* (which binds to one type of integrin α subunit). The clustered FAK molecules cross-phosphorylate each other on a specific tyrosine, creating a phosphotyrosine docking site for members of the Src family of cytoplasmic tyrosine kinases. In addition to phosphorylating other proteins at the adhesion sites, these kinases then phosphorylate FAK on additional tyrosines, creating docking sites for a variety of additional intracellular signaling proteins. In this way, outside-in signaling from integrins, via FAK and Src-family kinases, is relayed into the cell (as discussed in Chapter 15).

One way to analyze the function of FAK is to examine focal adhesions in cells from mutant mice that lack the protein. FAK-deficient fibroblasts still adhere to

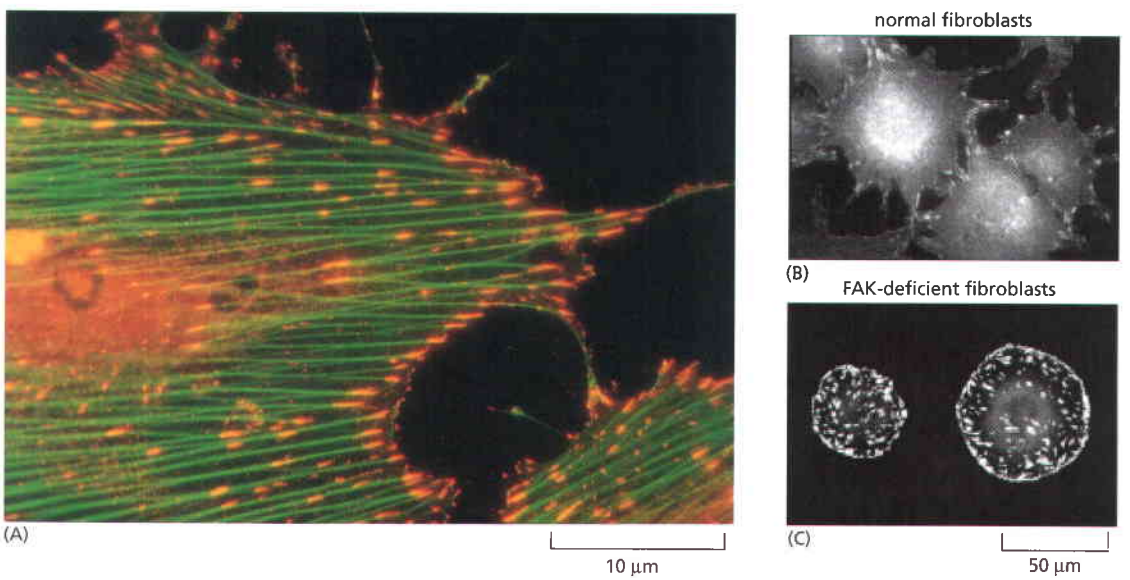


Figure 19–52 Focal adhesions and the role of focal adhesion kinase (FAK). (A) A fibroblast cultured on a fibronectin-coated substratum and stained with fluorescent antibodies: actin filaments are stained *green* and activated proteins that contain phosphotyrosine are *red*, giving *orange* where the two components overlap. The actin filaments terminate at focal adhesions, where the cell attaches to the substratum by means of integrins. Proteins containing phosphotyrosine are also concentrated at these sites, reflecting the local activation of FAK and other protein kinases. Signals generated at such adhesion sites help regulate cell division, growth, and survival. (B, C) The influence of FAK on formation of focal adhesions is shown by a comparison of normal and FAK-deficient fibroblasts, stained with an antibody against vinculin to reveal the focal adhesions. (B) The normal fibroblasts have fewer focal adhesions and have spread after 2 hours in culture. (C) At the same time point, the FAK-deficient fibroblasts have more focal adhesions and have not spread. (A, courtesy of Keith Burridge; B, C, from D. Ilic et al., *Nature* 377:539–544, 1995. With permission from Macmillan Publishers Ltd.)

fibronectin and form focal adhesions. In fact, they form too many focal adhesions; as a result, cell spreading and migration are slowed (Figure 19–52B and C). This unexpected finding suggests that FAK normally helps disassemble focal adhesions and that this loss of adhesions is required for normal cell migration. Many cancer cells have elevated levels of FAK, which may help explain why they are often more motile than their normal counterparts.

Integrins Can Produce Localized Intracellular Effects

Through FAK and other pathways, activated integrins, like other signaling receptors, can induce global cell responses, often including changes in gene expression. But the integrins are especially adept at stimulating localized changes in the cytoplasm close to the cell–matrix contact. We have already mentioned an important example in our discussion of epithelial cell polarity: it is through integrins that the basal lamina plays its part in directing the internal apico-basal organization of epithelial cells.

Localized intracellular effects may be a common feature of signaling by transmembrane cell adhesion proteins in general. In the developing nervous system, for example, the growing tip of an axon is guided mainly by its responses to local adhesive (and repellent) cues in the environment that are recognized by transmembrane cell adhesion proteins, as discussed in Chapter 22. The primary effects of the adhesion proteins are thought to result from the activation of intracellular signaling pathways that act locally in the axon tip, rather than through cell–cell adhesion itself or signals conveyed to the cell body. Through localized activation of the Rho family of small GTPases, for example (as discussed in Chapters 15 and 16), the transmembrane adhesion proteins can control motility and guide forward movement. In this and other ways, practically all the classes of cell-cell and cell-matrix adhesion molecules that we have mentioned, including integrins, are deployed to help guide axon outgrowth in the developing nervous system.

