Molecular biology of the cell
BIO 207

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CHAPTER

19

Cell Junctions and the Extracellular Matrix

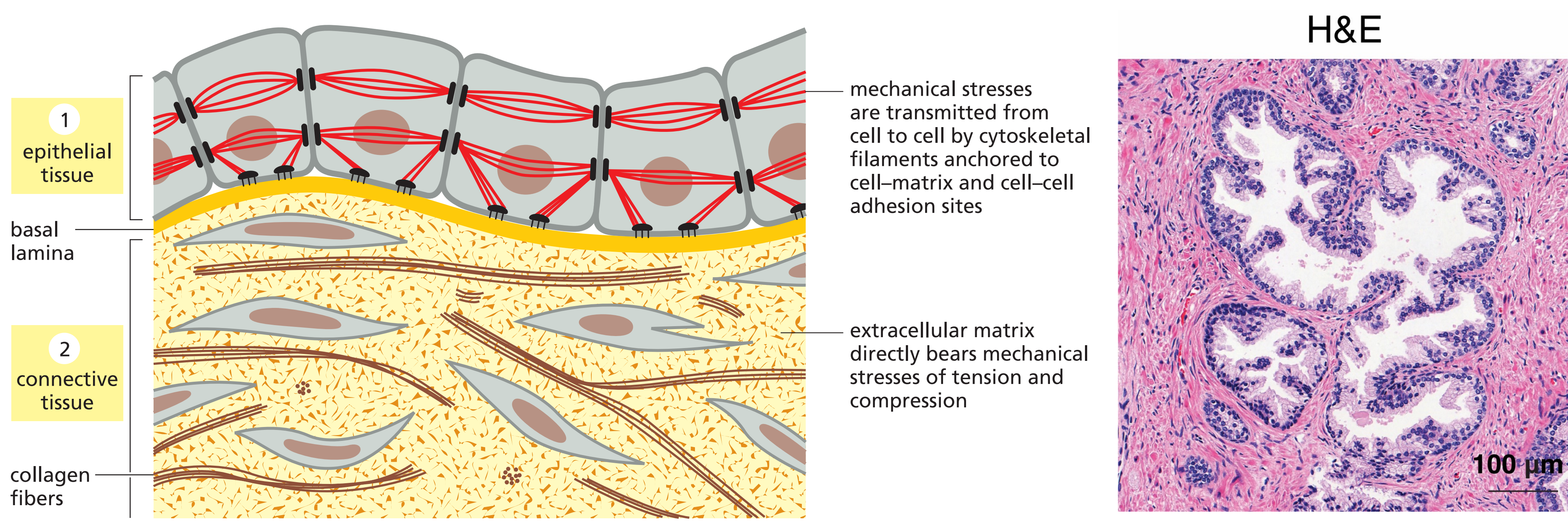
IN THIS CHAPTER

CELL–CELL JUNCTIONS

THE EXTRACELLULAR MATRIX OF ANIMALS

CELL–MATRIX JUNCTIONS

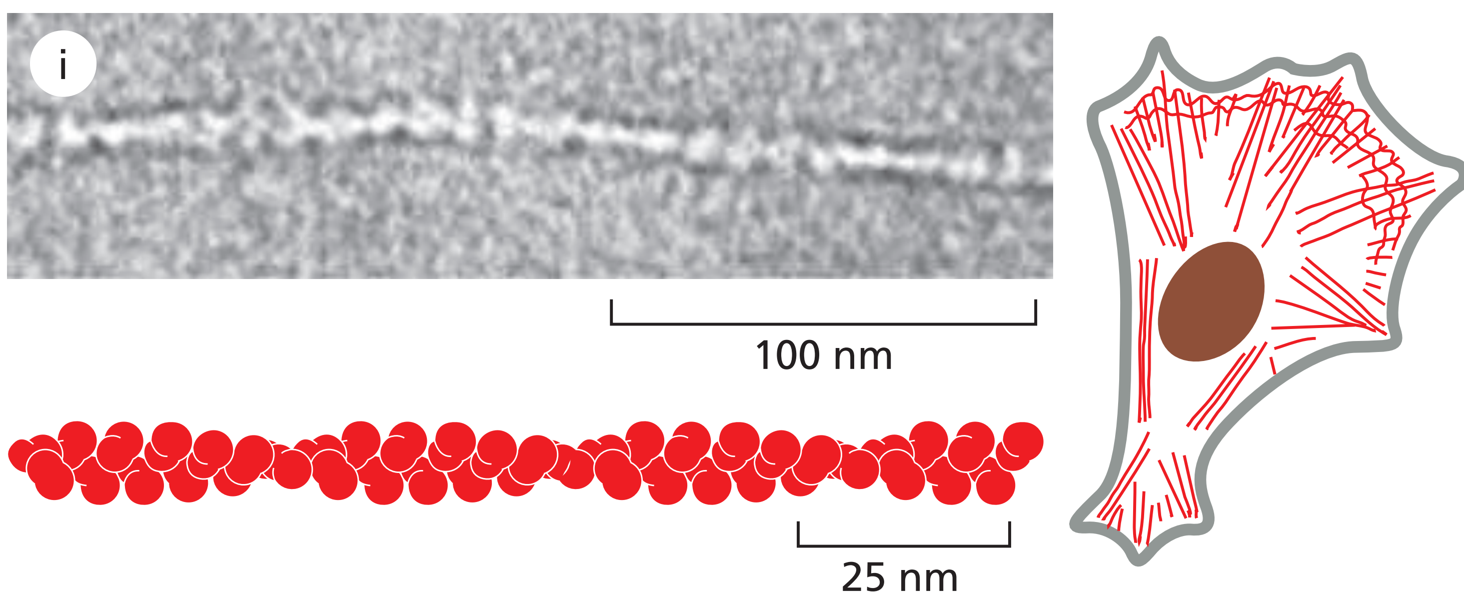
Our cells are organized in a complex system

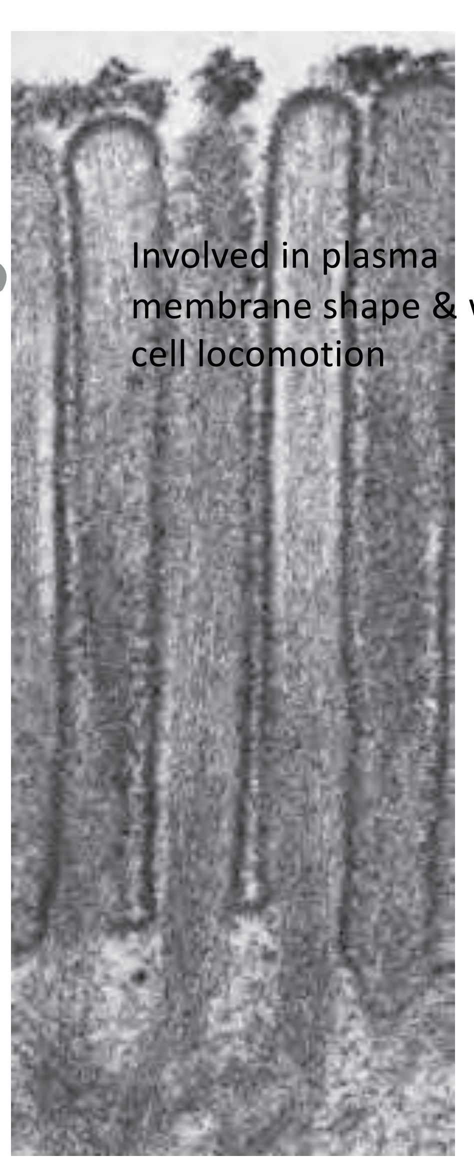


How does a cell sense its surroundings?

- Cell - Cell contacts
- Cell - Matrix Contacts
- Contact points with cytoskeletal proteins

ACTIN FILAMENTS





Actin filaments (also known as *microfilaments*) are helical polymers of the protein actin. They are flexible structures with a diameter of 8 nm that organize into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane. (i) Single actin filament; (ii) microvilli; (iii) stress fibers (*red*) terminating in focal adhesions (*green*); (iv) striated muscle.

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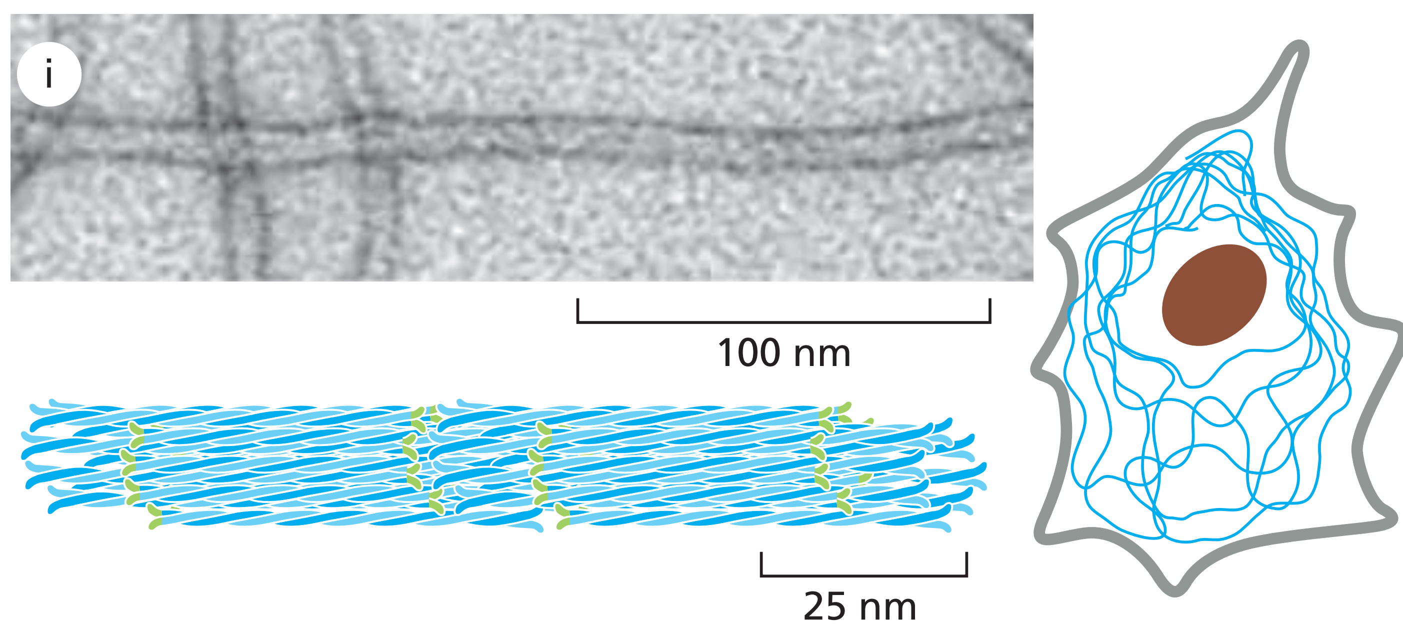
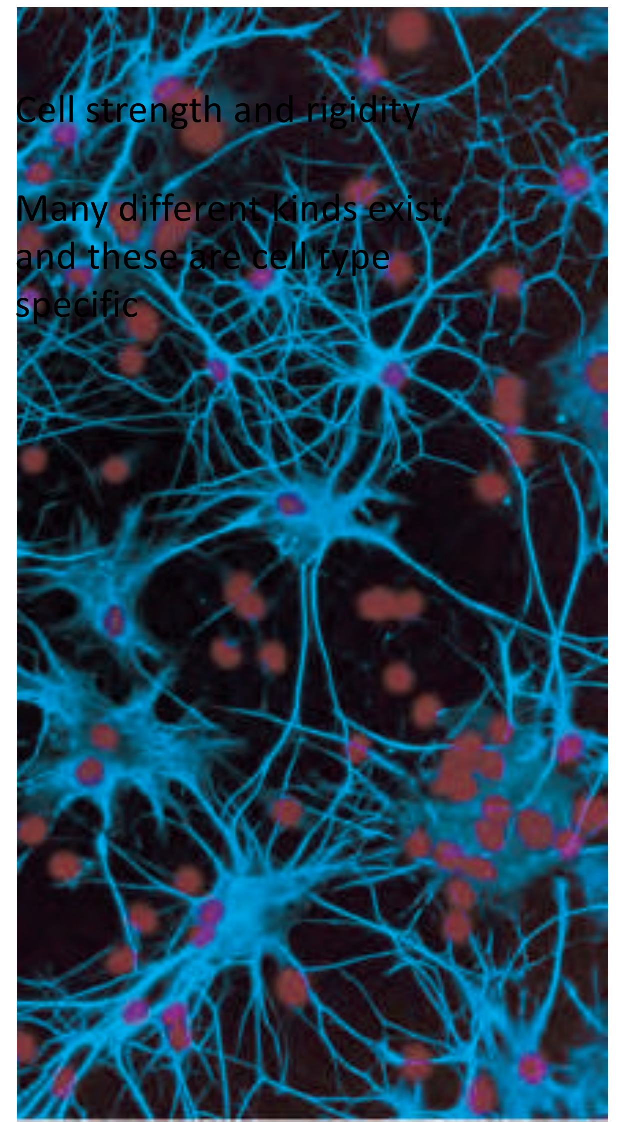
INTERMEDIATE FILAMENTS



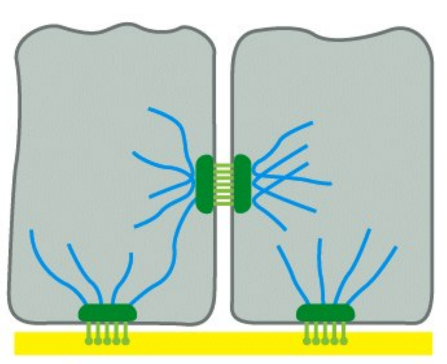
Intermediate filaments are ropelike fibers with a diameter of about 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell–cell junction to another, thereby strengthening the entire epithelium. (i) Individual intermediate filaments; (ii) Intermediate filaments (*blue*) in neurons and (iii) epithelial cell; (iv) nuclear lamina.

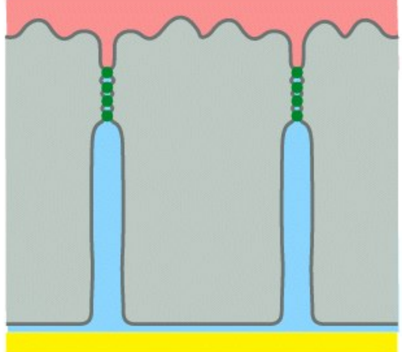


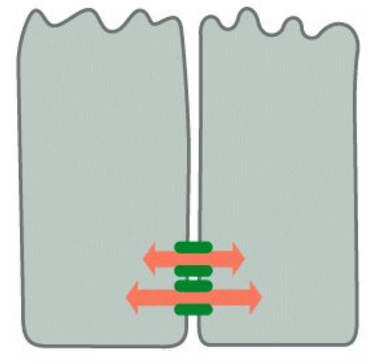
Cell strength and rigidity

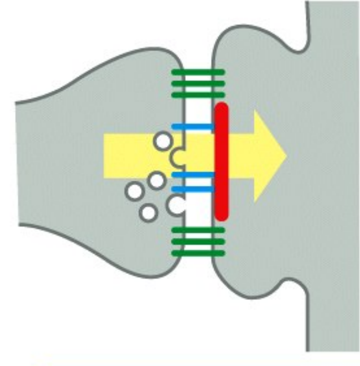
Many different kinds exist, and these are cell type specific

The main types and functions of cell-cell or cell-matrix junctions









(A) ANCHORING JUNCTIONS	(B) OCCLUDING JUNCTIONS	(C) CHANNEL-FORMING JUNCTIONS	(D) SIGNAL-RELAYING JUNCTIONS
cell-cell or cell-matrix adhesion	To block inter-cellular passage (impermeable or selectively permeable barrier)	To enable communication between two cell interiors	To enable communication between two adjacent cells
	Ex: intestinal epithelial cells – glucose absorption	Ex: GAP junctions – electrical conductance between two heart muscle cells	Ex: neurological synapse – propagation of an action potential by release of neurotransmitter

Figure 19-2 *Molecular Biology of the Cell* (© Garland Science 2008)

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**

(A) ANCHORING JUNCTIONS

(B) OCCLUDING JUNCTIONS

(C) CHANNEL-FORMING JUNCTIONS

(D) SIGNAL-RELAYING JUNCTIONS

Table 19-1 *Molecular Biology of the Cell* (© Garland Science 2008)

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There are many cell junctions in the same cell

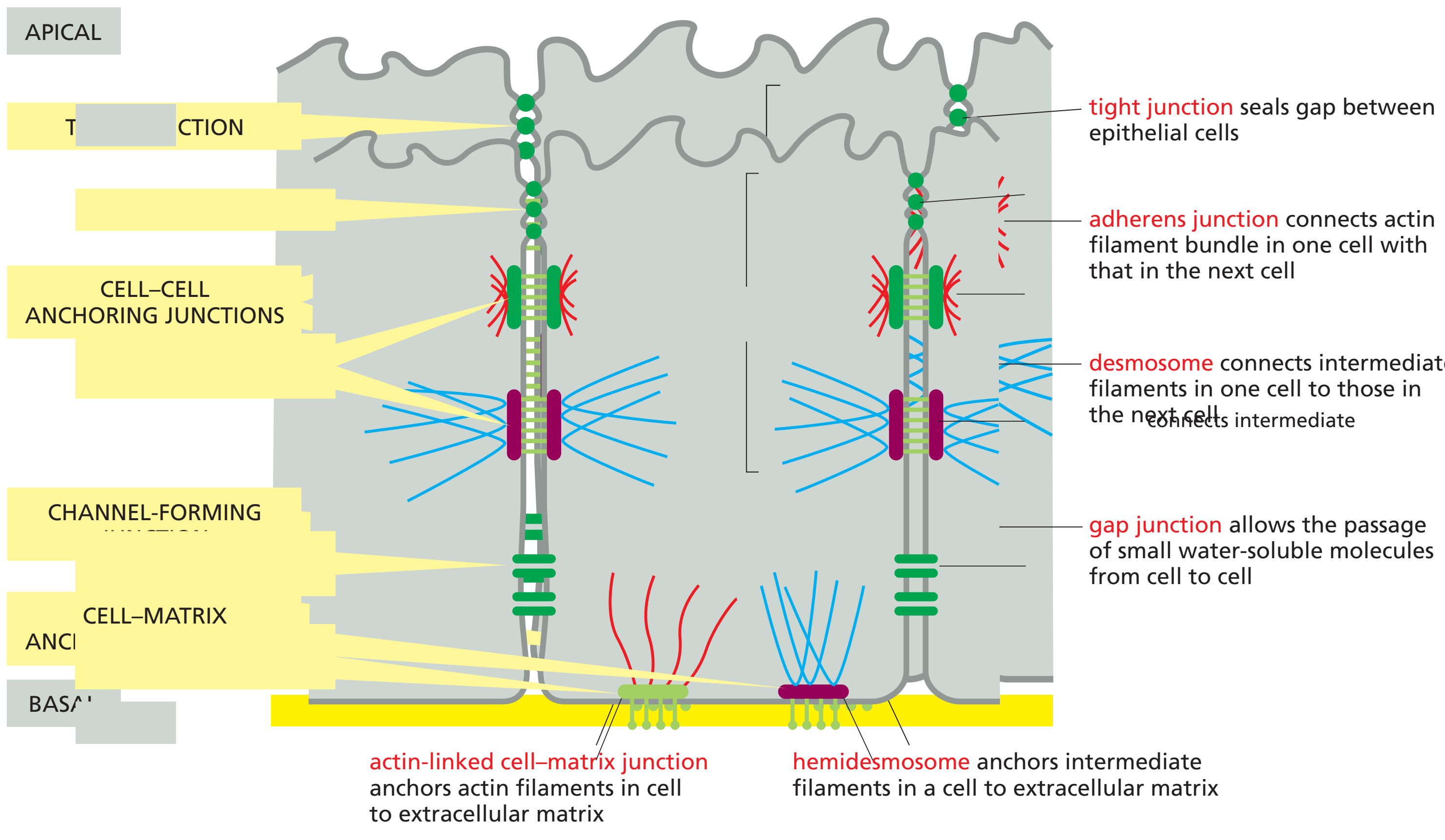

Figure 19–2 A summary of the various cell junctions found in a vertebrate epithelial cell, classified according to their primary functions. In the most apical portion of the cell, the relative positions of the junctions are the same in nearly all vertebrate epithelia. The tight junction occupies the 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. Two types of cell-matrix anchoring junctions tether the basal surface of the cell to the basal lamina. The drawing is based on epithelial cells of the small intestine.

Cell junctions can be classified on their function

There are different subtypes of these cell junctions, with different proteins involved

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Anchoring junctions

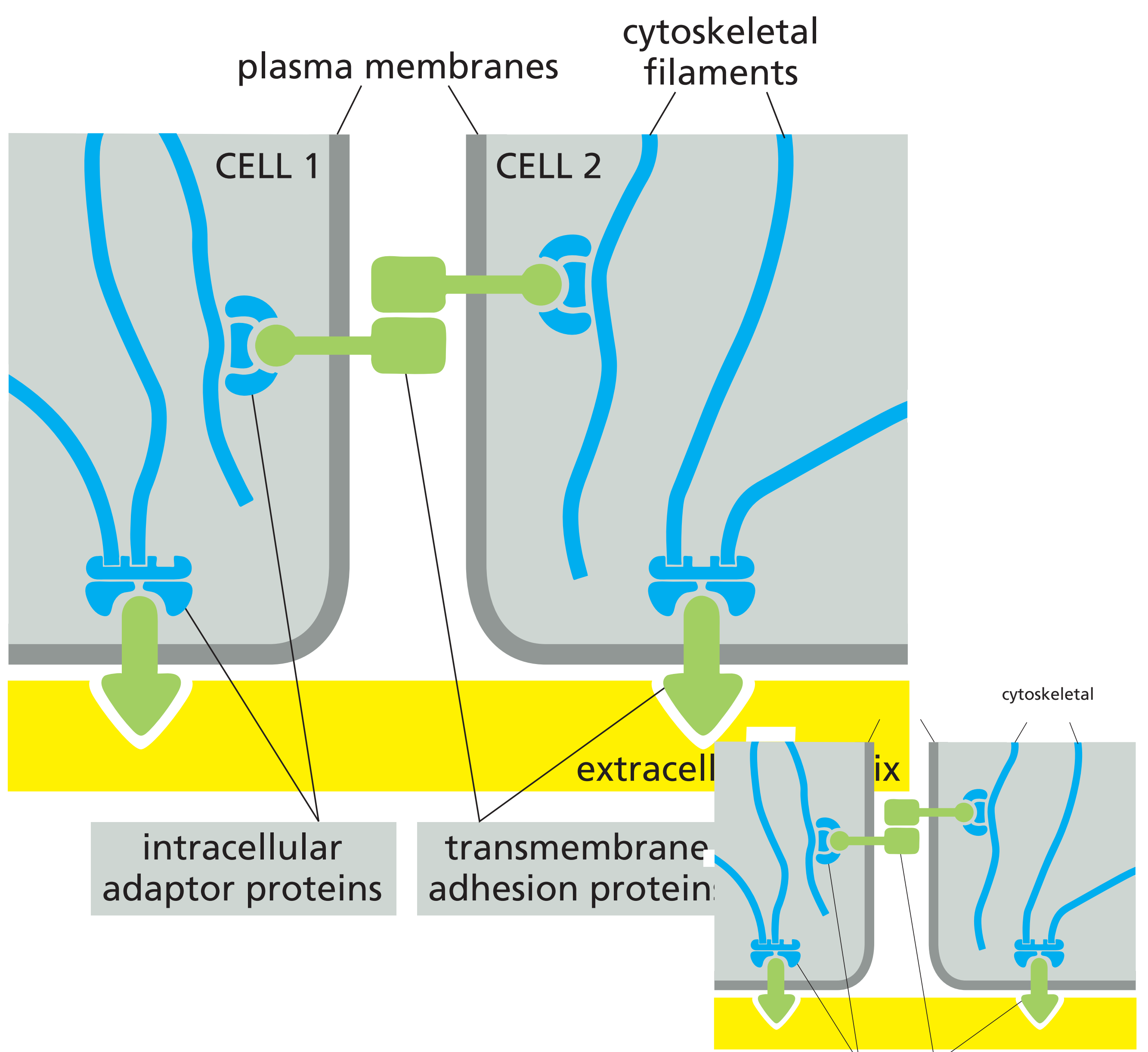
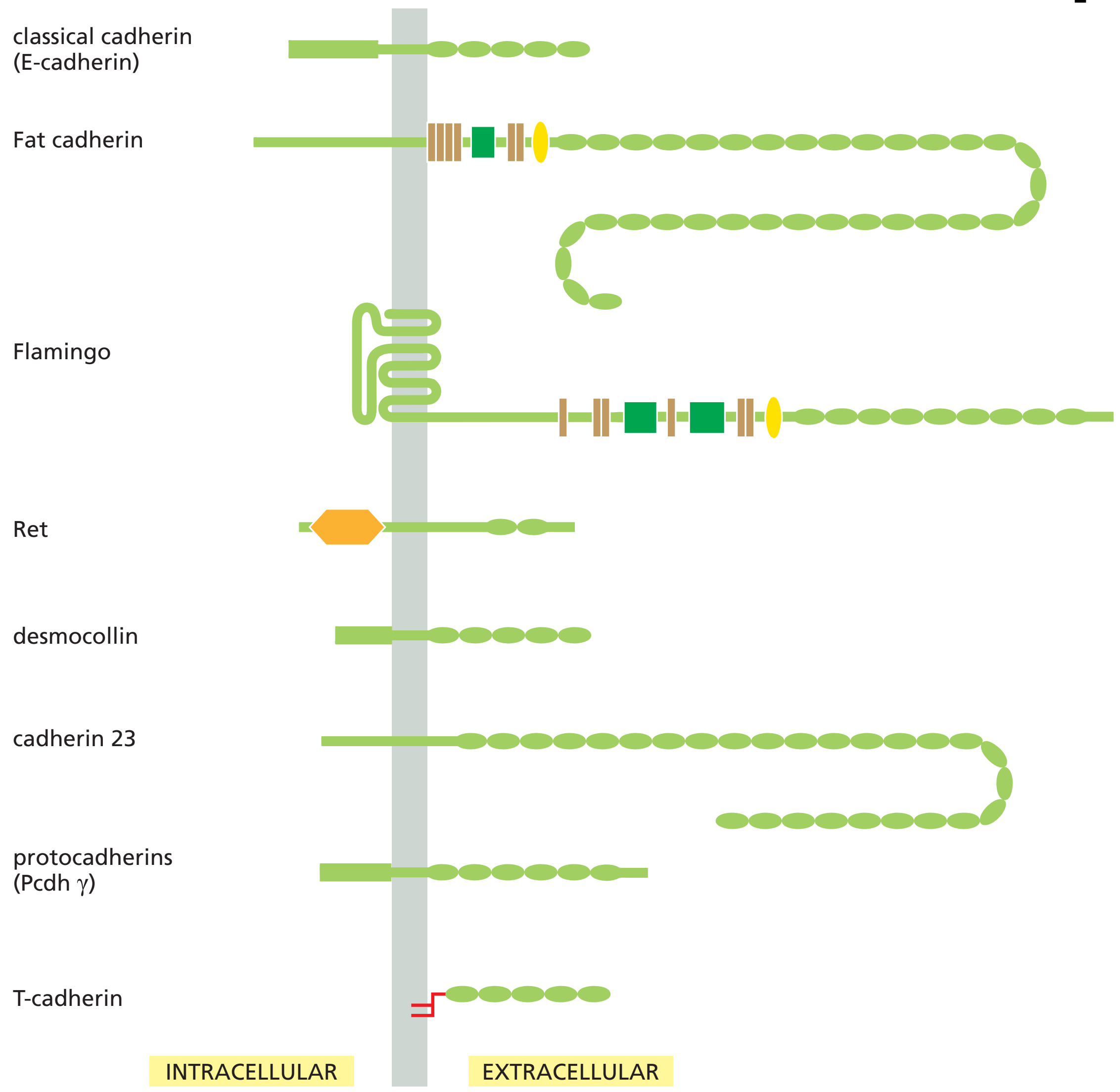


Figure 19–3 Transmembrane adhesion proteins link the cytoskeleton to extracellular structures. The external linkage may be either to other cells (cell–cell junctions, mediated typically by cadherins) or to extracellular matrix (cell–matrix junctions, mediated typically by integrins). The internal linkage to the cytoskeleton is generally indirect, via intracellular adaptor proteins, to be discussed later.

Different types

TABLE 19–1 Anchoring Junctions				
Junction	Transmembrane adhesion protein	Extracellular ligand	Intracellular cytoskeletal attachment	Intracellular adaptor proteins
Cell–Cell				
Adherens junction	Classical cadherins	Classical cadherin on neighboring cell	Actin filaments	α-Catenin, β-catenin, plakoglobin (γ-catenin), p120-catenin, vinculin
Desmosome	Nonclassical cadherins (desmoglein, desmocollin)	Desmoglein and desmocollin on neighboring cell	Intermediate filaments	Plakoglobin (γ-catenin), plakophilin, desmoplakin
Cell–Matrix				
Actin-linked cell–matrix junction	Integrin	Extracellular matrix proteins	Actin filaments	Talin, kindlin, vinculin, paxillin, focal adhesion kinase (FAK), numerous others
Hemidesmosome	$\alpha_6\beta_4$ Integrin, type XVII collagen	Extracellular matrix proteins	Intermediate filaments	Plectin, BP230

Cadherin superfamily



Structural related domains in the extracellular domain

Figure 19–4 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 extracellular cadherin domain *(green ovals)*. In the classical cadherins of vertebrates there are 5 of these domains, and in desmogleins and desmocollins there are 4 or 5, but some nonclassical cadherins have more than 30. The intracellular portions are more varied, reflecting interactions with a wide variety of intracellular ligands, including signaling molecules and adaptor proteins that connect the cadherin to the cytoskeleton. In some cases, such as T-cadherin, a transmembrane domain is not present and the protein is attached to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor. The differently colored motifs in Fat, Flamingo, and Ret represent conserved domains that are also found in other protein families.

Interactions of cadherins

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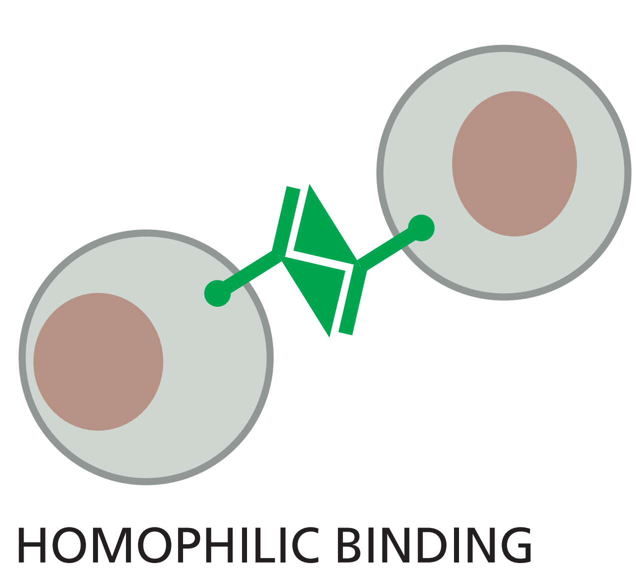

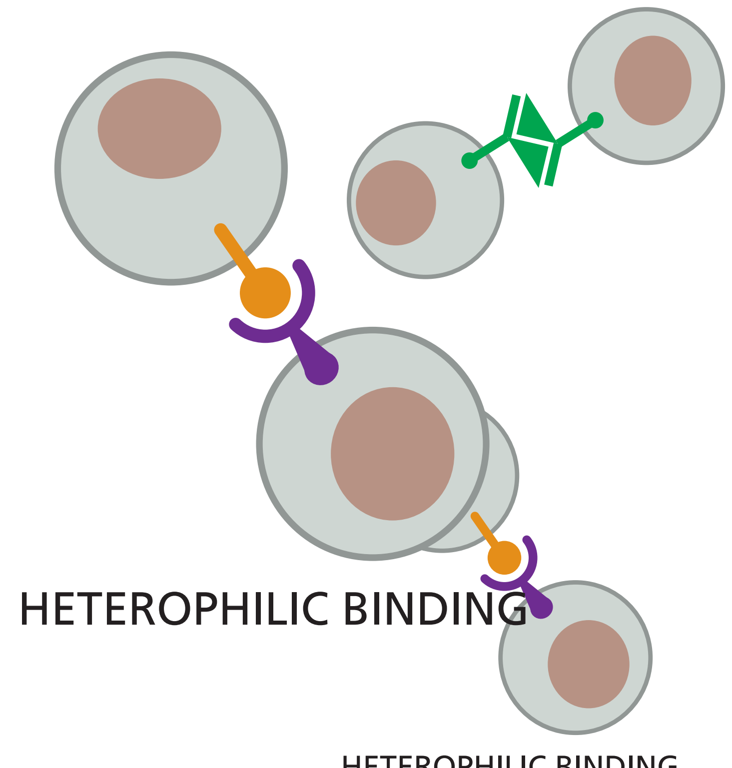

Figure 19–5 Homophilic versus heterophilic binding. Cadherins in general bind homophilically; some other cell adhesion molecules, discussed later, bind heterophilically.

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N-terminal cadherin interaction

38.5 nm

N

N

C

C

plasma membrane of cell 1

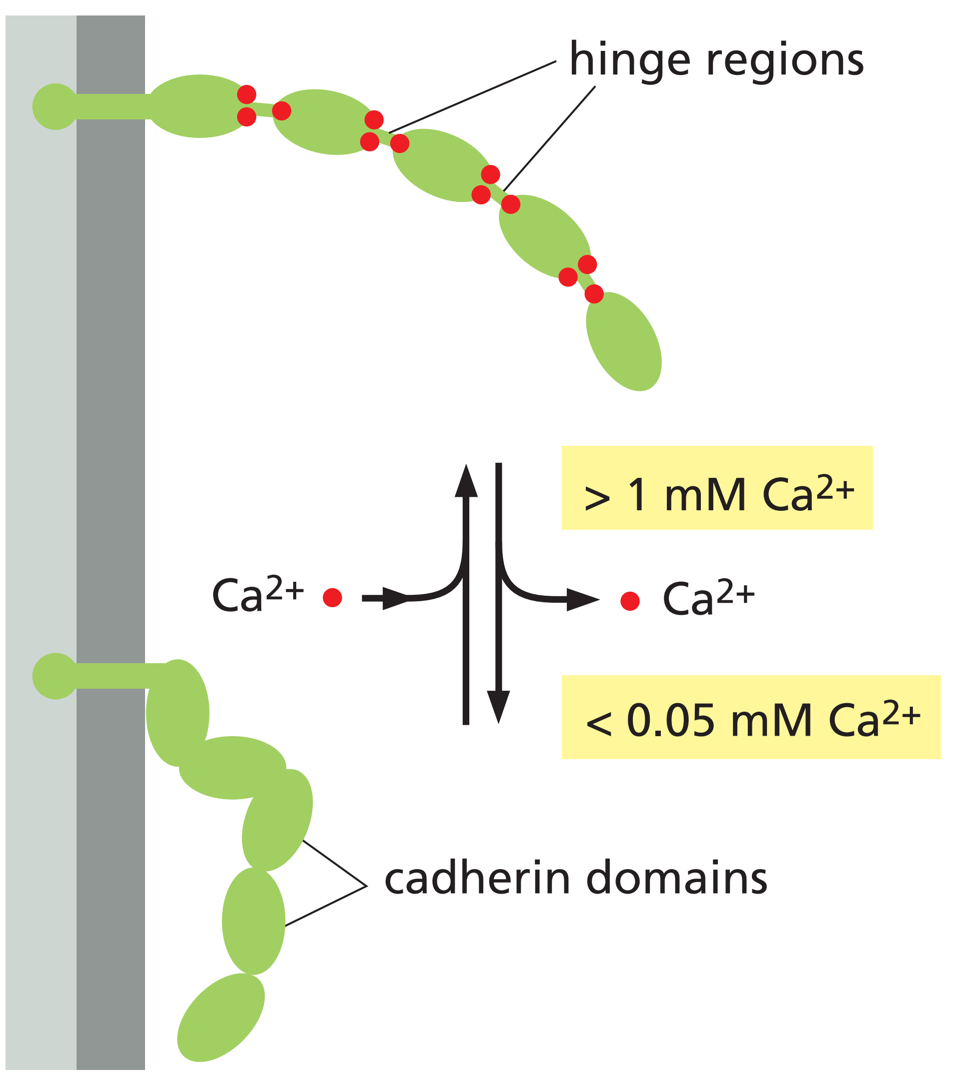
plasma membrane of cell 2

Ca^{2+}

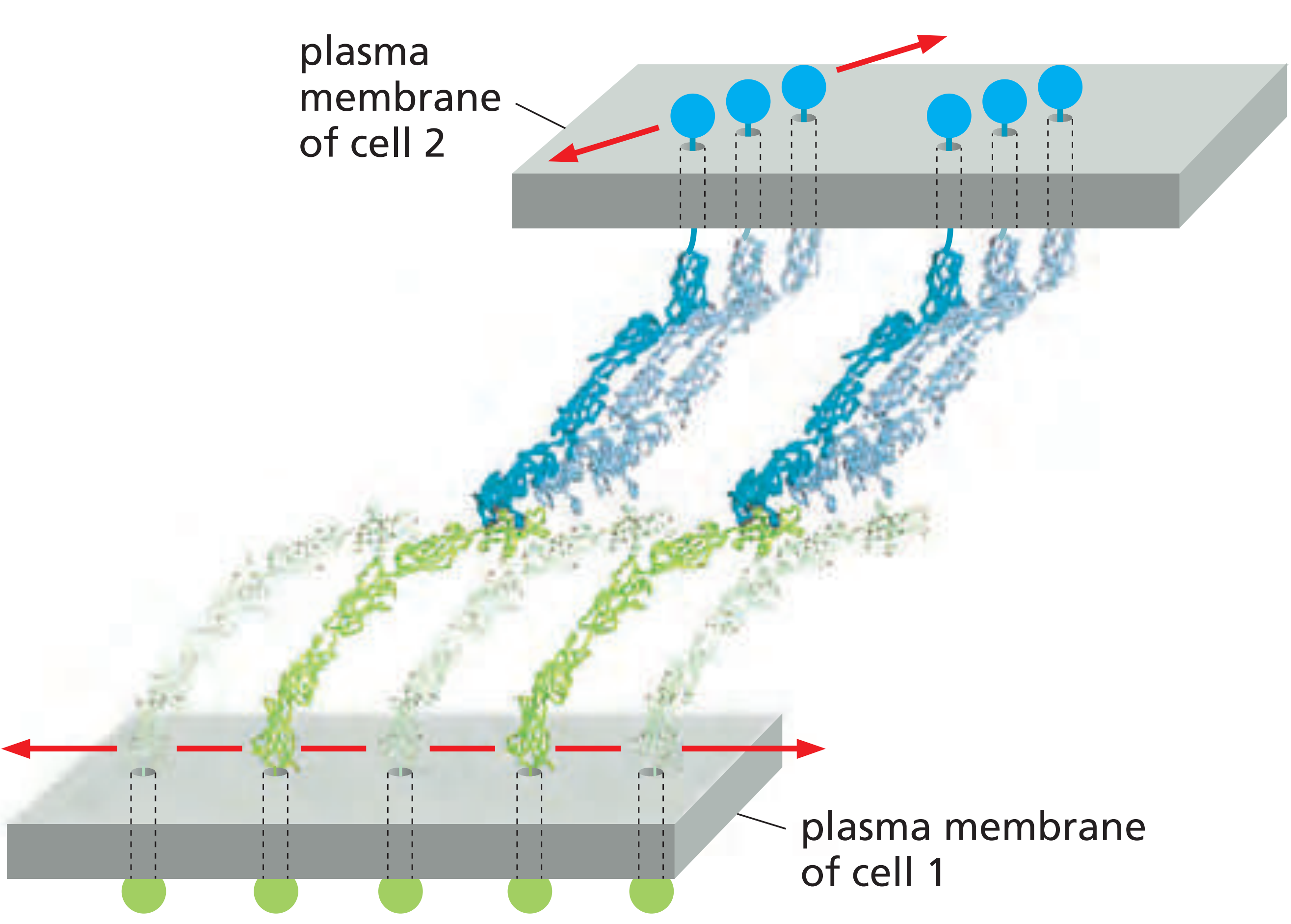
flexible hinge region

N-terminal cadherin domains

Cadherin structure is calcium dependent



Cadherin interaction between cells



Cadherin interaction

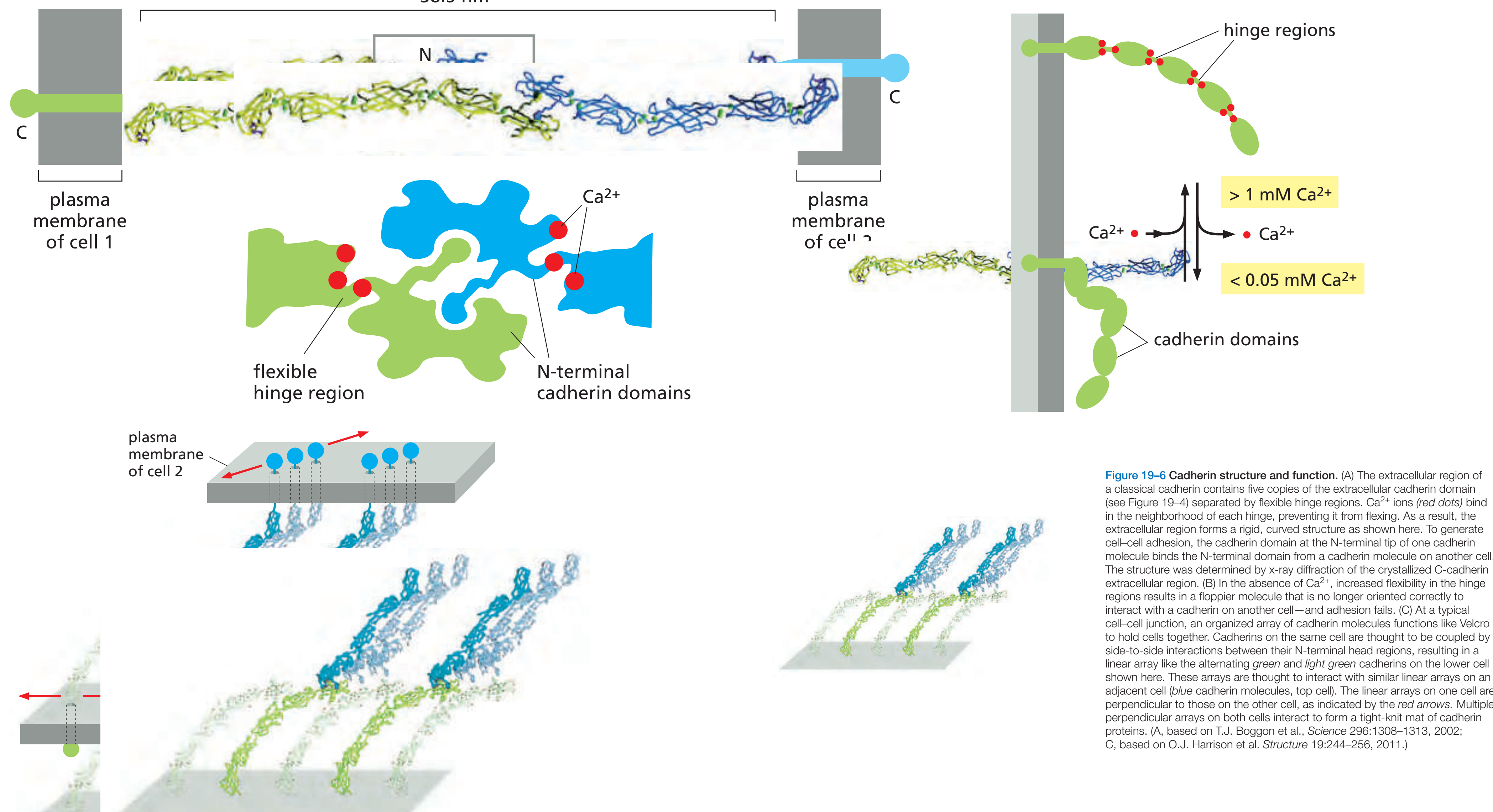
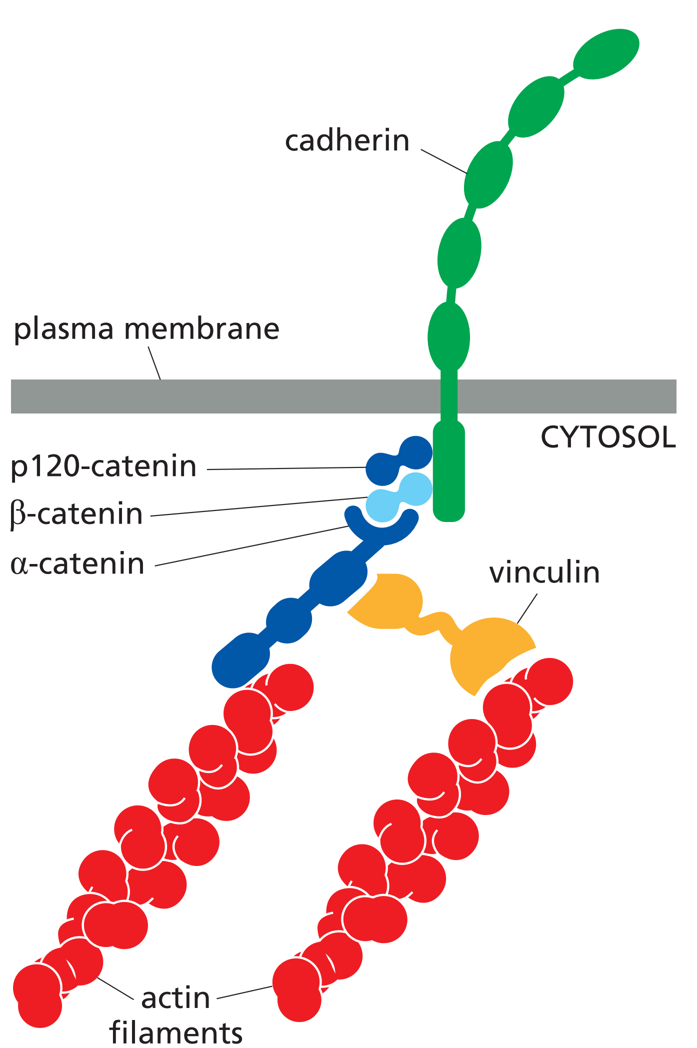


Figure 19–6 Cadherin structure and function. (A) The extracellular region of a classical cadherin contains five copies of the extracellular cadherin domain (see Figure 19–4) separated by flexible hinge regions. Ca^{2+} ions *(red dots)* bind in the neighborhood of each hinge, preventing it from flexing. As a result, the extracellular region forms a rigid, curved structure as shown here. To generate cell–cell adhesion, the cadherin domain at the N-terminal tip of one cadherin molecule binds the N-terminal domain from a cadherin molecule on another cell. The structure was determined by x-ray diffraction of the crystallized C-cadherin extracellular region. (B) In the absence of Ca^{2+}, increased flexibility in the hinge regions results in a floppier molecule that is no longer oriented correctly to interact with a cadherin on another cell—and adhesion fails. (C) At a typical cell–cell junction, an organized array of cadherin molecules functions 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, resulting in a linear array like the alternating *green* and *light green* cadherins on the lower cell shown here. These arrays are thought to interact with similar linear arrays on an adjacent cell (*blue* cadherin molecules, top cell). The linear arrays on one cell are perpendicular to those on the other cell, as indicated by the *red arrows.* Multiple perpendicular arrays on both cells interact to form a tight-knit mat of cadherin proteins. (A, based on T.J. Boggon et al., *Science* 296:1308–1313, 2002; C, based on O.J. Harrison et al. *Structure* 19:244–256, 2011.)

Classical cadherins interact with the cytoskeleton



Interaction with the cytoskeleton is mediated by an adaptor complex

B-Catenin, is also involved in WNT signaling!

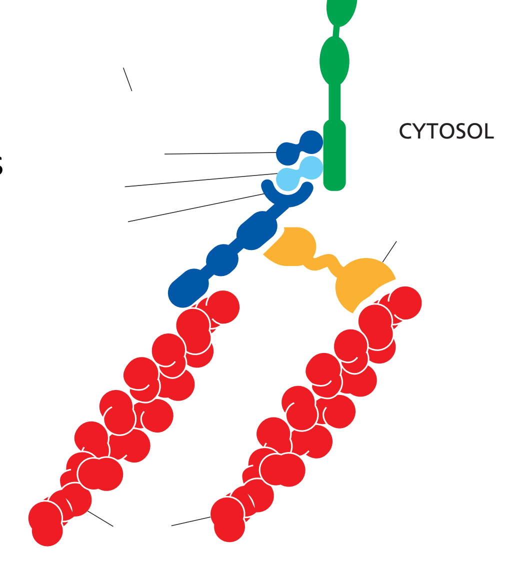


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Figure 19–10 The linkage of classical cadherins to actin filaments. The cadherins are coupled indirectly to actin filaments through an adaptor protein complex containing p120-catenin, β-catenin, and α-catenin. Other proteins, including vinculin, associate with α-catenin and help provide the linkage to actin. β-Catenin has a second, and very important, function in intracellular signaling, as we discuss in Chapter 15 (see Figure 15–60). For clarity, this diagram does not show the cadherin of the adjacent cell in the junction.