Table 19–5 summarizes the categories of cell adhesion molecules that we have considered in this chapter. In the next section, we turn from the adhesion molecules in cell membranes to look in detail at the extracellular matrix that surrounds cells in connective tissues.

Table 19–5 Cell Adhesion Molecule Families

	SOME FAMILY MEMBERS	Ca^{2+} OR Mg^{2+} DEPENDENCE	HOMOPHILIC OR HETEROPHILIC	CYTOSKELETON ASSOCIATIONS	CELL JUNCTION ASSOCIATIONS
Cell–Cell Adhesion					
Classical cadherins	E, N, P, VE	yes	homophilic	actin filaments (via catenins)	adherens junctions, synapses
Desmosomal cadherins	desmoglein, desmocollin	yes	homophilic	intermediate filaments (via desmoplakin, plakoglobin, and plakophilin)	desmosomes
Ig family members	N-CAM, ICAM	no	both	unknown	neuronal and immunological synapses
Selectins (blood cells and endothelial cells only)	L-, E-, and P-selectins	yes	heterophilic	actin filaments	(no prominent junctional structure)
Integrins on blood cells	αLβ2 (LFA1)	yes	heterophilic	actin filaments	immunological synapses
Cell–Matrix Adhesion					
Integrins	many types	yes	heterophilic	actin filaments (via talin, paxillin, filamin, α-actinin, and vinculin)	focal adhesions
	α6β4	yes	heterophilic	intermediate filaments (via plectin and dystonin)	hemidesmosomes
Transmembrane proteoglycans	syndecans	no	heterophilic	actin filaments	(no prominent junctional structure)

Summary

Integrins are the principal receptors used by animal cells to bind to the extracellular matrix: they function as transmembrane linkers between the extracellular matrix and the cytoskeleton connecting usually to actin, but to intermediate filaments for the specialized integrins at hemidesmosomes. Integrin molecules are heterodimers, and the binding of ligands is associated with dramatic changes of conformation. This creates an allosteric coupling between binding to matrix outside the cell and binding to the cytoskeleton inside it, allowing the integrin to convey signals in both directions across the plasma membrane—from inside to out and from outside to in. Binding of the intracellular anchor protein talin to the tail of an integrin molecule tends to drive the integrin into an extended conformation with increased affinity for its extracellular ligand. Conversely, binding to an extracellular ligand, by promoting the same conformational change, leads to binding of talin and formation of a linkage to the actin cytoskeleton. Complex assemblies of proteins become organized around the intracellular tails of integrins, producing intracellular signals that can influence almost any aspect of cell behavior, from proliferation and survival, as in the phenomenon of anchorage dependence, to cell polarity and guidance of migration.

THE EXTRACELLULAR MATRIX OF ANIMAL CONNECTIVE TISSUES

We have already discussed the basal lamina as an archetypal example of extracellular matrix, common to practically all multicellular animals and an essential feature of epithelial tissues. We now turn to the much more varied and bulky forms of extracellular matrix found in connective tissues (**Figure 19–53**). Here, the extracellular matrix is generally more plentiful than the cells it surrounds, and it determines the tissue's physical properties.

The classes of macromolecules constituting the extracellular matrix in animal tissues are broadly similar, whether we consider the basal lamina or the other forms that matrix can take, but variations in the relative amounts of these different classes of molecules and in the ways in which they are organized give rise to an amazing diversity of materials. The matrix can become calcified to form the rock-hard structures of bone or teeth, or it can form the transparent substance of the cornea, or it can adopt the ropelike organization that gives tendons their enormous tensile strength. It forms the jelly in a jellyfish. Covering the body of a beetle or a lobster, it forms a rigid carapace. Moreover, the extracellular matrix is more than a passive scaffold to provide physical support. It has an active and complex role in regulating the behavior of the cells that touch it, inhabit it, or crawl through its meshes, influencing their survival, development, migration, proliferation, shape, and function.

In this section, we focus our discussion on the extracellular matrix of connective tissues in vertebrates, but bulky forms of extracellular matrix play an

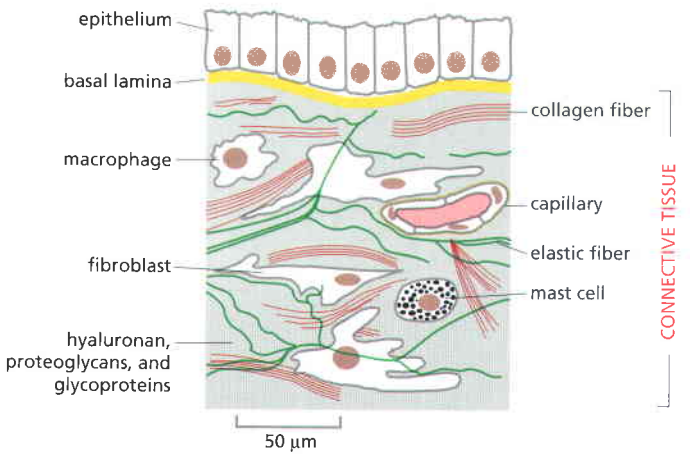


Figure 19–53 The connective tissue underlying an epithelium. This tissue contains a variety of cells and extracellular matrix components. The predominant cell type is the fibroblast, which secretes abundant extracellular matrix.