The adaptor complex undergoes a conformational change upon cadherin interaction

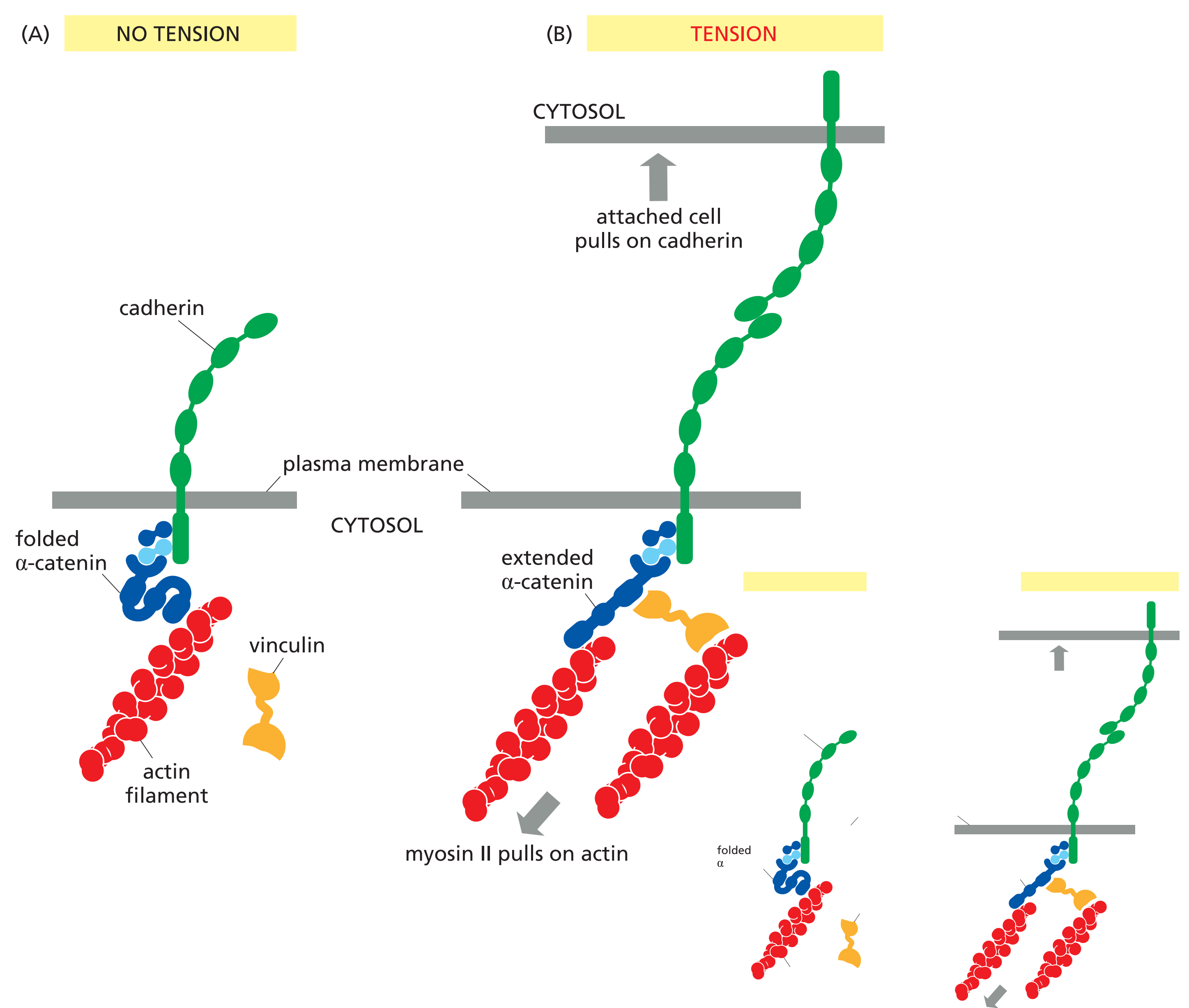

Figure 19–12 Mechanotransduction in an adherens junction. (A) Cell–cell junctions are able to sense increased tension and respond by strengthening their actin linkages. Tension sensing is thought to depend in part on α-catenin (see Figure 19–10). (B) When actin filaments are pulled from within the cell by non-muscle myosin II, the resulting force unfolds a domain in α-catenin, thereby exposing an otherwise hidden binding site for the adaptor protein vinculin. Vinculin then promotes additional actin recruitment, strengthening the linkages between the junction and the cytoskeleton.

These inteactions organize the cytoskeleton and cells

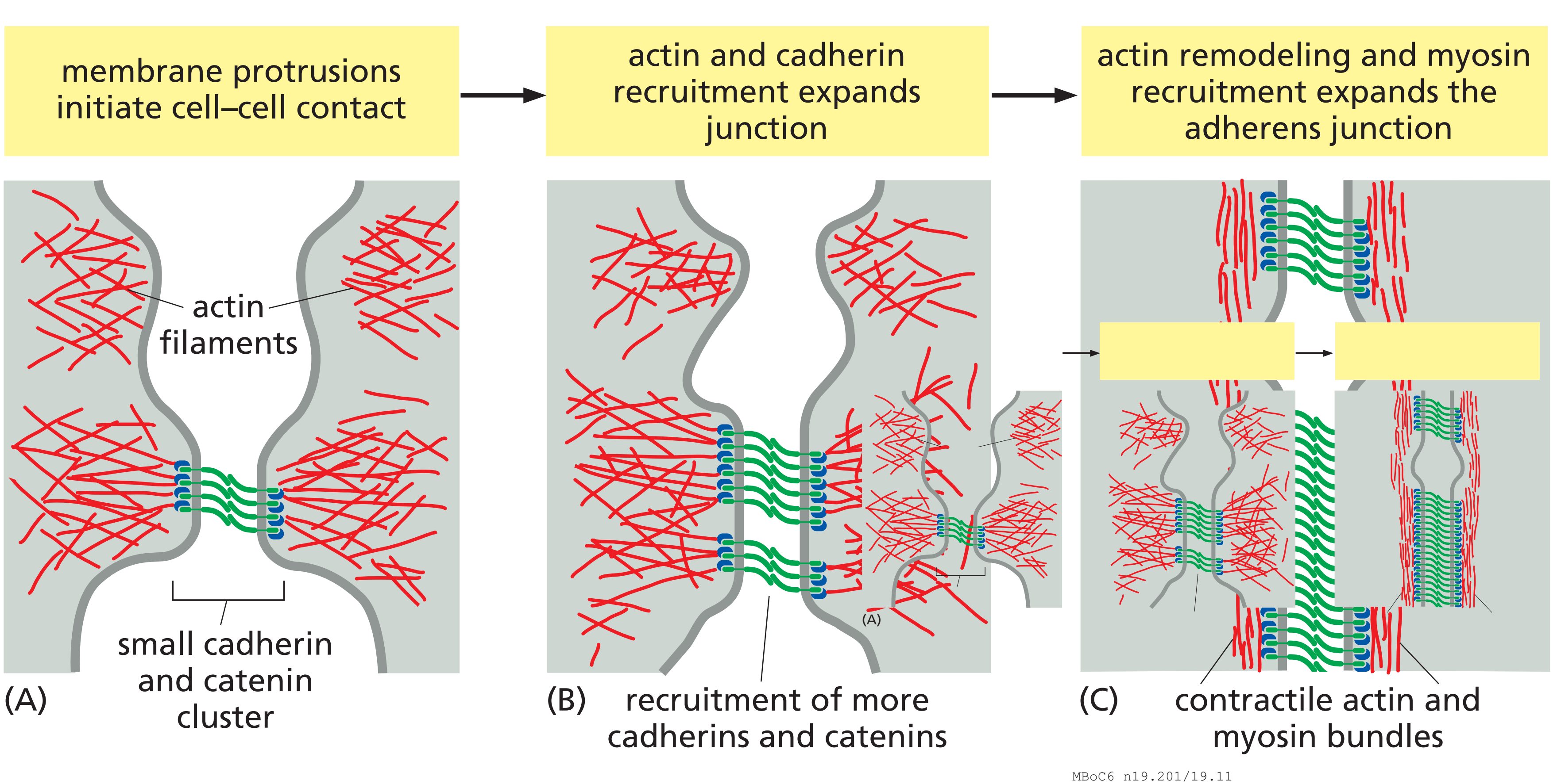

Figure 19–11 Assembly of an adherens junction. (A) Assembly begins when two unattached epithelial cell precursors explore their surroundings with membrane protrusions, generated by local nucleation of actin networks. When the cells make contact, small cadherin and catenin clusters take shape at the contact sites and associate with actin, leading to activation of the small monomeric GTPase Rac (not shown), an important actin regulator (see Figure 16–85). (B) Rac promotes additional actin protrusions in the vicinity, expanding the size of the contact zone and thereby promoting further recruitment of cadherins and their associated catenin proteins. (C) Eventually, Rac is inactivated and replaced by the related GTPase Rho (not shown), which shifts actin remodeling toward the assembly of linear, contractile filament bundles. Rho also promotes the assembly of myosin II filaments that associate with bundles of actin filaments to generate contractile activity. This contractile activity generates tension that stimulates further actin recruitment and expansion of the junction, in part through the mechanisms illustrated in Figure 19–12.

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Parallel organization of the cytoskeleton in neighboring cells

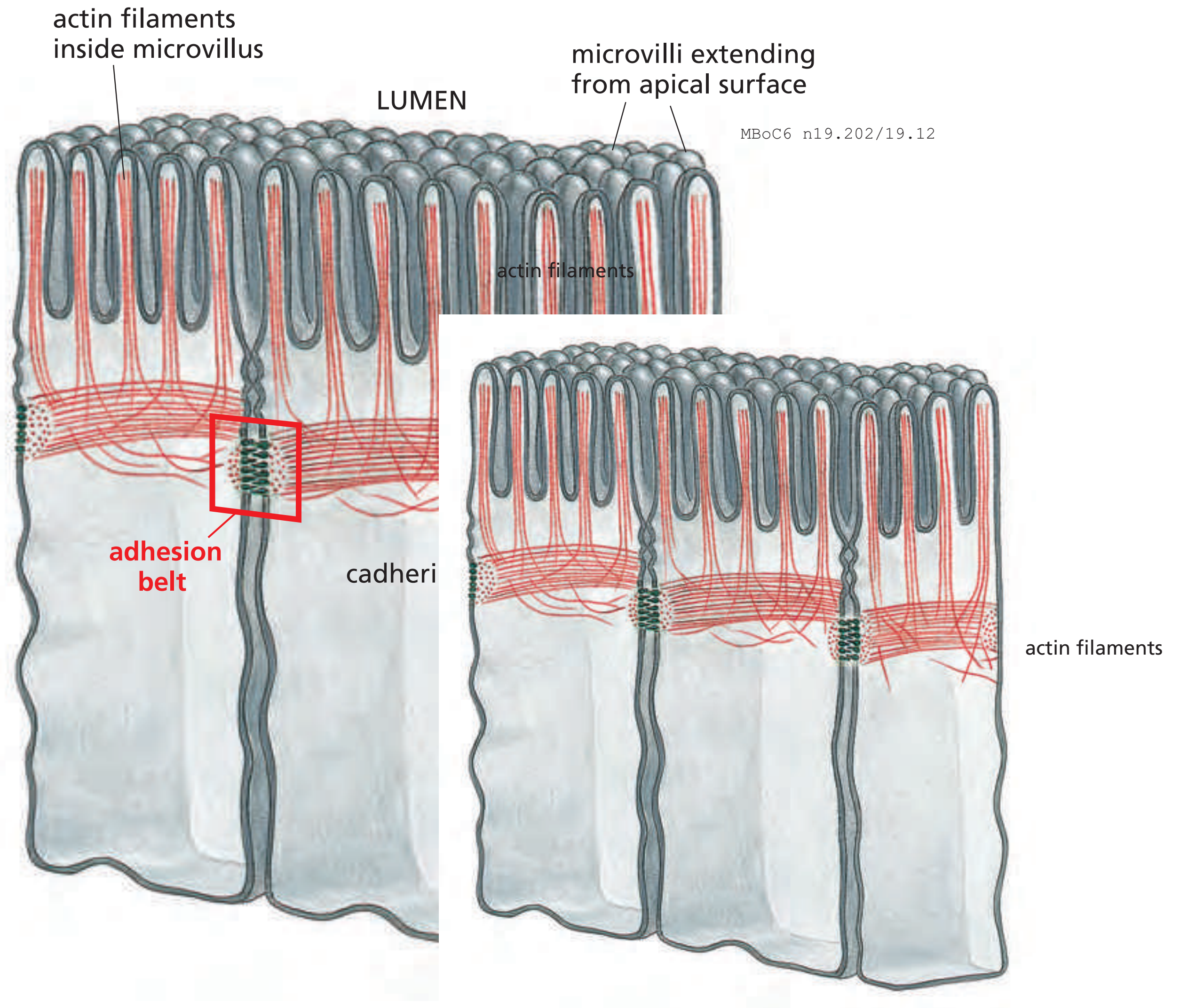

Figure 19–13 Adherens junctions between epithelial cells in the small intestine. These cells are specialized for absorption of nutrients; at their apex, facing the lumen of the gut, they have many microvilli (protrusions that increase the absorptive surface area). The adherens junction takes the form 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 proteins to cadherins, which bind to cadherins on the adjacent cell. In this way, the actin filament bundles in adjacent cells are tied together. For clarity, this drawing does not show most of the other cell–cell and cell–matrix junctions of epithelial cells (see Figure 19–2).

These interactions are important for patterning and development

sheet of epithelial cells

adhesion belt with
associated actin filaments

INVAGINATION OF EPITHELIAL SHEET CAUSED
BY AN ORGANIZED TIGHTENING OF
ADHESION BELTS IN SELECTED REGIONS OF
CELL SHEET

EPITHELIAL TUBE PINCHES OFF
FROM OVERLYING SHEET OF CELLS

sheet of epithelial cells

epithelial
tube

TION OF EPITHELIAL SHEET CAUSED

adhesion belt with
associated actin filaments

Figure 19–14 **The folding of an epithelial sheet to form an epithelial tube.** The oriented contraction of the bundles of actin and myosin 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 19–8).

Classic cad unmixing

Artificially mixed cells of a developing frog embryo

There is a selective re-association according to the cells' origin

mesoderm cells

neural plate cells

ectoderm cells

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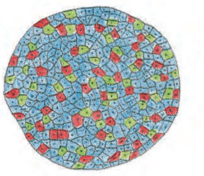
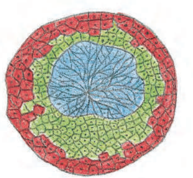
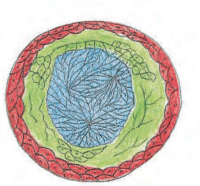
Figure 19-7 Molecular Biology of the Cell 6e (© Garland Science 2015)

This selective cell-cell associ
of cadherin interactions, and







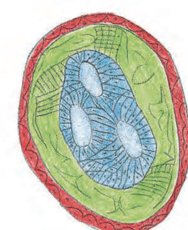


Figure 19–7 Sorting out. Cells from different layers of an early amphibian embryo will sort out according to their origins. In the classical experiment shown here, mesoderm cells *(green)*, neural plate cells *(blue)*, and epidermal cells *(red)* have been disaggregated and then reaggregated in a random 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.)

Cadherins in development

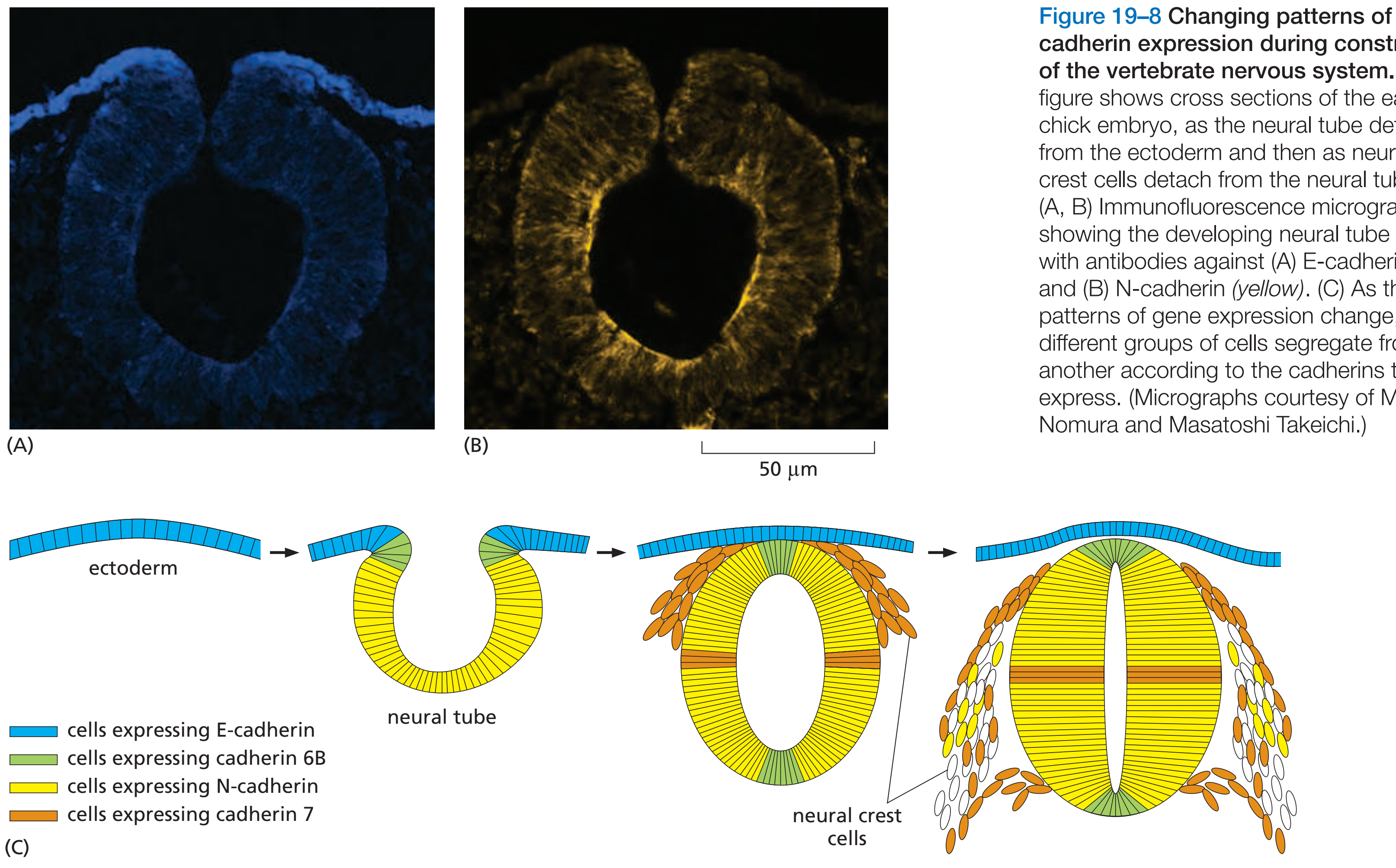
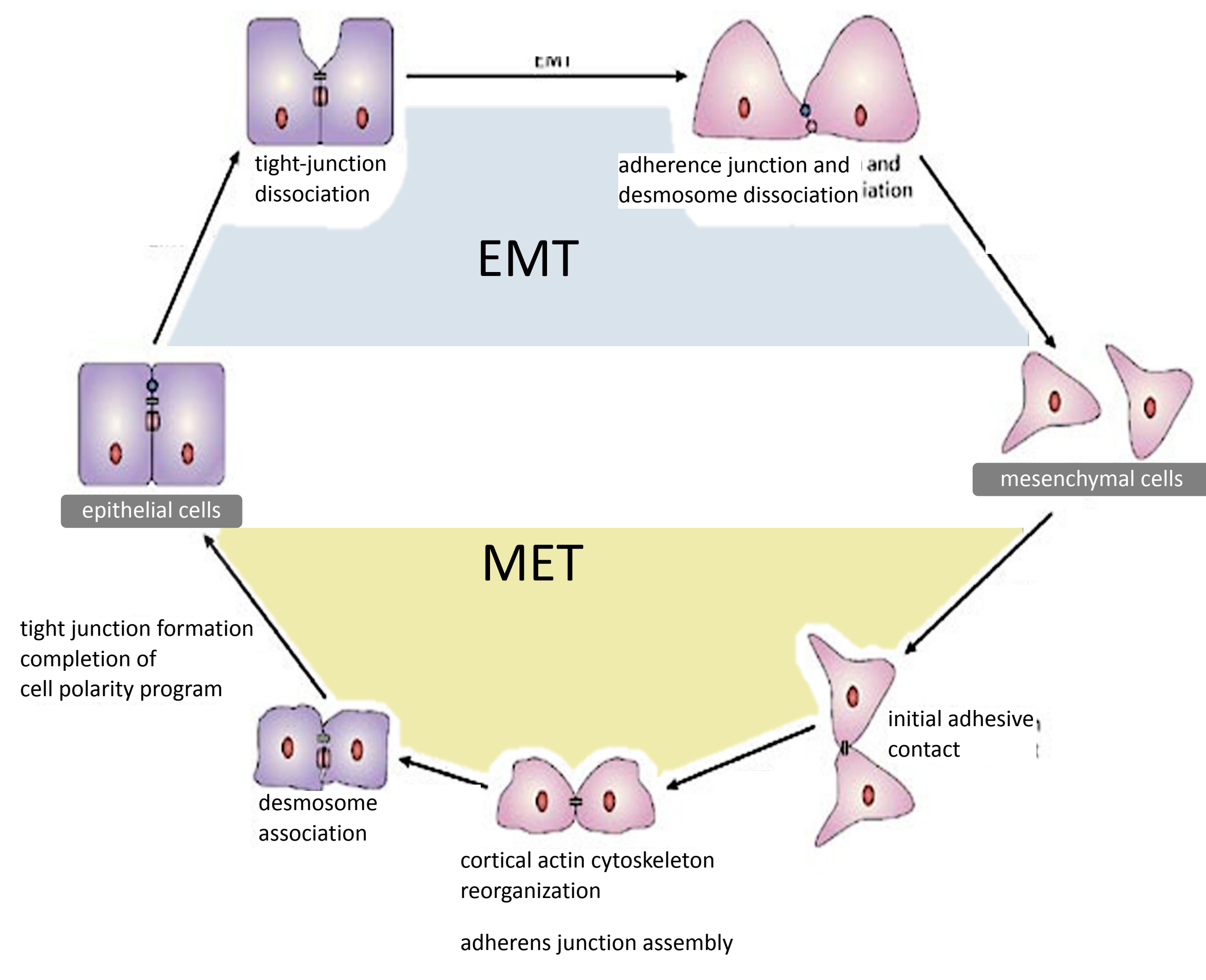


Figure 19–8 Changing patterns of cadherin expression during construction of the vertebrate nervous system. The figure shows cross sections of the early chick embryo, as the neural tube detaches from the ectoderm and then as neural crest cells detach from the neural tube. (A, B) Immunofluorescence micrographs showing the developing neural tube labeled with antibodies against (A) E-cadherin *(blue)* and (B) N-cadherin *(yellow)*. (C) As the patterns of gene expression change, the different groups of cells segregate from one another according to the cadherins they express. (Micrographs courtesy of Miwako Nomura and Masatoshi Takeichi.)

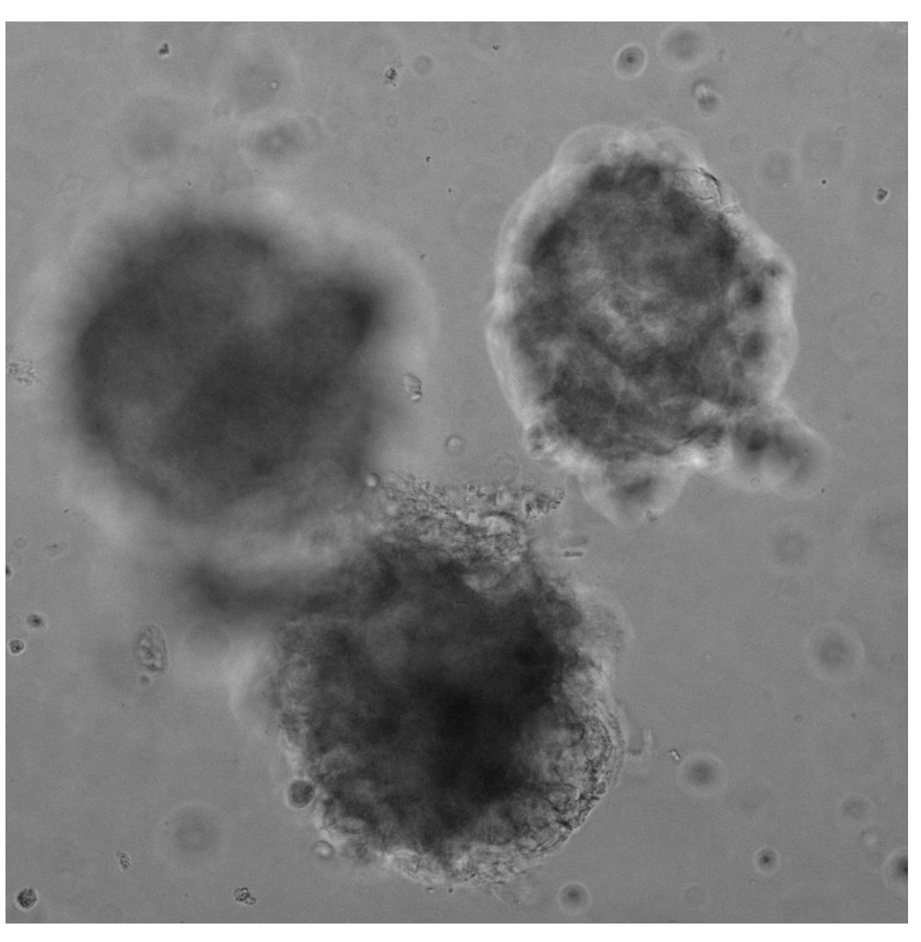
cell expressing

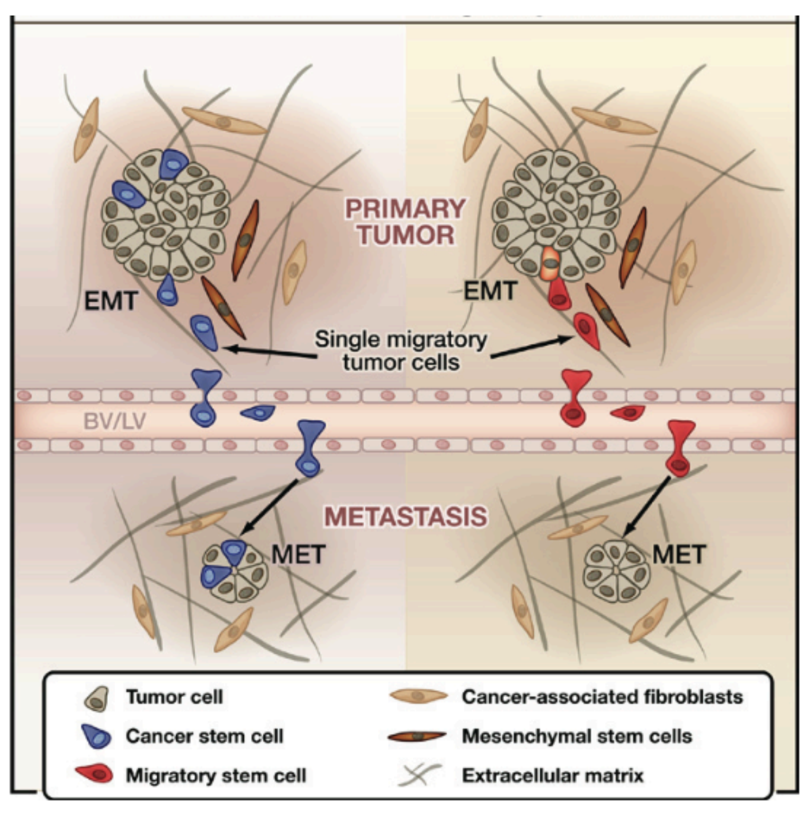
Transitions between epithelial and mesenchymal phenotypes



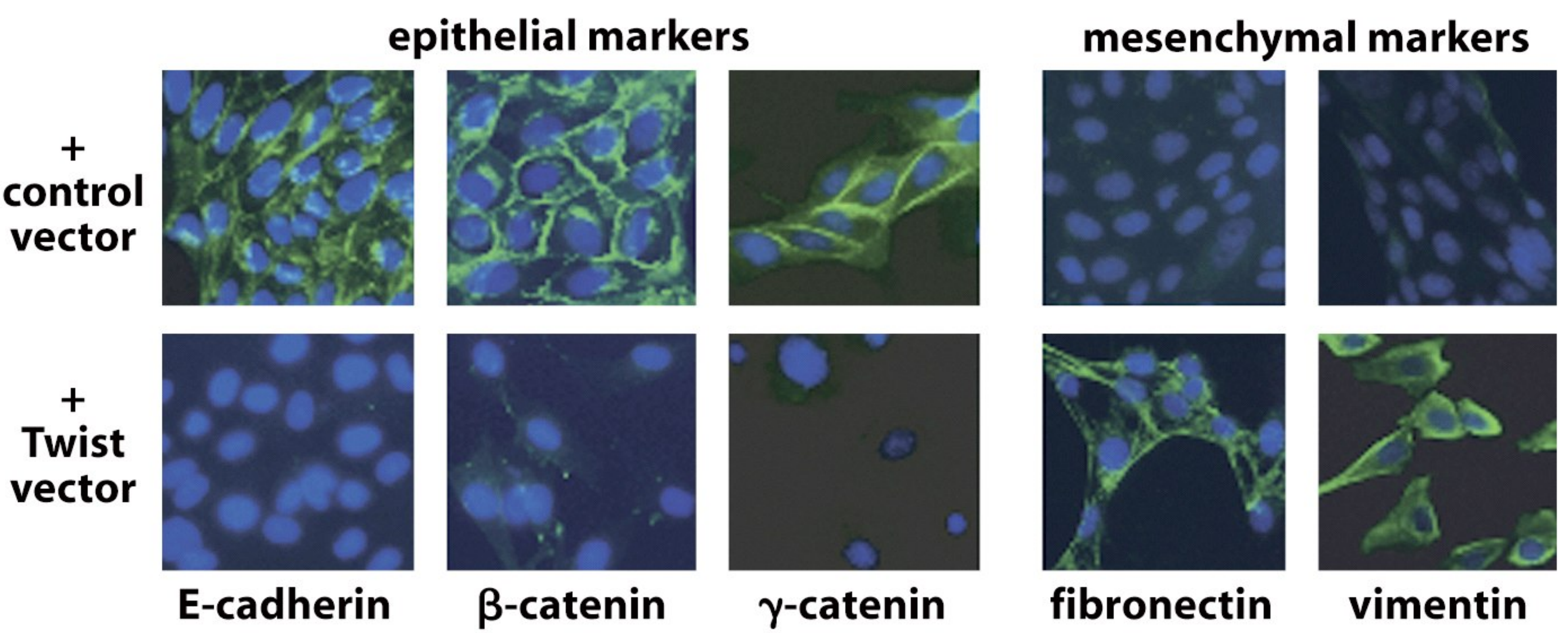
such transitions play crucial roles in embryo development, differentiation of multiple tissues and in tissue repair

EMT is also involved in wound healing and cancer



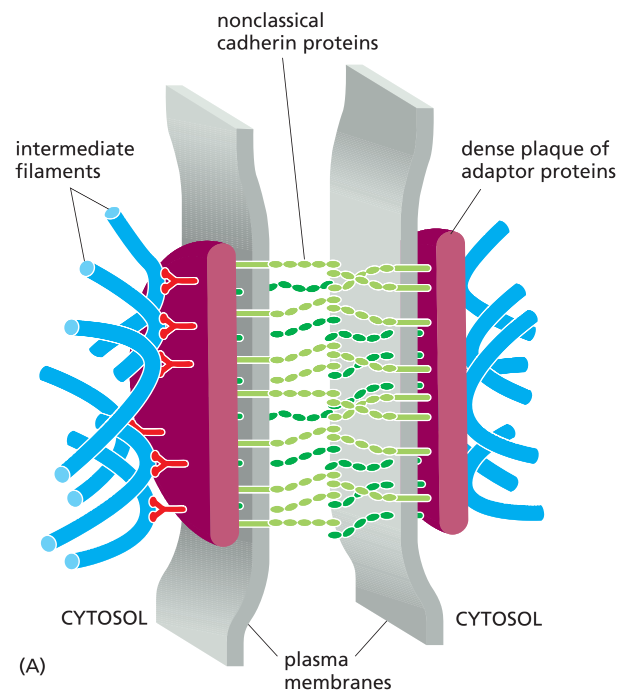


EMT is regulated by transcription factors such as Twist



Snail, Slug and Zeb are other TF's known to induce EMT

Desmosomes



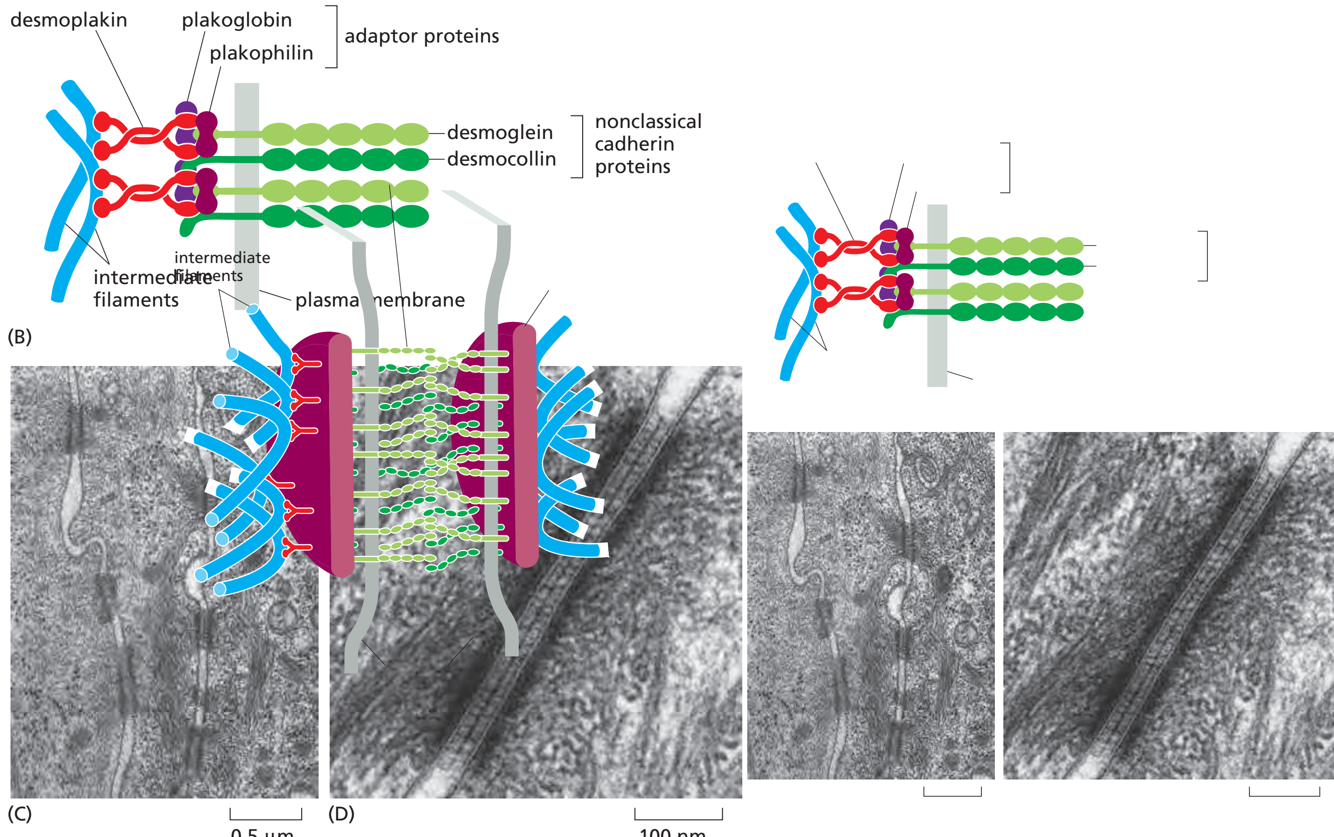


Figure 19–16 Desmosomes. (A) The structural components of a desmosome. On the cytoplasmic surface of each interacting plasma membrane is a dense plaque composed of a mixture of intracellular adaptor proteins. A bundle of keratin intermediate filaments is attached to the surface of each plaque. Transmembrane nonclassical cadherins bind to the plaques and interact through their extracellular domains to hold the adjacent membranes together. (B) Some of the molecular components of a desmosome. Desmoglein and desmocollin are nonclassical cadherins. 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 three 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.)

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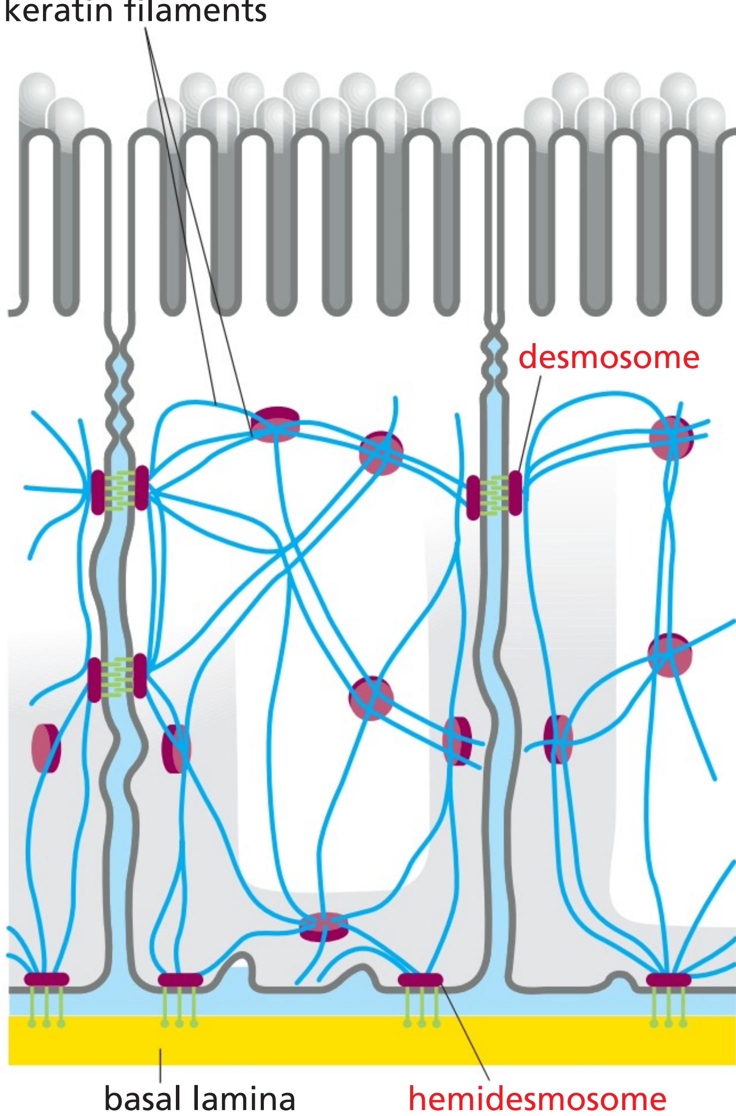
Interaction with intermediate filaments



Desmosomes are cell to cell
Hemidesmosomes are cell to extracellular matrix (basal lamina)

These interactions give rigidity!

Figure 19–17 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.

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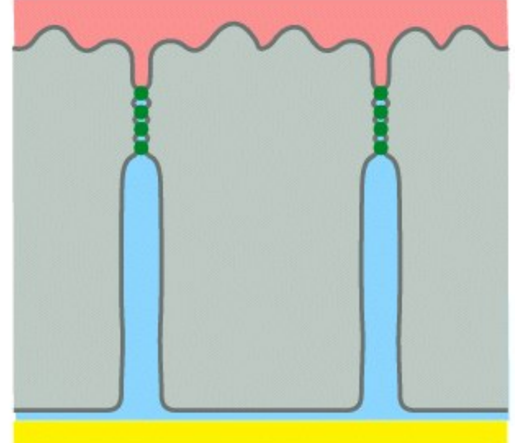
Tight junctions

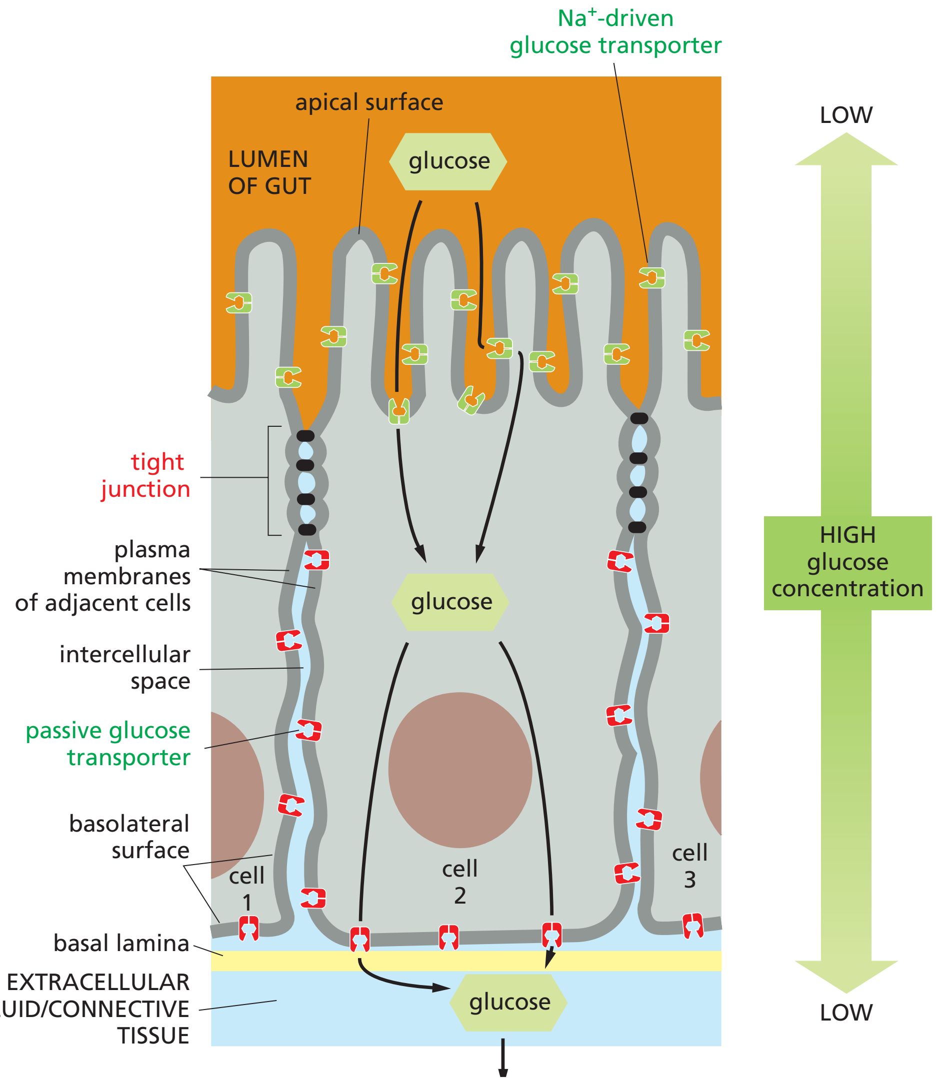


(B) **OCCLUDING JUNCTIONS**

To block inter-cellular passage (impermeable or selectively permeable barrier)

Ex: intestinal epithelial cells – glucose absorption

Tight junctions in the intestine



Tight junctions are everywhre, like the tracts urinary system

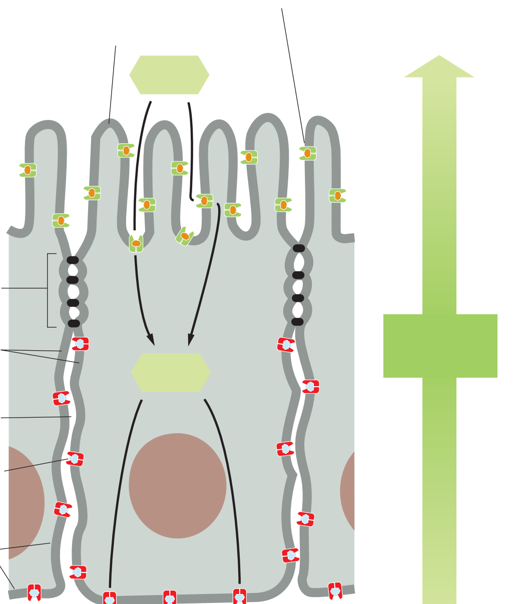


Figure 19–18 The role of tight junctions in transcellular transport. For clarity, only the tight junctions are shown. Transport proteins are confined to different regions of the plasma membrane in epithelial cells of the small intestine. This segregation permits a vectorial transfer of nutrients across the epithelium from the gut lumen to the blood. In the example shown, glucose is actively transported into the cell by Na^+-driven glucose transporters at its apical surface, and it leaves the cell through passive glucose transporters 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 (see Movie 11.2).

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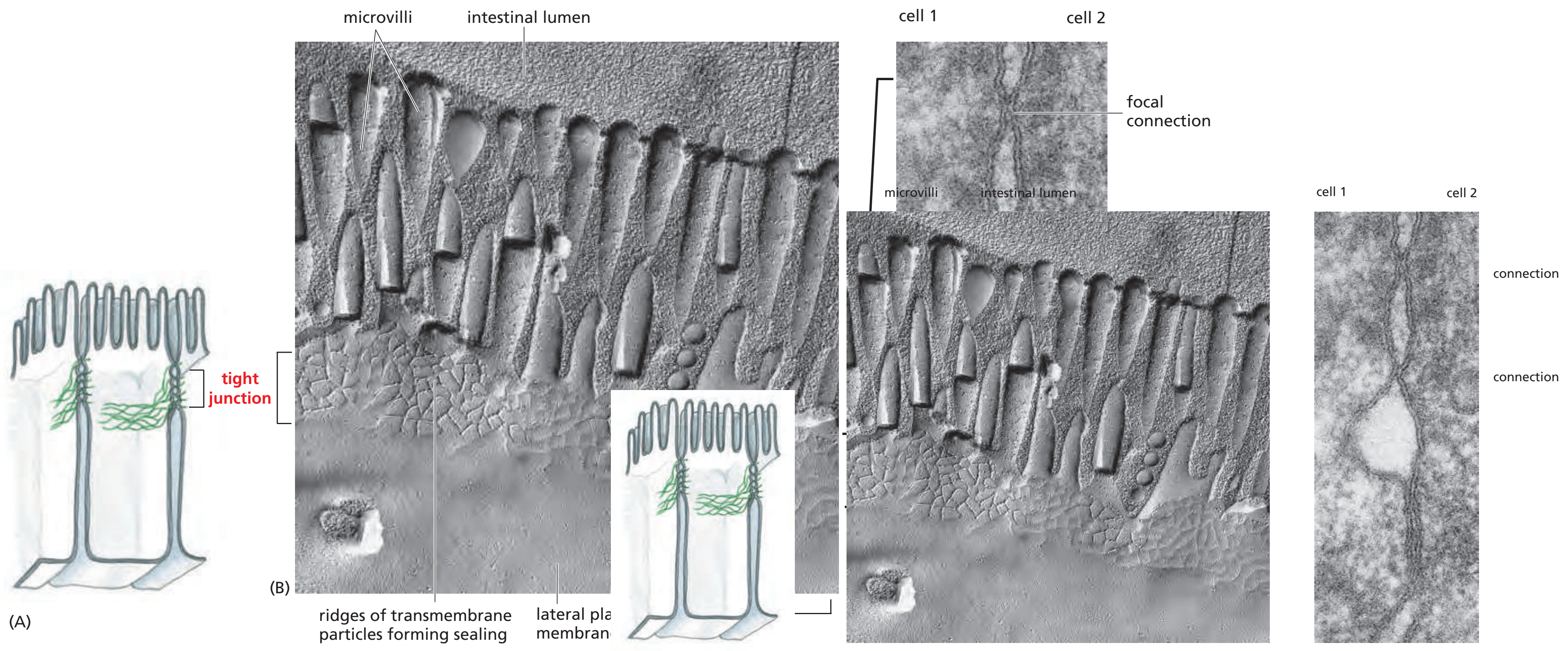

Figure 19–20 The structure of a tight junction between epithelial cells of the small intestine. The junctions are shown (A) schematically, (B) in a freeze-fracture electron micrograph, and (C) in a conventional electron micrograph. In (B), the plane of the micrograph is parallel to the plane of the membrane, and the tight junction appears as a band of branching sealing strands that encircle each cell in the epithelium (see Figure 19–21A). 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.)

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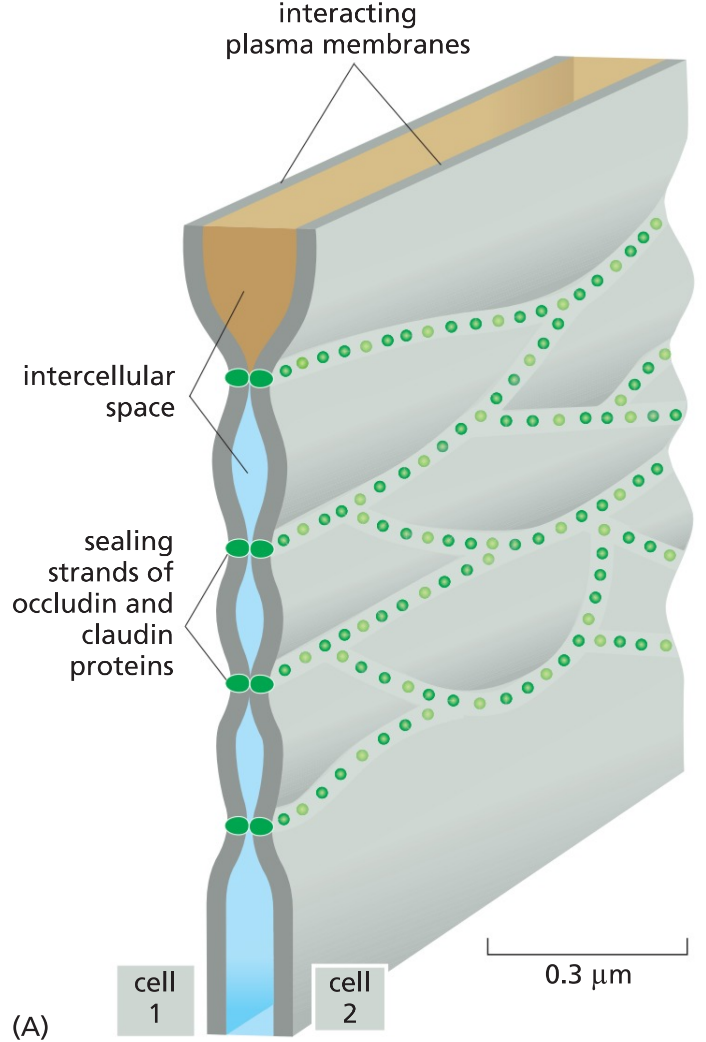
Tight junctions



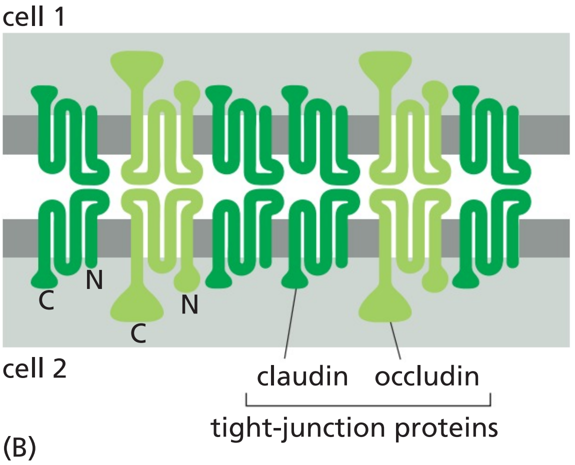
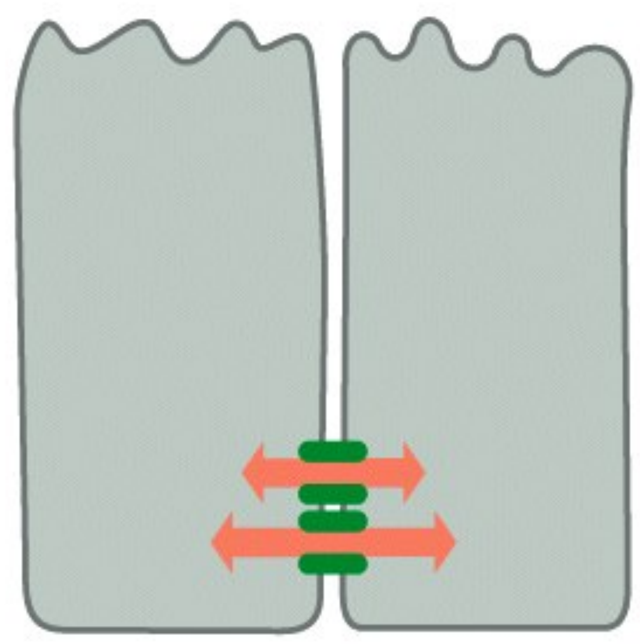


Figure 19–21 **A model of a tight junction.** (A) The sealing strands hold adjacent plasma membranes together. The strands are composed of transmembrane proteins that make contact across the intercellular space and create a seal. (B) The molecular composition of a sealing strand. The major extracellular components of the tight junction are members of a family of proteins with four transmembrane domains. One of these proteins, claudin, is the most important for the assembly and structure of the sealing strands, whereas the related protein occludin has the less critical role of determining junction permeability. The two termini of these proteins are both on the cytoplasmic side of the membrane, where they interact with large scaffolding proteins that organize the sealing strands and link the tight junction to the actin cytoskeleton (not shown here, but see Figure 19–22).

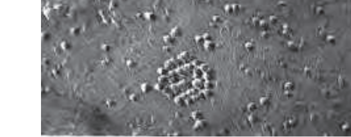
Channel forming or Gap junctions



(C) **CHANNEL-FORMING JUNCTIONS**

To enable communication between two cell interiors

(A)



Gap junction size defines which molecules are shared

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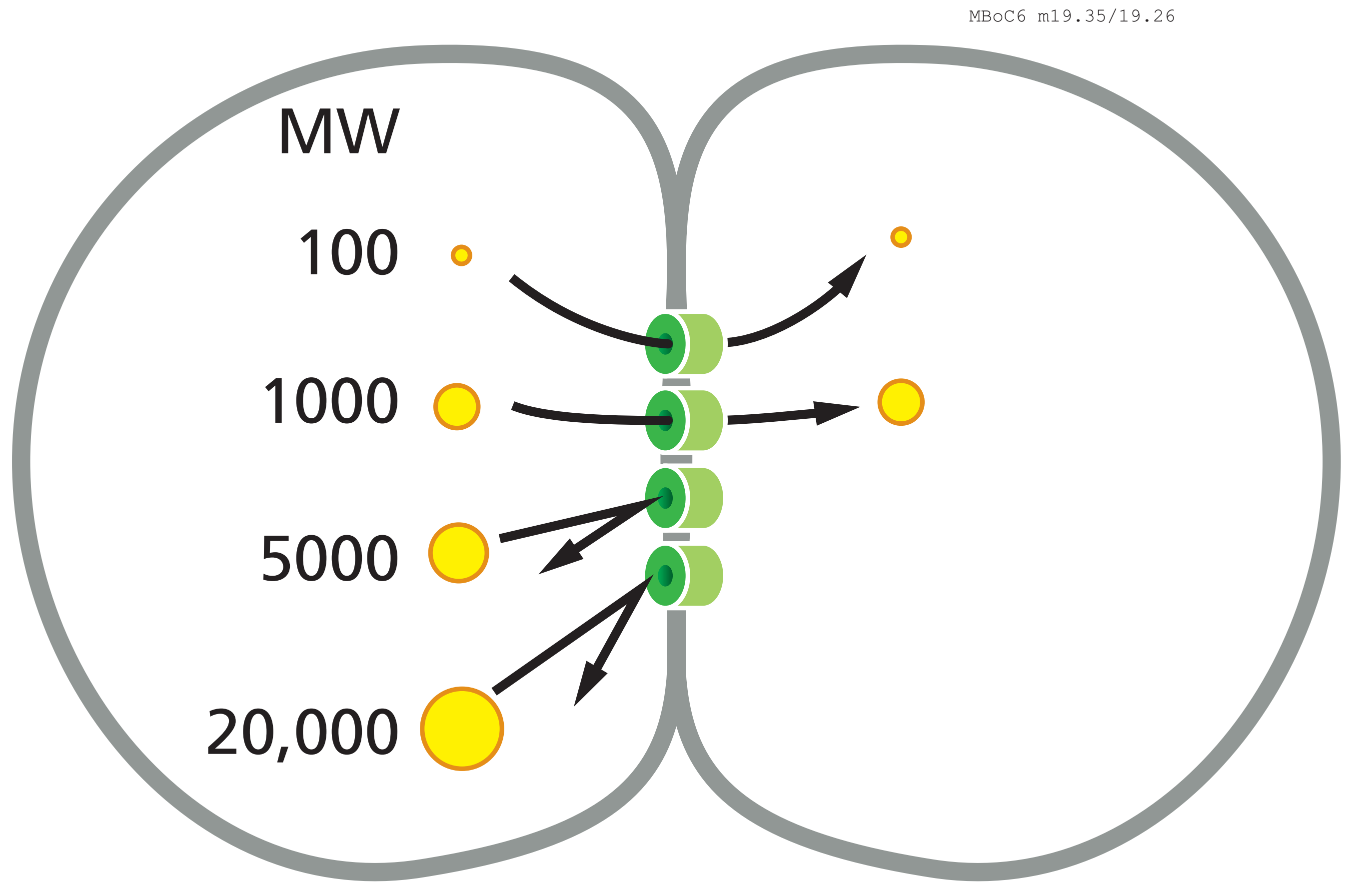

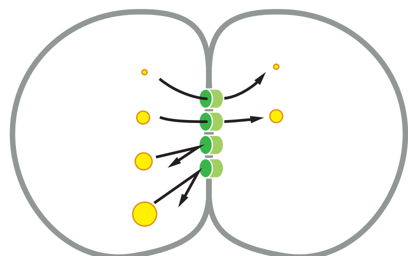

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Figure 19–24 Determining the size of a gap-junction channel. When fluorescent molecules of various sizes are injected into one of two cells coupled by gap junctions, molecules with a molecular weight (MW) of less than about 1000 daltons can pass into the other cell, but larger molecules cannot. Thus, the coupled cells share their small molecules (such as inorganic ions, sugars, amino acids, nucleotides, vitamins, and the intracellular signaling molecules cyclic AMP and inositol trisphosphate) but not their macromolecules (proteins, nucleic acids, and polysaccharides).

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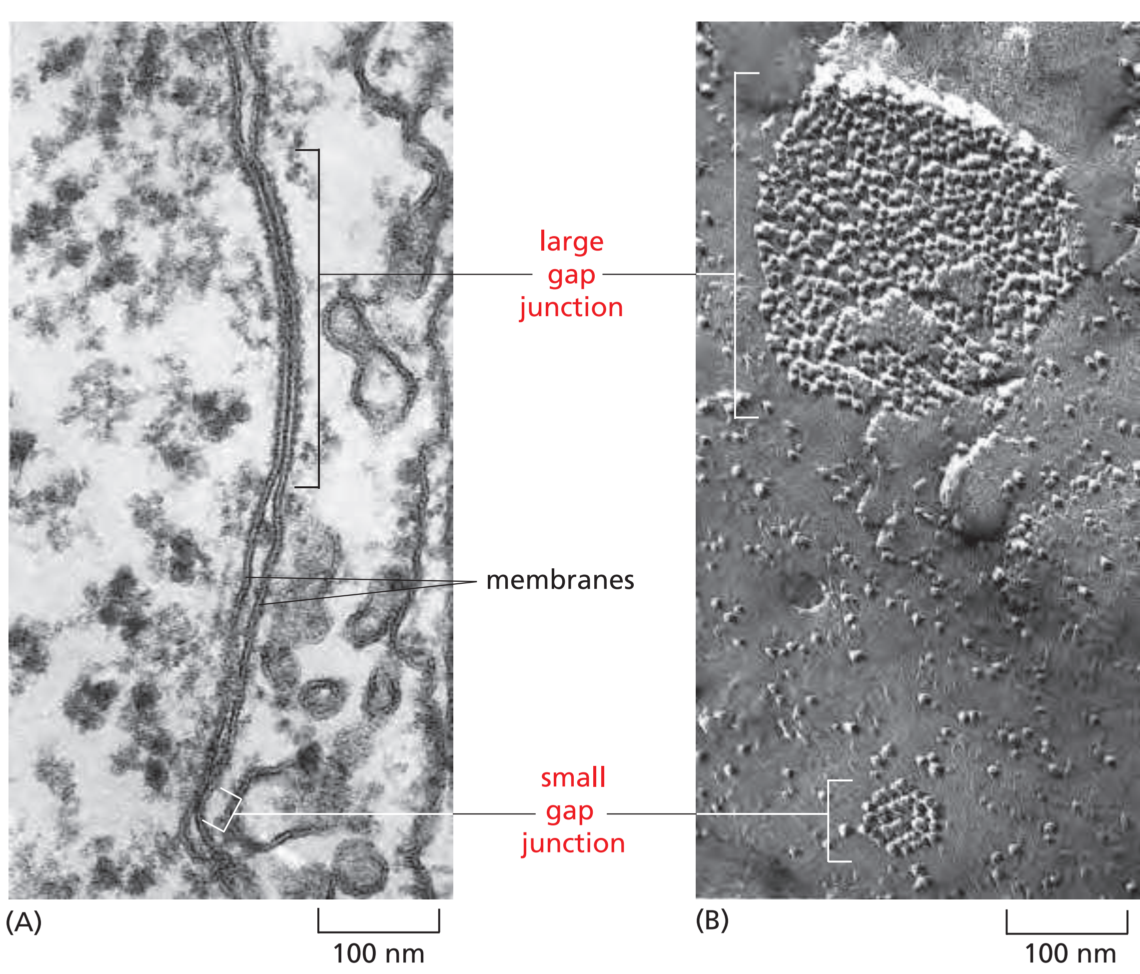


Figure 19–23 Gap junctions as seen in the electron microscope. (A) Thin-section and (B) freeze-fracture electron micrographs of a large and a small gap-junction plaque between fibroblasts in culture. In (B), each gap junction is seen as a cluster of homogeneous intramembrane particles. Each intramembrane particle corresponds to a connexon (see Figure 19–25). (From N.B. Gilula, in Cell Communication [R.P. Cox, ed.], pp. 1–29. New York: Wiley, 1974.)

Gap junction structure

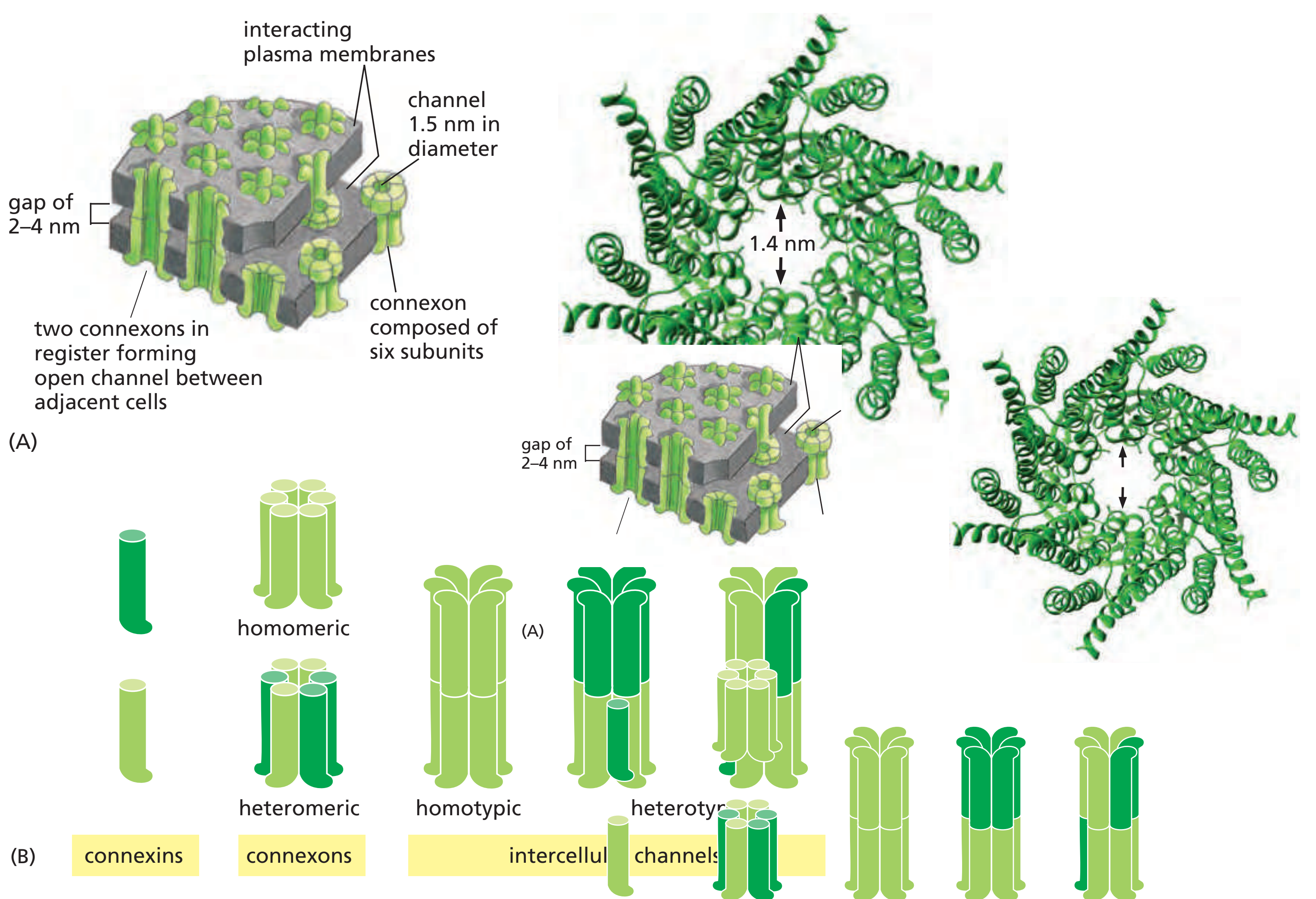
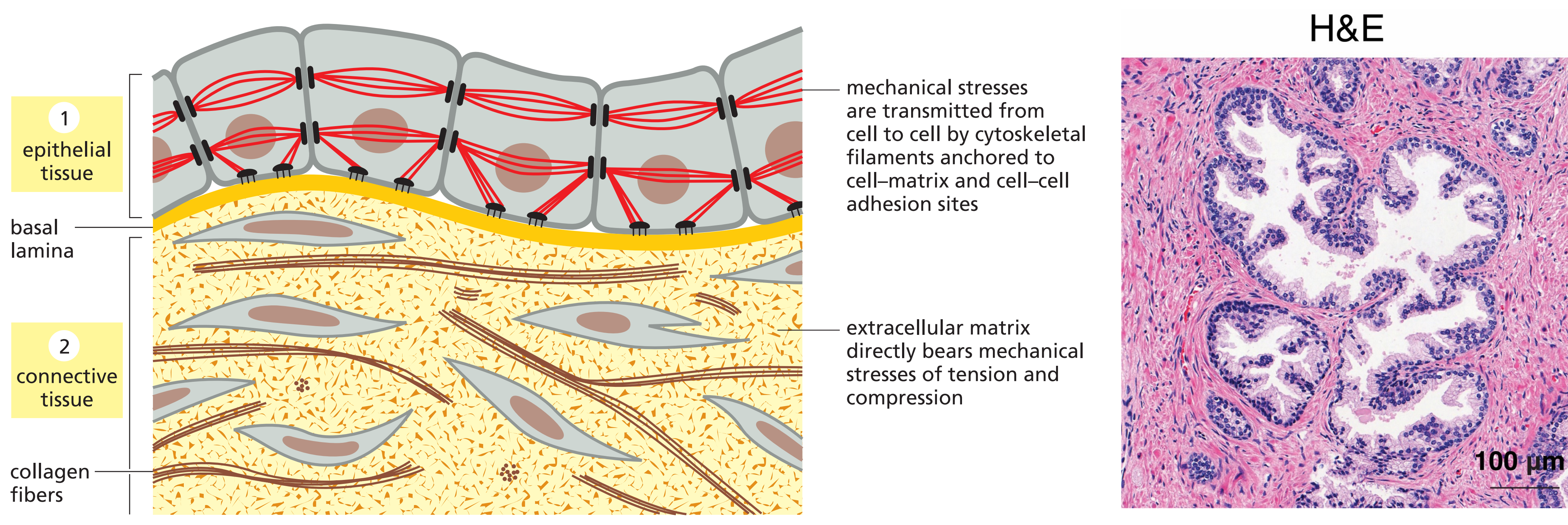


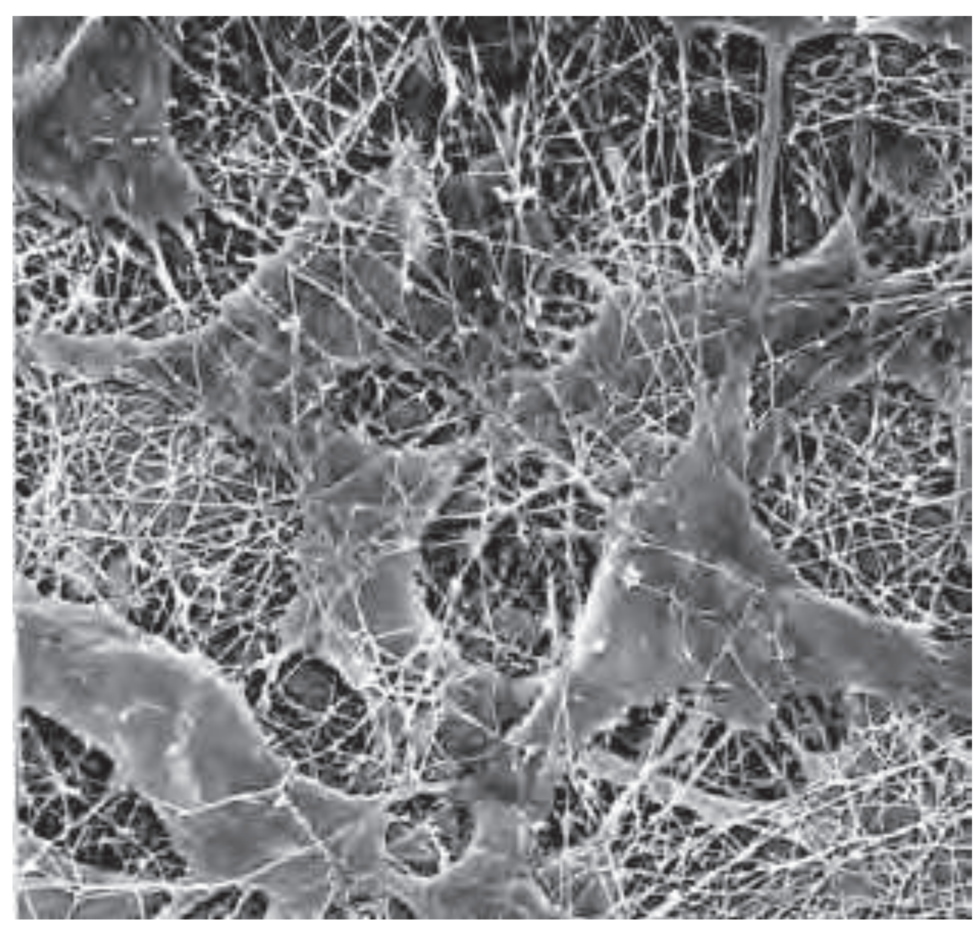
Figure 19–25 **Gap junctions.** (A) A drawing of the interacting plasma membranes of two adjacent cells connected by gap junctions. Each lipid bilayer is shown as a pair of *red* sheets. Protein assemblies called connexons *(green)*, each of which is formed by six connexin subunits, penetrate the apposed lipid bilayers. 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. (C) The high-resolution structure of a homomeric gap-junction channel, determined by x-ray crystallography of human connexin 26. In this view, we are looking down on the pore, formed from six connexin subunits. The structure illustrates the general features of the channel and suggests a pore size of about 1.4 nm, as predicted from studies of gap-junction permeability with molecules of various sizes (see Figure 19–24). (PDB code: 2ZW3.)

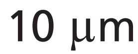
The extracellular matrix

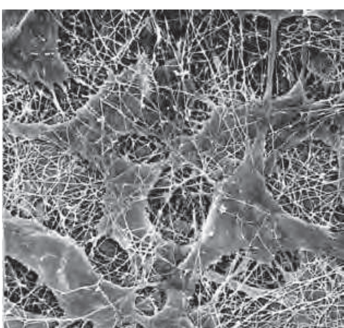
Our cells are organized in a complex system



Looks a little chaotic?







μ

Figure 19–30 **Fibroblasts in connective tissue.** This scanning electron micrograph shows tissue from the cornea of a rat. The extracellular matrix surrounding the fibroblasts is here composed largely of collagen fibrils. The glycoproteins, hyaluronan, and proteoglycans, which normally form a hydrated gel filling the interstices of the fibrous network, have been removed by enzyme and acid treatment. (Courtesy of T. Nishida.)

Major componenst of the ECM

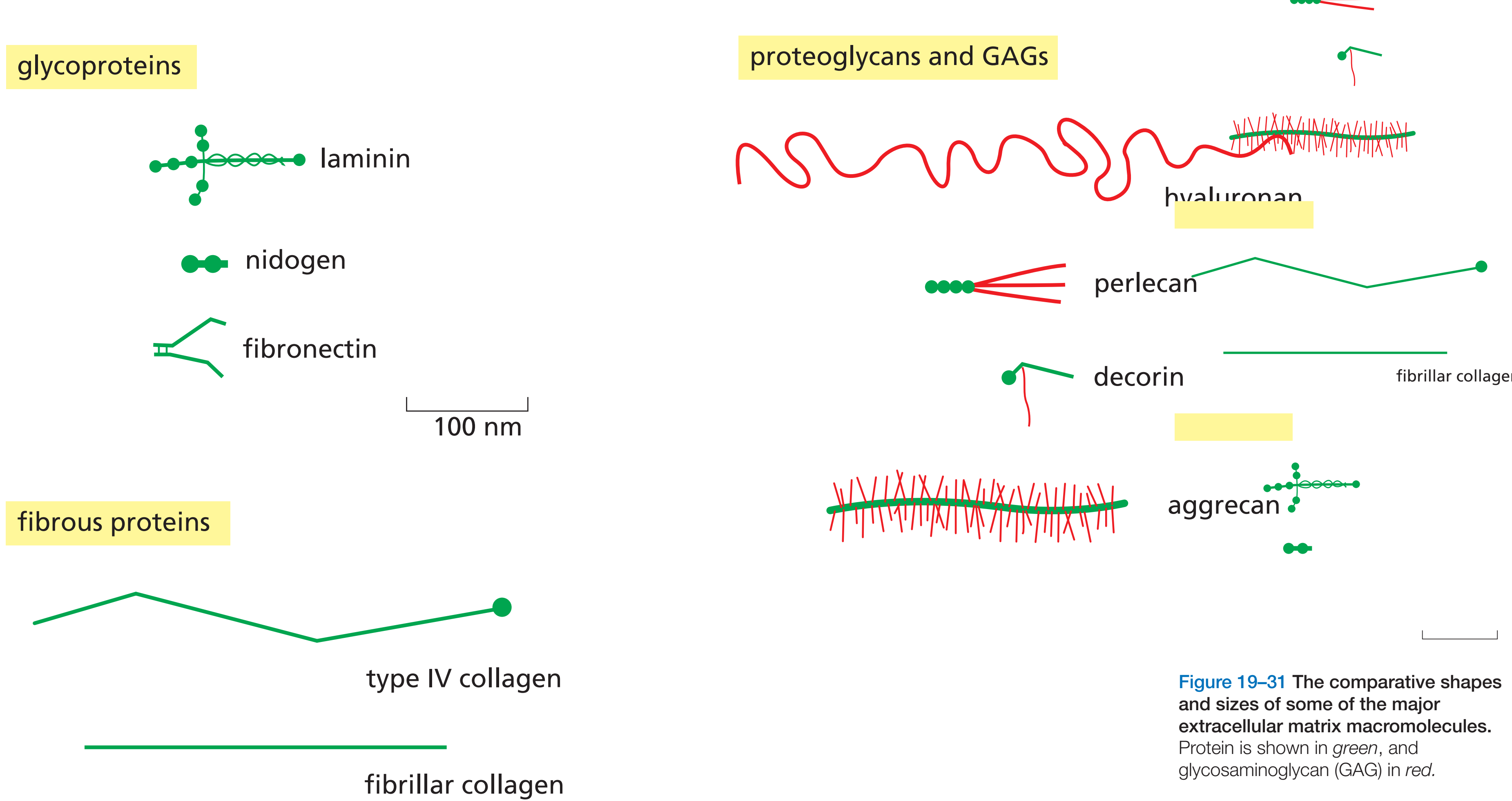


Figure 19–31 The comparative shapes and sizes of some of the major extracellular matrix macromolecules. Protein is shown in *green*, and glycosaminoglycan (GAG) in *red*.

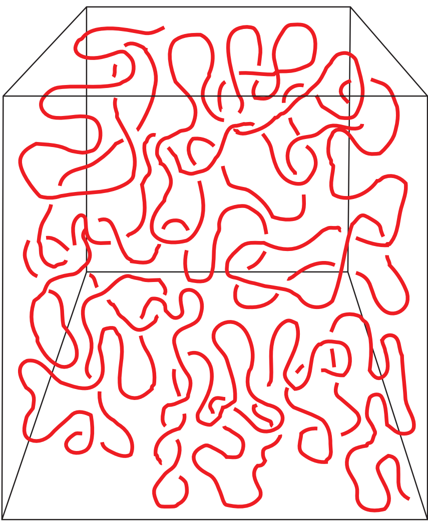
The sizes of ECM proteins are highly variable

globular protein (MW 50,000)

glycogen (MW ~400,000)

spectrin (MW 460,000)

collagen (MW 290,000)



hyaluronan (MW 8 x 10^6)

300 nm

globular protein (MW 50,000)

glycogen (MW ~400,000)

spectrin (MW 460,000)

collagen (MW 290,000)

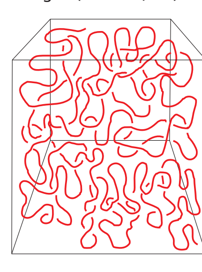


Figure 19–33 The relative dimensions and volumes occupied by various macromolecules. Several proteins, a glycogen granule, and a single hydrated molecule of hyaluronan are shown.

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Glycosylation is important for the function of many ECM proteins

fibrillar collagen

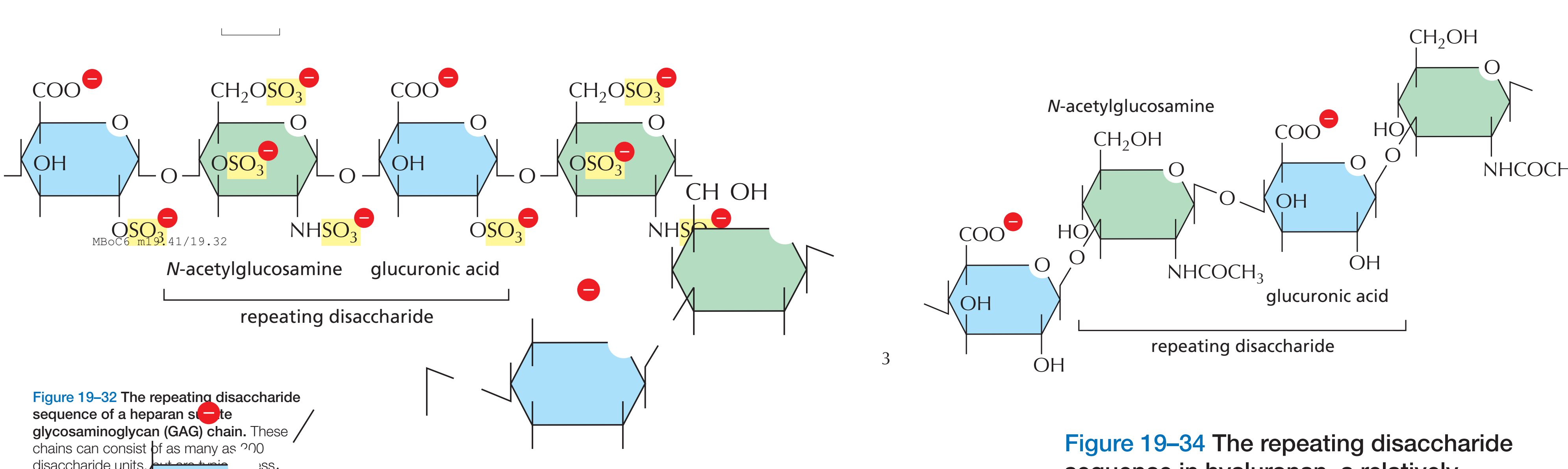


Figure 19–32 The repeating disaccharide sequence of a heparan sulfate glycosaminoglycan (GAG) chain. These chains can consist of as many as 200 disaccharide units, but are typically less than half that size. ... density of negative charge ... due to the presence of ... sulfate groups. The molecule ... with its maximal number of sulfate groups. *In vivo,* the proportion of sulfated and nonsulfated groups is variable. Heparin typically has >70% sulfation, while heparan sulfate has <50%.

Figure 19–34 The repeating disaccharide sequence in hyaluronan, a relatively simple GAG. This ubiquitous molecule in vertebrates consists of a single long chain of up to 25,000 sugar monomers. Note the absence of sulfate groups.

Sulfation can be variable between ECMs

GAG are added on a serine

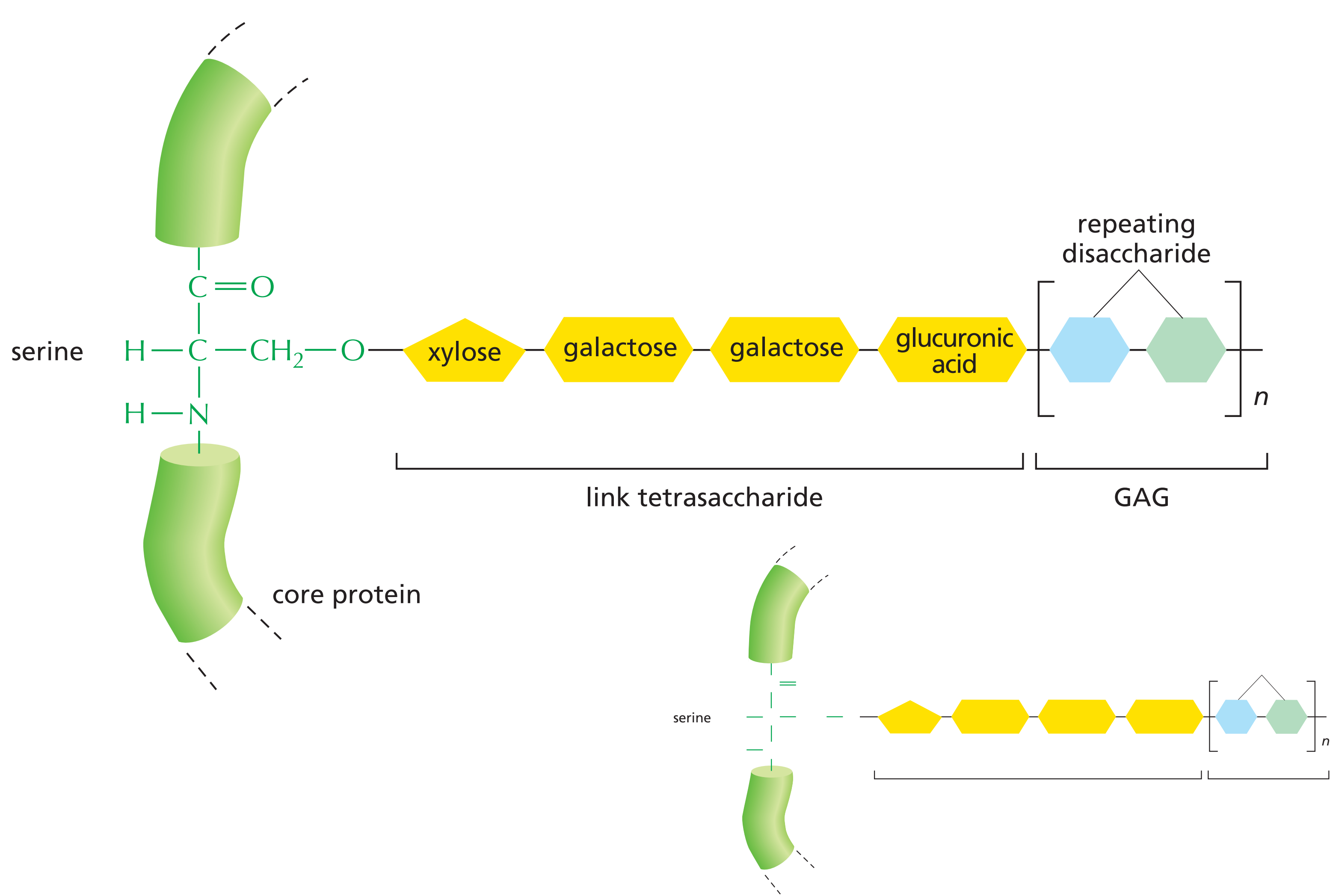


Figure 19–35 The linkage between a GAG chain and its core protein in a proteoglycan molecule. A specific link tetrasaccharide is first assembled on a serine side chain. The rest of the GAG chain, consisting mainly of a repeating disaccharide unit, is then synthesized, with one sugar being added at a time. In chondroitin sulfate, the disaccharide is composed of D-glucuronic acid and *N*-acetyl-D-galactosamine; in heparan sulfate, it is either D-glucuronic acid or L-iduronic acid and *N*-acetyl-D-glucosamine; in keratan sulfate, it is D-galactose and *N*-acetyl-D-glucosamine.

Glycosylation is variable in size and absolute number

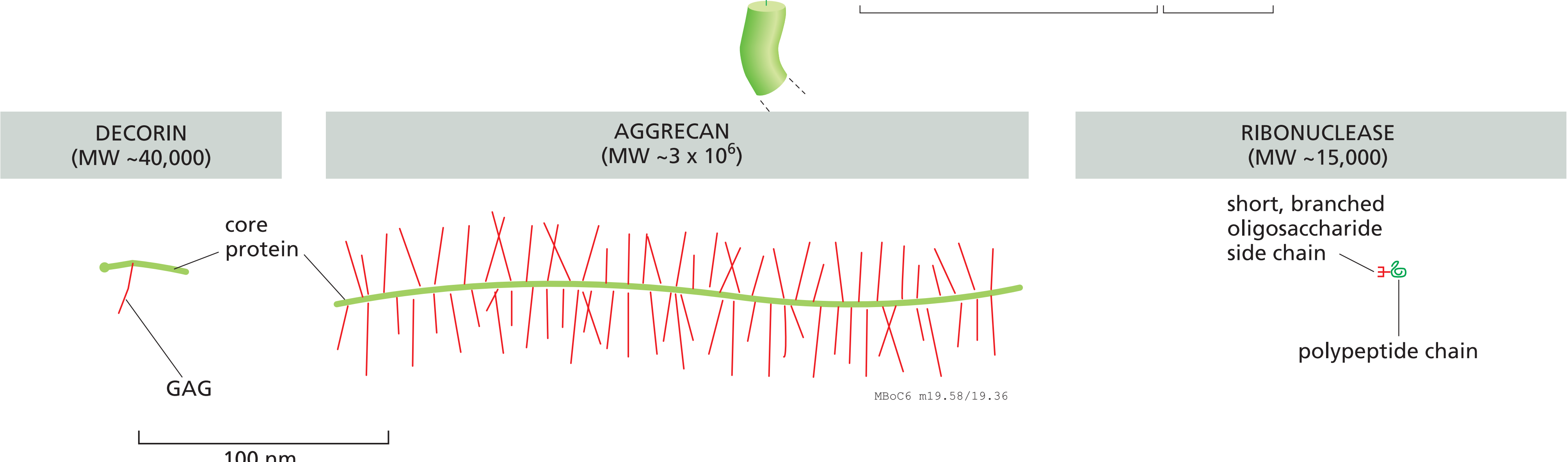
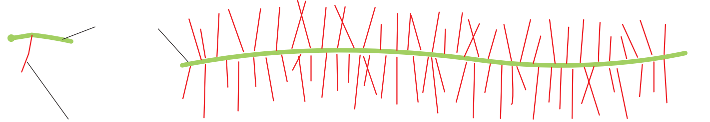
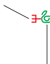


Figure 19–36 Examples of a small (decorin) and a large (aggrecan) proteoglycan found in the extracellular matrix. The figure compares these two proteoglycans with a typical secreted glycoprotein molecule, pancreatic ribonuclease B. All three are drawn to scale. The core proteins of both aggrecan and decorin contain oligosaccharide chains as well as the GAG chains, but these are not shown. Aggrecan typically consists of about 100 chondroitin sulfate chains and about 30 keratan sulfate chains linked to a serine-rich core protein of almost 3000 amino acids. Decorin "decorates" the surface of collagen fibrils, hence its name.

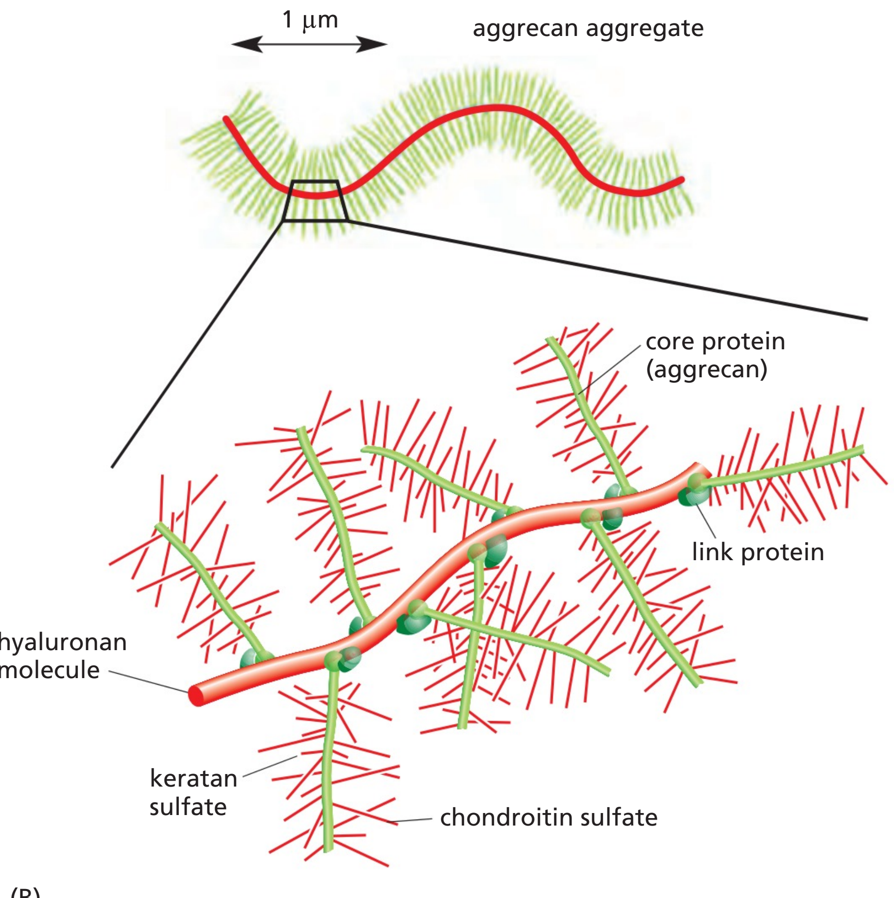




Aggrecan

(A)

1 μm



(B)

Figure 19–37 An aggrecan aggregate from fetal bovine cartilage. (A) An electron micrograph of an aggrecan aggregate shadowed with platinum. Many free aggrecan molecules are also visible. (B) A drawing of the giant aggrecan aggregate shown in (A). It consists of about 100 aggrecan monomers (each like the one shown in Figure 19–36) noncovalently bound through the N-terminal domain of the core protein to a single hyaluronan chain. A link protein binds both to the core protein of the proteoglycan and to the hyaluronan chain, thereby stabilizing the aggregate. The link proteins are members of a family of hyaluronan-binding proteins, some of which are cell-surface proteins. The molecular mass of such a complex can be 10^8 daltons or more, and it occupies a volume equivalent to that of a bacterium, which is about 2×10^{-12} cm^3. (A, courtesy of Lawrence Rosenberg.)

Collagens

Collagens

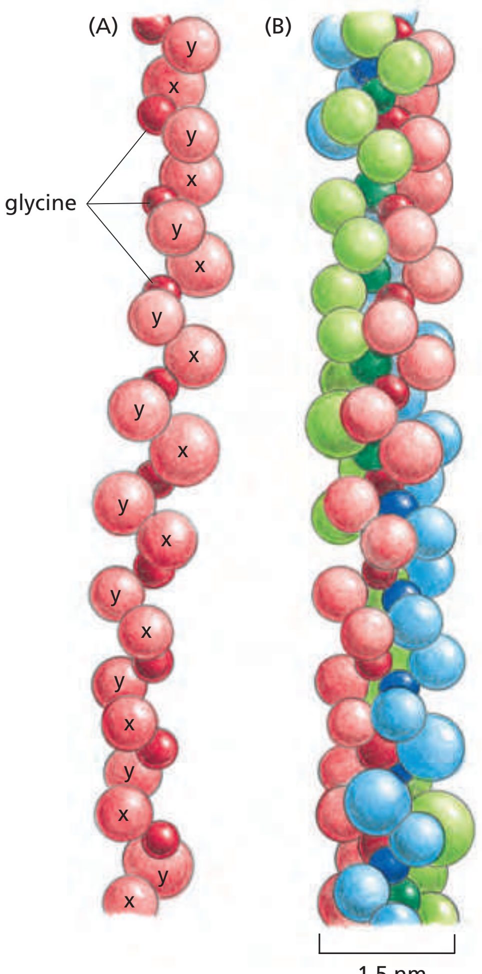
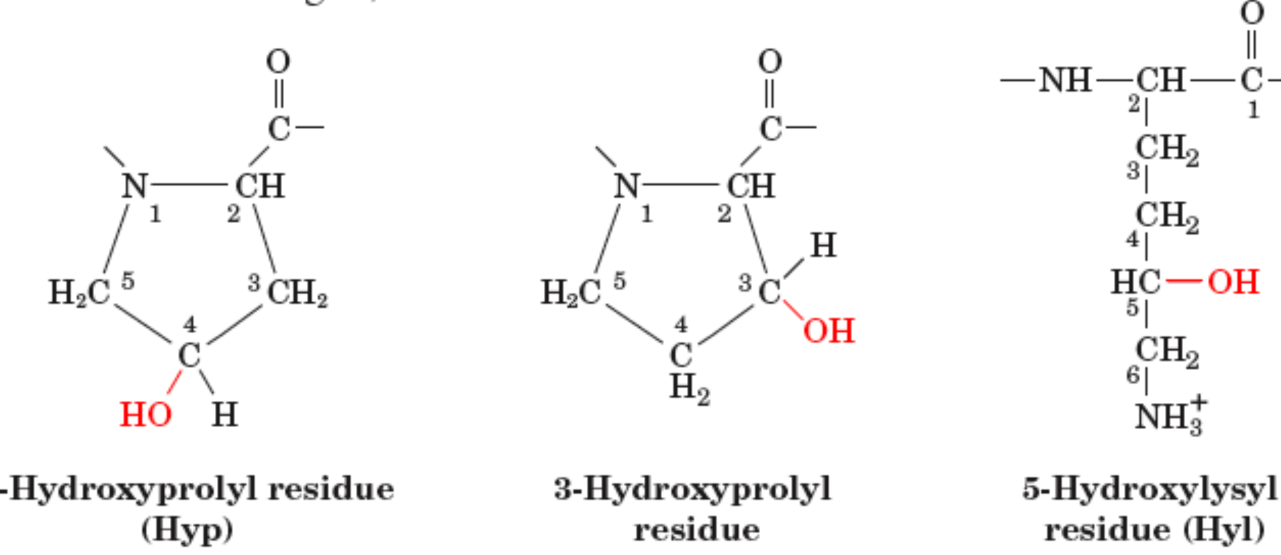


Figure 19–39 The structure of a typical collagen molecule. (A) A model o part of a single collagen α chain, in which each amino acid is represented by a sphere. The chain is about 1000 amino acids long. It is arranged as a left-handed helix, with three amino acids per turn and with glycine as every third amino acid. Therefore, an α chain is composed of a series of triplet Gly-X-Y sequences, in which X and Y can be any amino acid (although X is common proline and Y is commonly hydroxyproline, a form of proline that is chemicall modified during collagen synthesis in the cell). (B) A model of part of a collagen molecule, in which three α chains, each shown in a different color, are wrapped around one another to form a triple-stranded helical rod. Glycin is the only amino acid small enough to occupy the crowded interior of the triple helix. Only a short length of the molecule is shown; the entire molecule is 300 nm long. (From a model by B.L. Trus.)

Collagen amino acids

Collagen has a distinctive amino acid composition: Nearly one-third of its residues are Gly; another 15 to 30% of its residues are Pro and **4-hydroxyprolyl (Hyp). 3-Hydroxyprolyl** and **5-hydroxylysyl (Hyl)** residues also occur in collagen, but in smaller amounts.



4-Hydroxyprolyl residue (Hyp)

3-Hydroxyprolyl residue

5-Hydroxylysyl residue (Hyl)

These hydroxylation reactions are vitamin C dependent, it is a co-factor for the enzymes lysyl and prolyl hydroxylase

Connectuve tissue

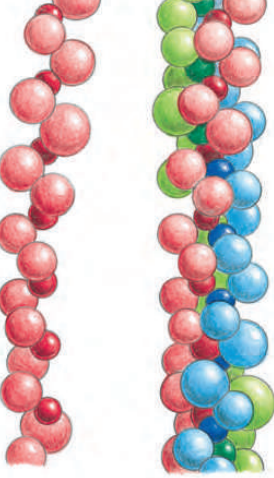


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Figure 19–40 A fibroblast surrounded by collagen fibrils in the connective tissue of embryonic chick skin. In this electron micrograph, the fibrils are organized into bundles that run approximately at right angles to one another. Therefore, some bundles are oriented longitudinally, whereas others are seen in cross section. The collagen fibrils are produced by fibroblasts. (From C. Ploetz, E.I. Zycband and D.E. Birk, *J. Struct. Biol.* 106:73–81, 1991. With permission from Elsevier.)

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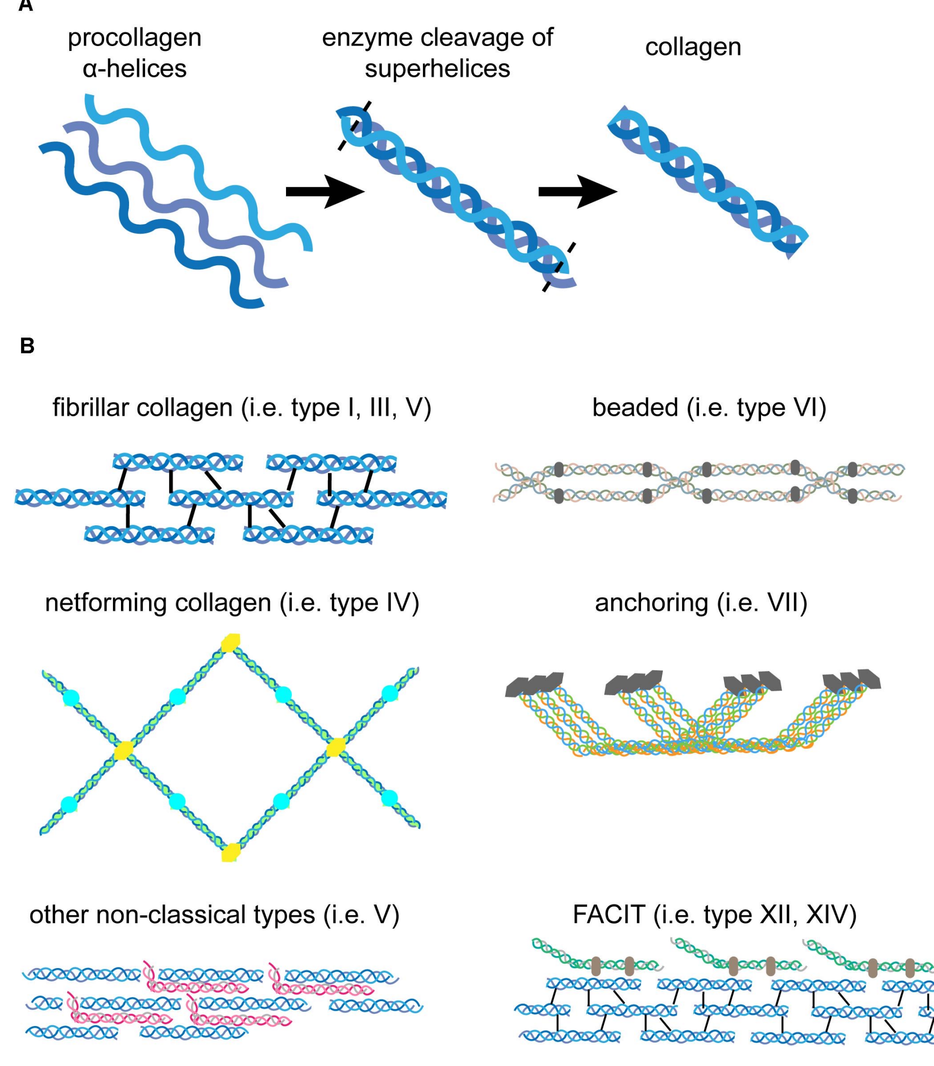
Collagen types

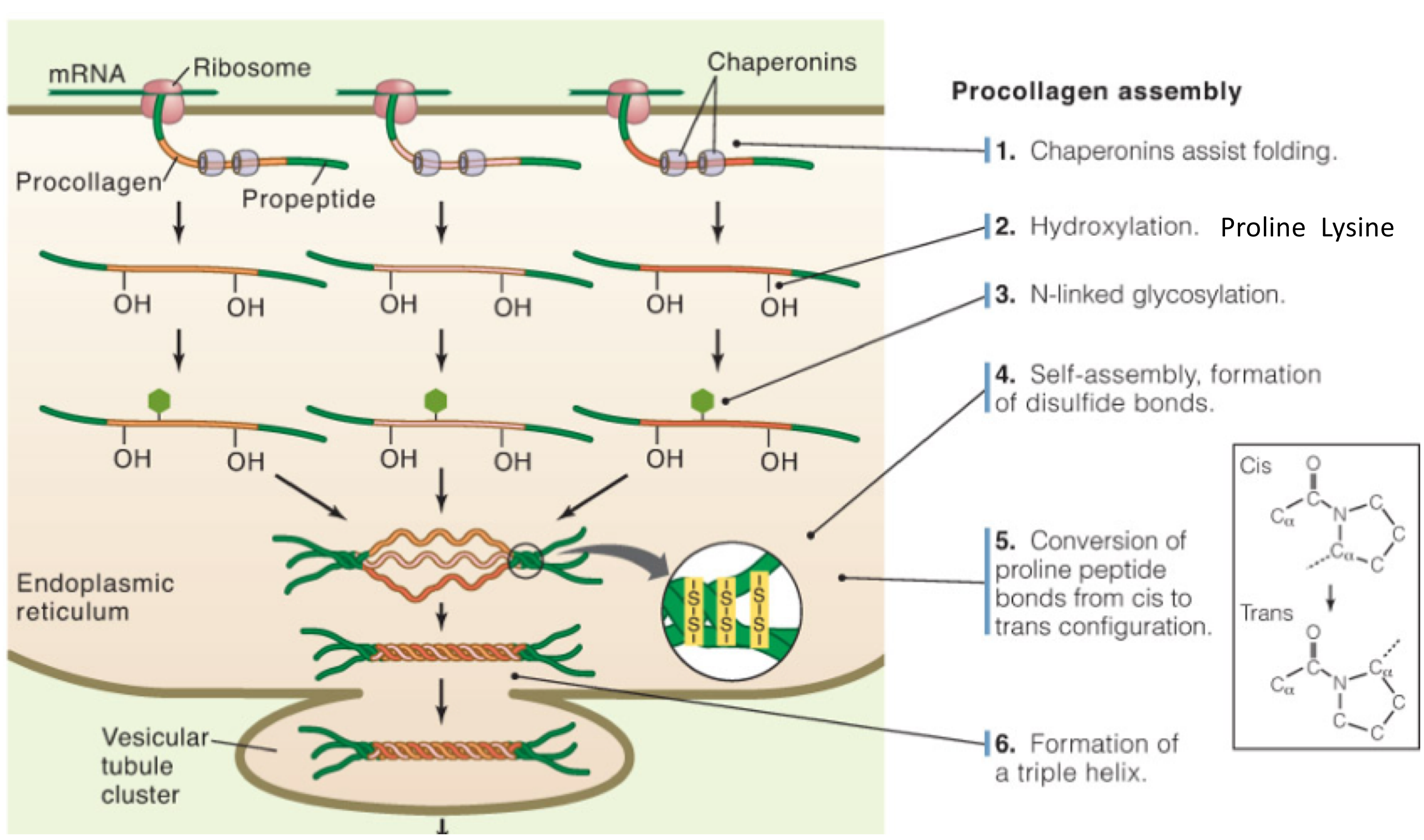
TABLE 19–2 Some Types of Collagen and Their Properties				
	Type	Polymerized form	Tissue distribution	Mutant phenotype
Fibril-forming (fibrillar)	I	Fibril	Bone, skin, tendons, ligaments, cornea, internal organs (accounts for 90% of body collagen)	Severe bone defects, fractures (*osteogenesis imperfecta*)
	II	Fibril	Cartilage, intervertebral disc, notochord, vitreous humor of the eye	Cartilage deficiency, dwarfism (*chondrodysplasia*)
	III	Fibril	Skin, blood vessels, internal organs	Fragile skin, loose joints, blood vessels prone to rupture (*Ehlers–Danlos syndrome*)
	V	Fibril (with type I)	As for type I	Fragile skin, loose joints, blood vessels prone to rupture
	XI	Fibril (with type II)	As for type II	Myopia, blindness
Fibril-associated	IX	Lateral association with type II fibrils	Cartilage	Osteoarthritis
Network-forming	IV	Sheetlike network	Basal lamina	Kidney disease (glomerulonephritis), deafness
	VII	Anchoring fibrils	Beneath stratified squamous epithelia	Skin blistering
Transmembrane	XVII	Nonfibrillar	Hemidesmosomes	Skin blistering
Proteoglycan core protein	XVIII	Nonfibrillar	Basal lamina	Myopia, detached retina, hydrocephalus

Note that types I, IV, V, IX, and XI are each composed of two or three types of α chains (distinct, nonoverlapping sets in each case), whereas types II, III, VII, XVII, and XVIII are composed of only one type of α chain each.

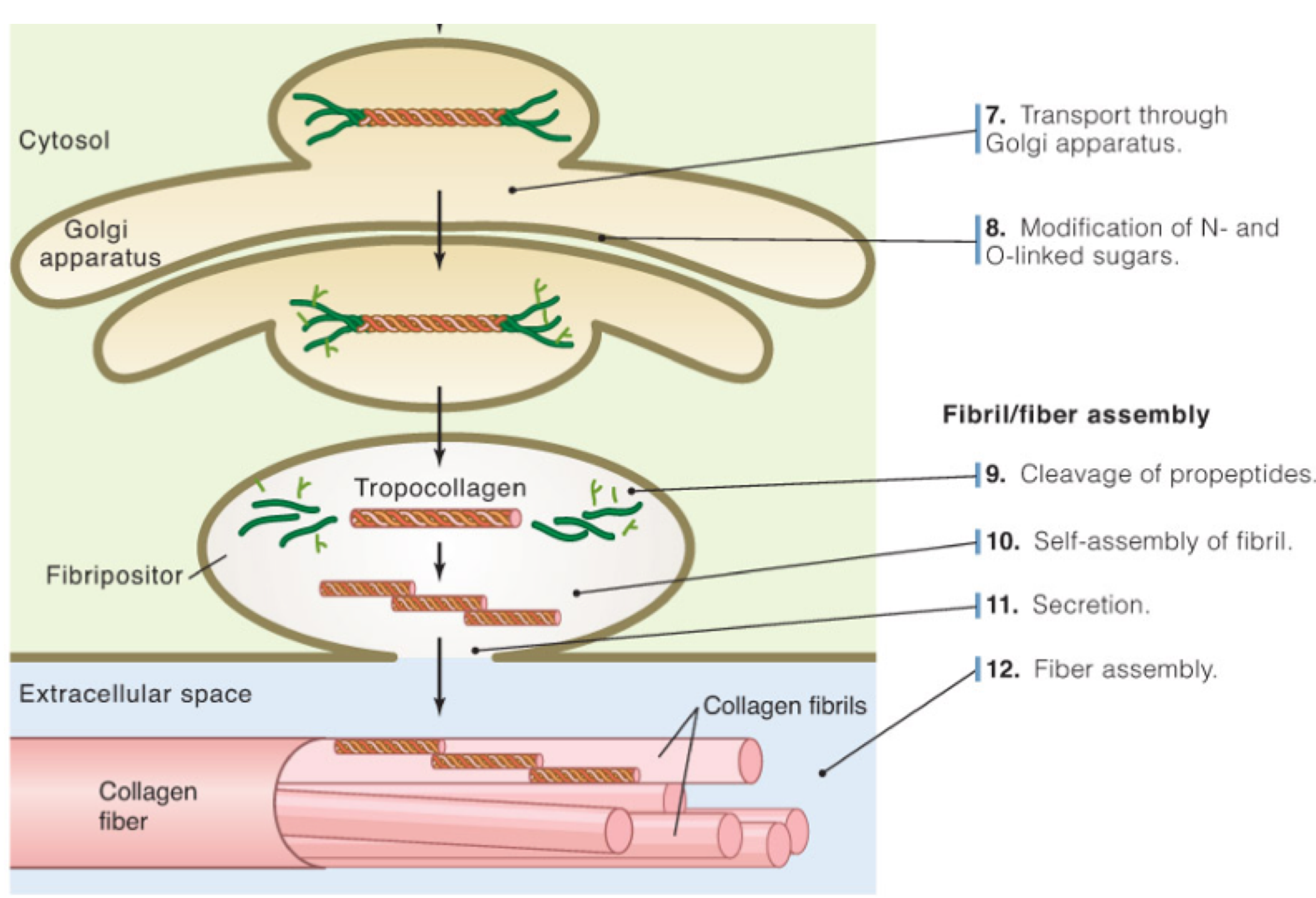
Collagen types



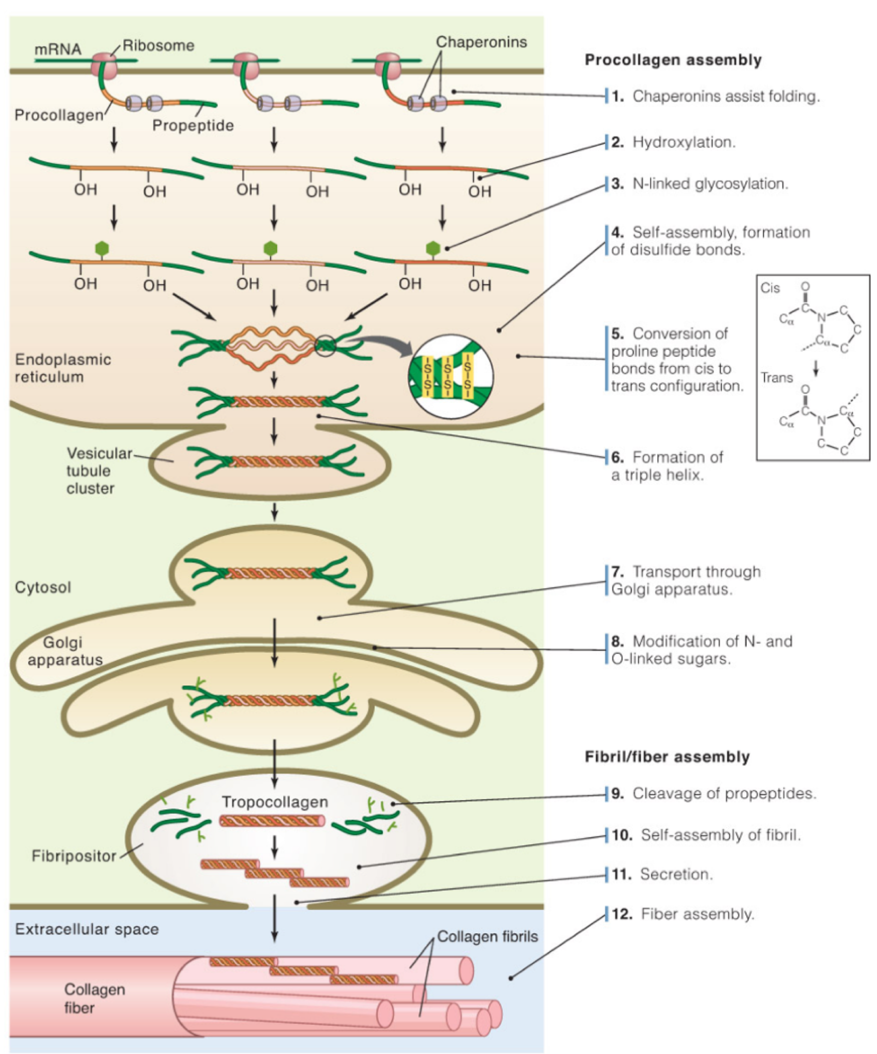
Collagen I synthesis



Collagen I synthesis



Collagen I synthesis



Collagen fibrils

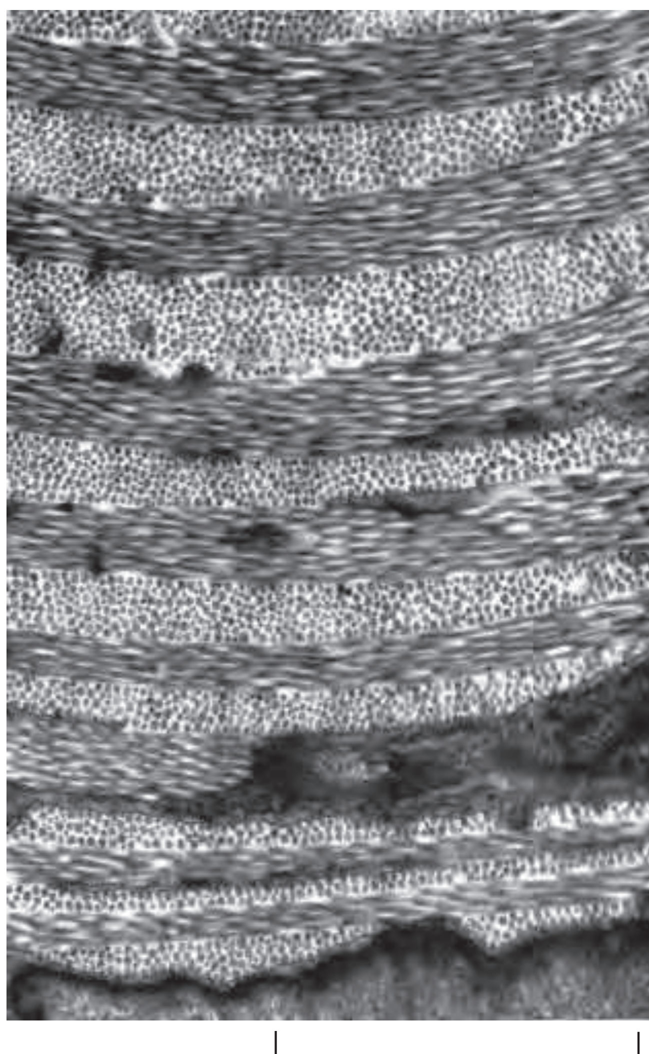
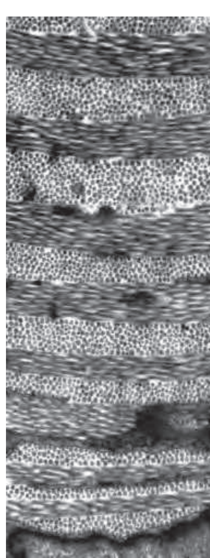
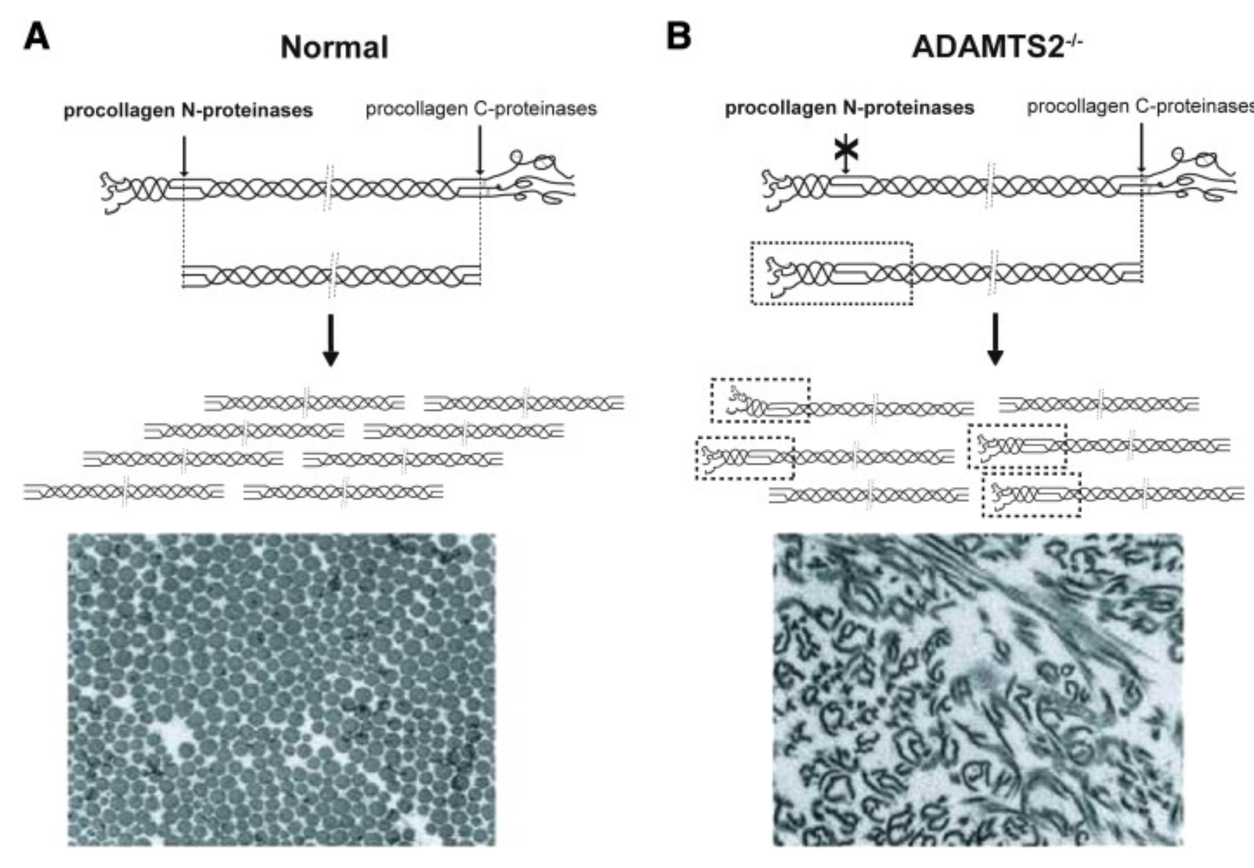


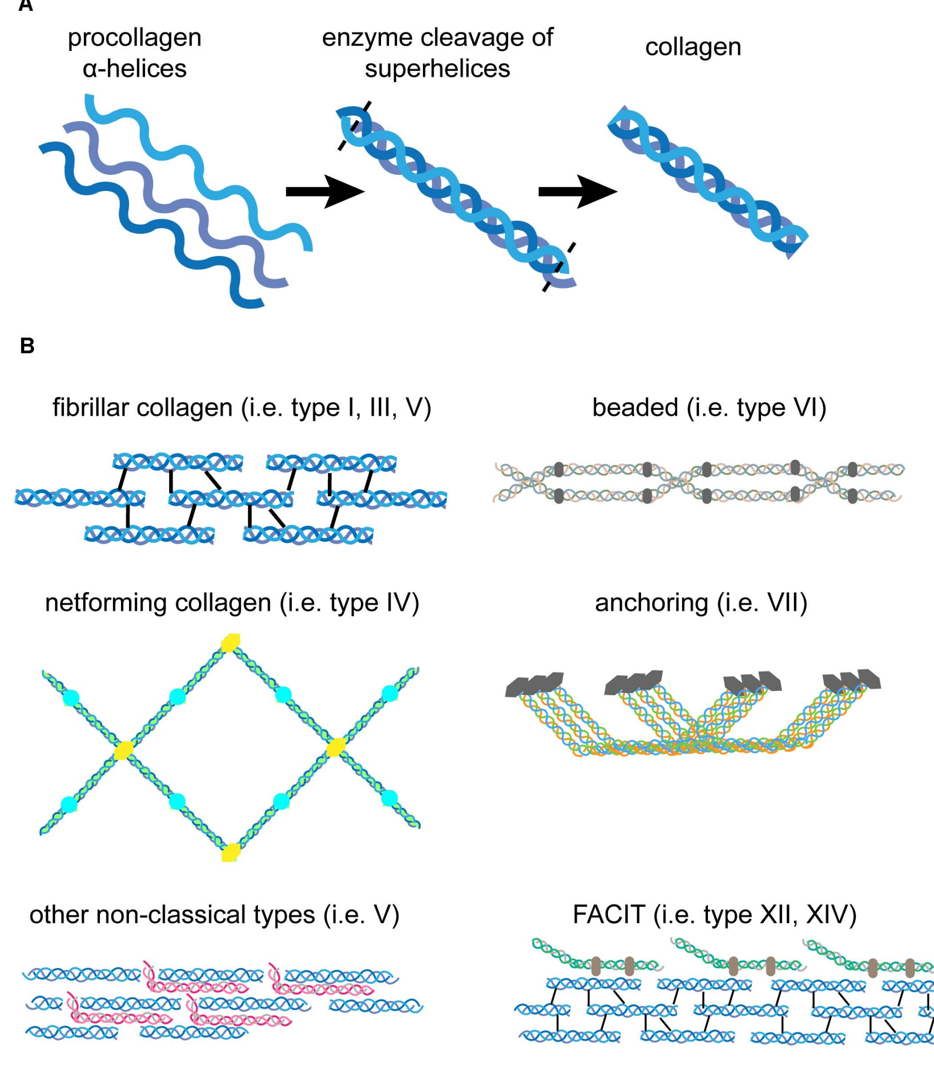
Figure 19–41 **Collagen fibrils in the tadpole skin.** This electron micrograph shows the plywoodlike arrangement of the fibrils: successive layers of fibrils are laid down nearly at right angles to each other. This organization is also found in mature bone and in the cornea. (Courtesy of Jerome Gross.)



Defective collagen synthesis has dire consequences



Collagen types



The ECM needs to be able to stretch

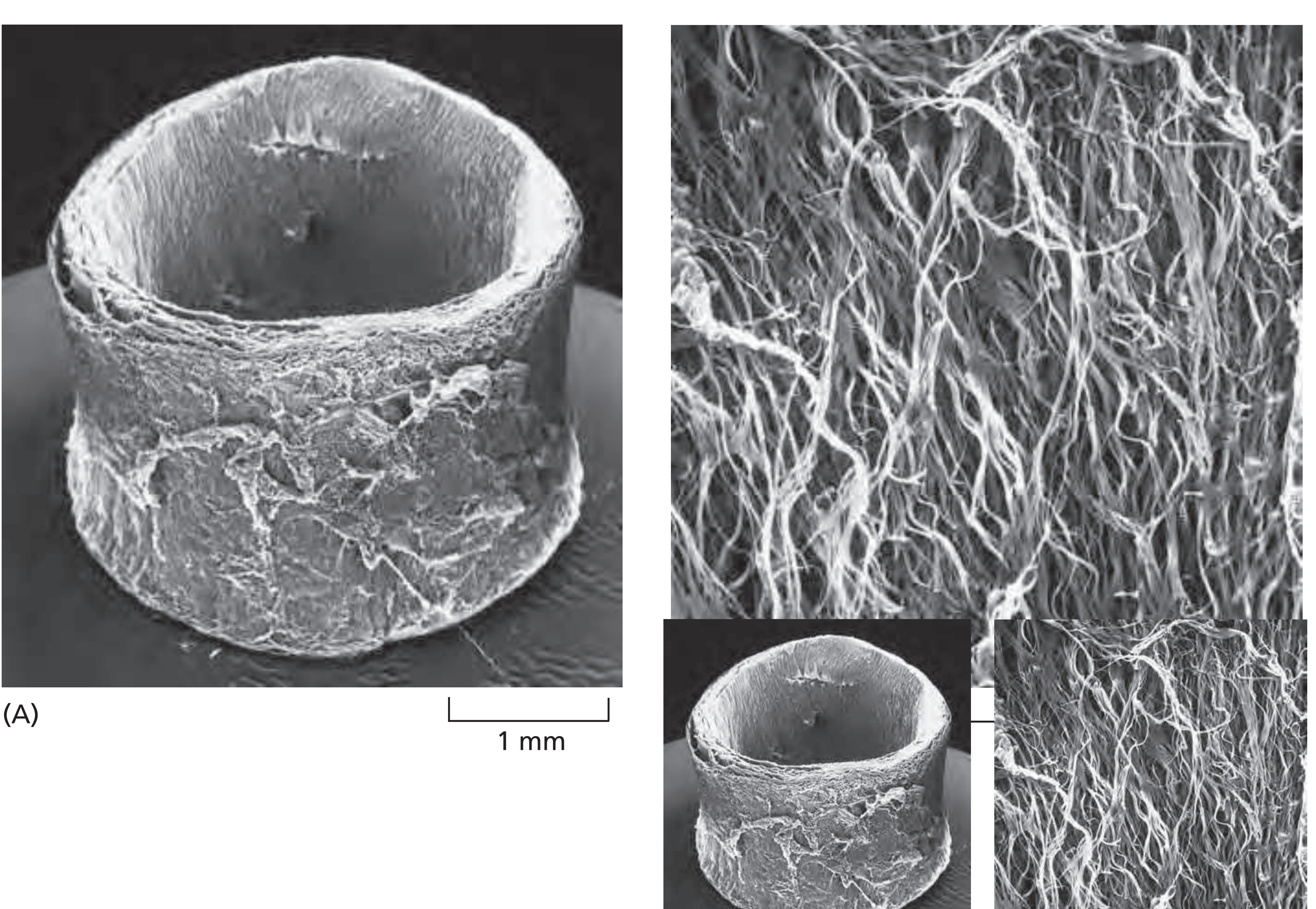
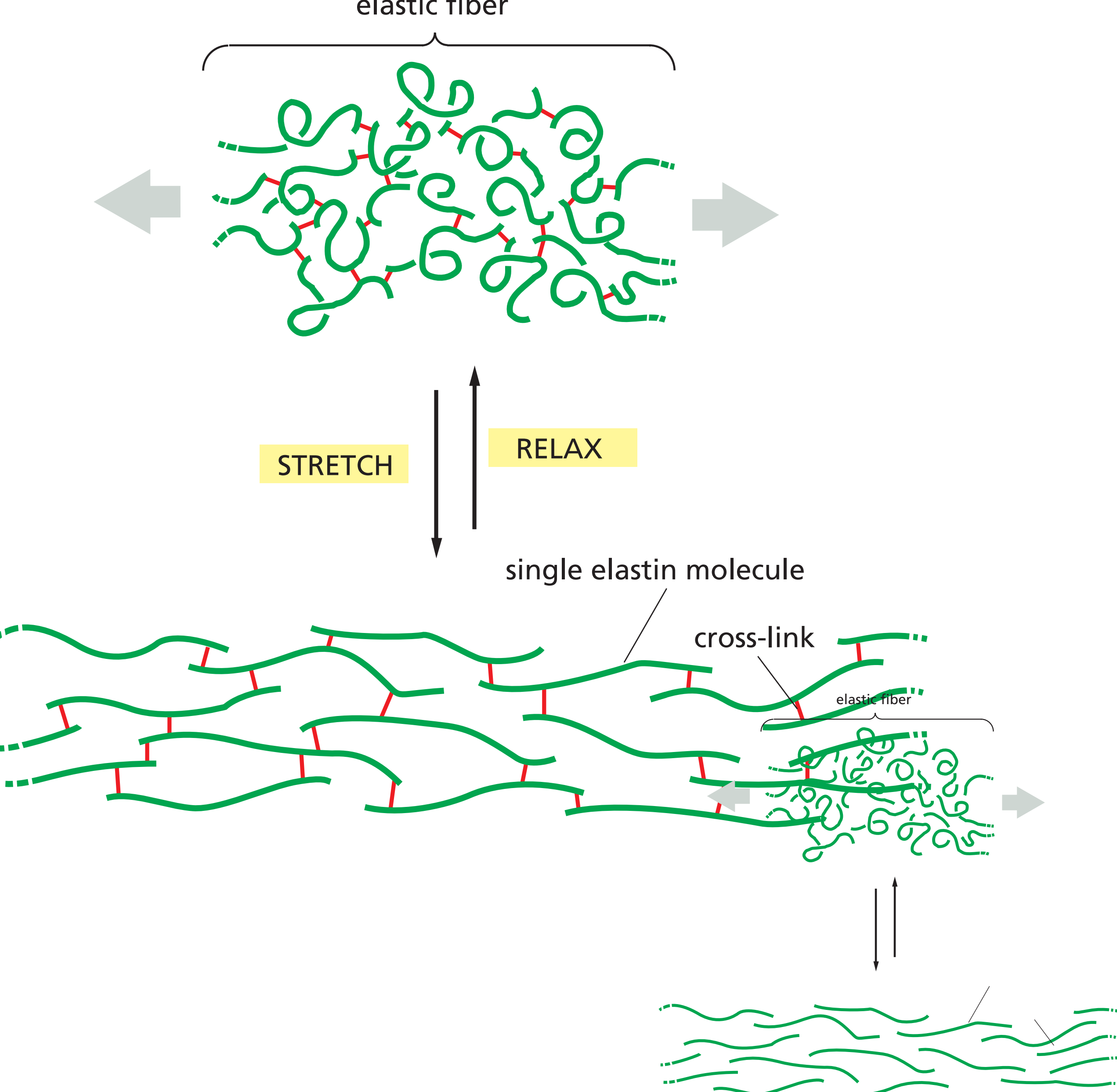


Figure 19–44 **Elastic fibers.** These scanning electron micrographs show (A) a low-power view of a segment of a dog's aorta and (B) a high-power view of the dense network of longitudinally oriented elastic fibers in the outer layer of the same blood vessel. All the other components have been digested away with enzymes and formic acid. (From K.S. Haas et al., *Anat. Rec.* 230:86–96, 1991. With permission from Wiley-Liss.)

The ECM needs to be able to stretch



Elastin also has a very long half life ~ 40 years

Figure 19–45 Stretching a network of elastin molecules. The molecules are joined together by covalent bonds *(red)* to generate a cross-linked network. In this model, each elastin molecule in the network can extend and contract in a manner resembling a random coil, so that the entire assembly can stretch and recoil like a rubber band.

Complex glycoproteins in the ECM

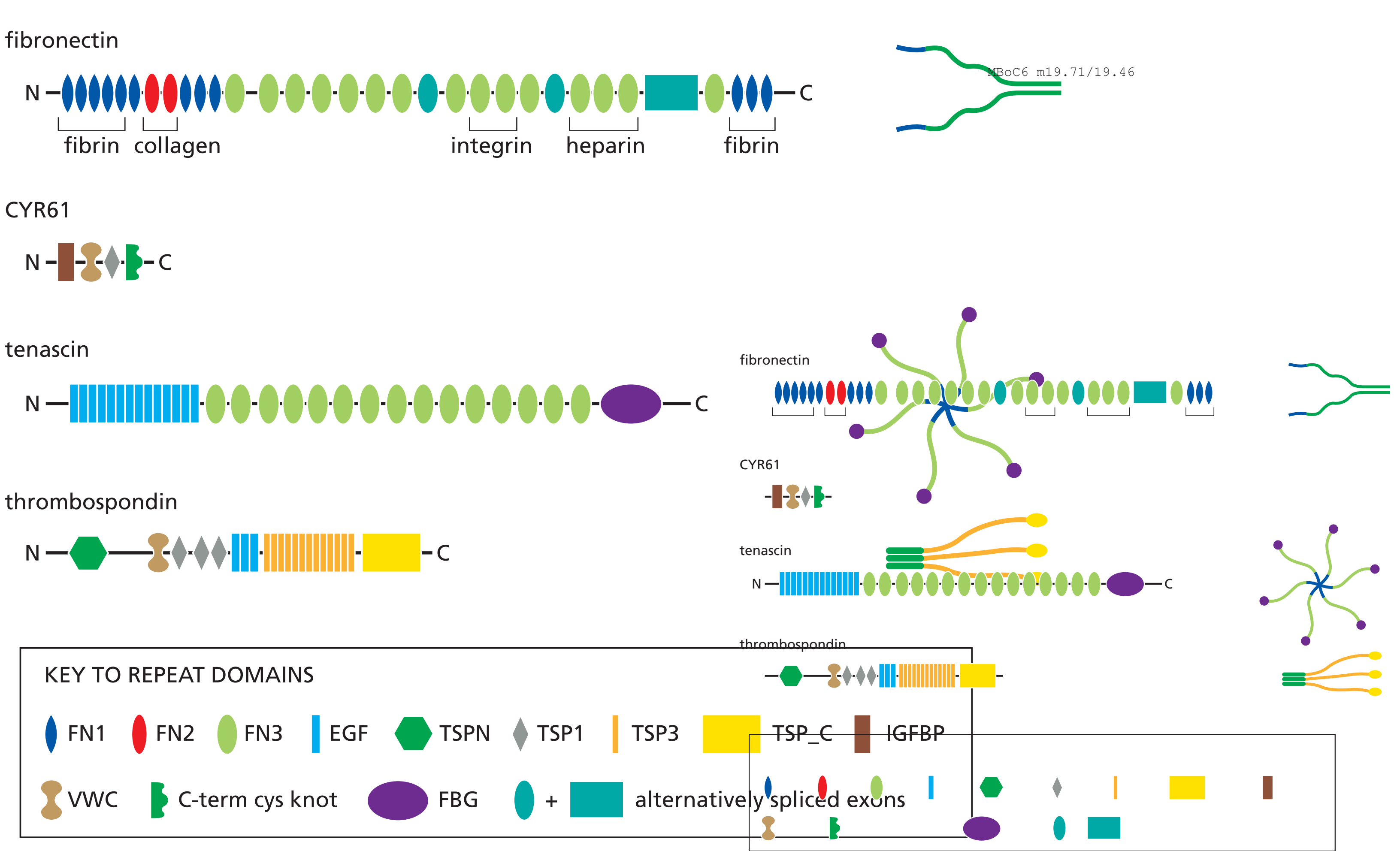


Figure 19–46 Complex glycoproteins of the extracellular matrix. Many matrix glycoproteins are large scaffold proteins containing multiple copies of specific protein-interaction domains. Each domain is folded into a discrete globular structure, and many such domains are arrayed along the protein like beads on a string. This diagram shows four representative proteins among the roughly 200 matrix glycoproteins that are found in mammals. Each protein contains multiple repeat domains, with the names listed in the key at the bottom. Fibronectin, for example, contains numerous copies of three different *fibronectin repeats* (types I–III, labeled here as FN1, FN2, and FN3). Two type III repeats near the C-terminus contain important binding sites for cell-surface integrins, whereas other FN repeats are involved in binding fibrin, collagen, and heparin, as indicated (see Figure 19–47). Other matrix proteins contain repeated sequences resembling those of epidermal growth factor (EGF), a major regulator of cell growth and proliferation; these repeats might serve a similar signaling function in matrix proteins. Other proteins contain domains, such as the insulin-like growth factor-binding protein (IGFBP) repeat, that bind and regulate the function of soluble growth factors. To add more structural diversity, many of these proteins are encoded by RNA transcripts that can be spliced in different ways, adding or removing exons, such as those in fibronectin. Finally, the scaffolding and regulatory functions of many matrix proteins are further expanded by assembly into multimeric forms, as shown at the right: fibronectin forms dimers linked at the C-termini, whereas tenascin and thrombospondin form N-terminally linked hexamers and trimers, respectively. Other domains include four repeats from thrombospondin (TSPN, TSP1, TSP3, TSP_C). VWC, von Willebrand type C; FBG, fibrinogen-like. (Adapted from R.O. Hynes and A. Naba, *Cold Spring Harb. Perspect. Biol.* 4:a004903, 2012.)

MBoC6 n19.205/19.47

Fibronectin

100 nm

(A)

N

collagen binding

N

integrin binding

heparin binding

S-S

S-S

C C

(B)

(A)

synergy sequence

Arg

Gly

Asp

RGD sequence

(C)

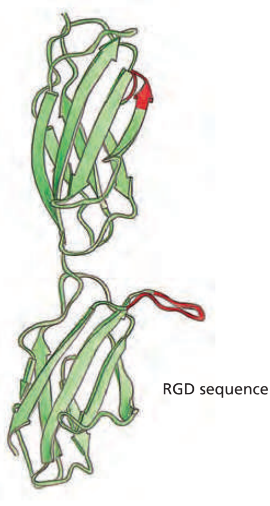


Figure 19–47 The structure of a fibronectin dimer. (A) Electron micrographs of individual fibronectin dimer molecules shadowed with platinum; *red arrows* mark the joined C-termini. (B) The two polypeptide chains are similar but generally not identical (being made from the same gene but from differently spliced mRNAs). They are joined by two disulfide bonds near the C-termini. Each chain is almost 2500 amino acids long and is folded into multiple domains (see Figure 19–46). As indicated, some domains are specialized for binding to a particular molecule. For simplicity, not all of the known binding sites are shown. (C) The three-dimensional structure of the ninth and tenth type III fibronectin repeats, as determined by x-ray crystallography. Both the Arg-Gly-Asp (RGD) and the "synergy" sequences shown in *red* are important for binding to integrins on cell surfaces. (A, from J. Engel et al., *J. Mol. Biol.* 150:97–120, 1981. With permission from Academic Press; C, from Daniel J. Leahy, *Annu. Rev. Cell Dev. Biol.* 13:363–393, 1997. With permission from Annual Reviews.)

Fibronectin can unfold when pulled upon

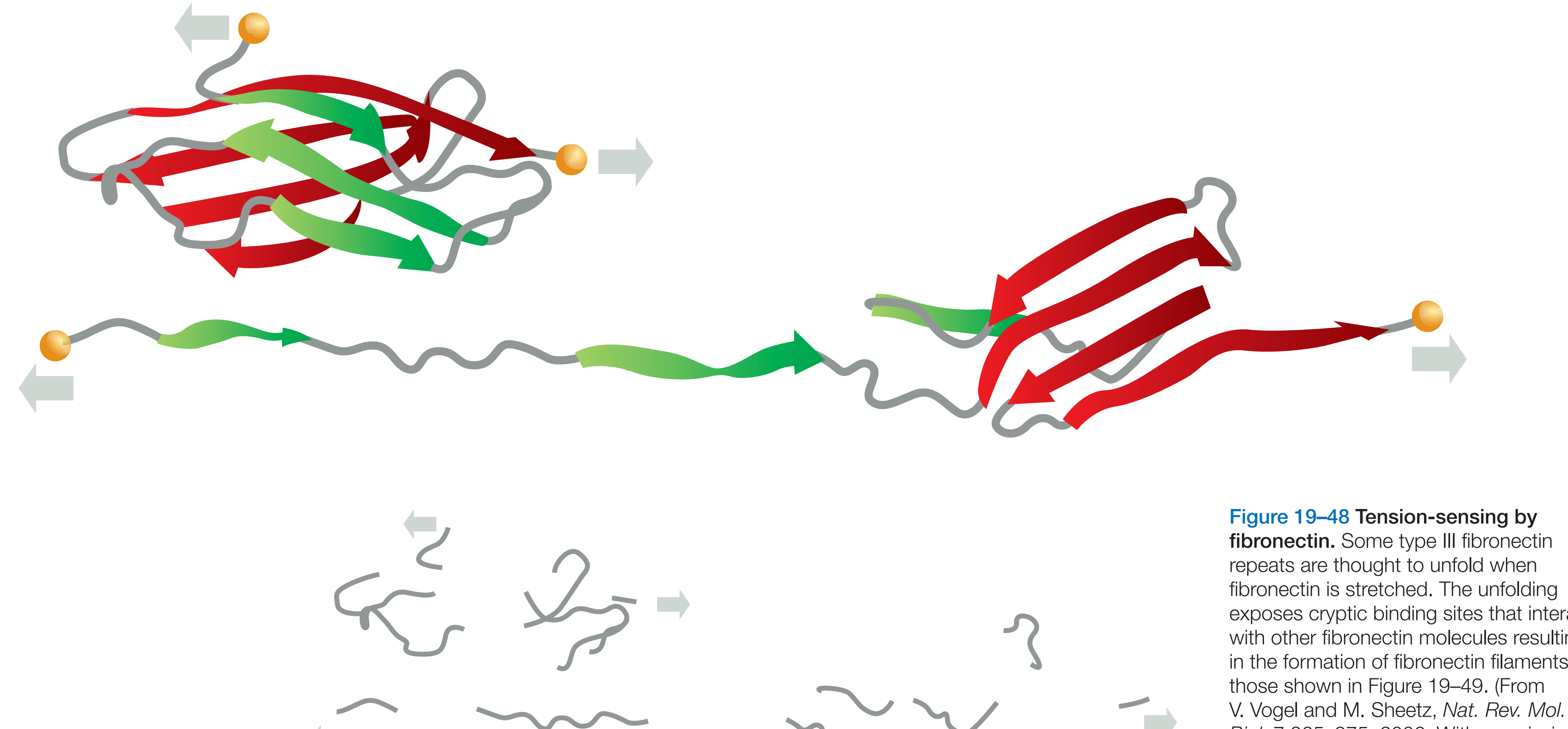


Figure 19–48 **Tension-sensing by fibronectin.** Some type III fibronectin repeats are thought to unfold when fibronectin is stretched. The unfolding exposes cryptic binding sites that interact with other fibronectin molecules resulting in the formation of fibronectin filaments like those shown in Figure 19–49. (From V. Vogel and M. Sheetz, *Nat. Rev. Mol. Cell Biol.* 7:265–275, 2006. With permission from Macmillan Publishers Ltd.)

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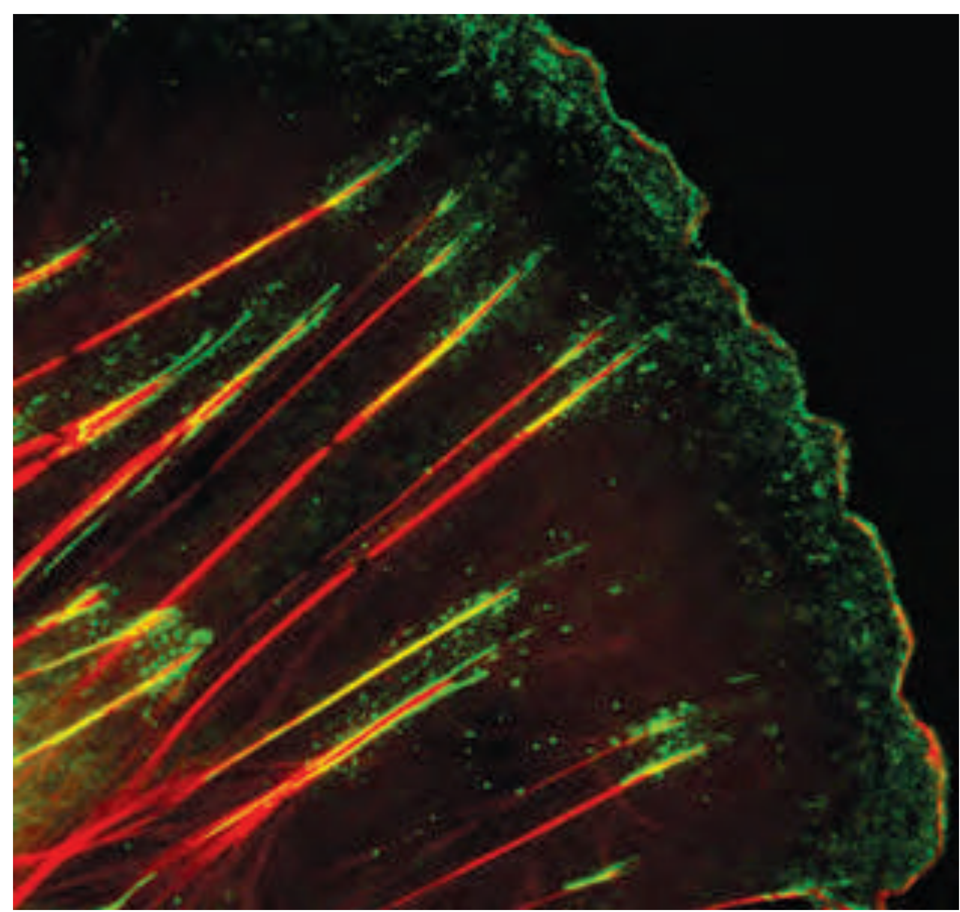
Fibronectin and the cytoskeleton align



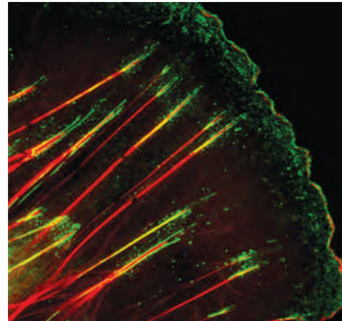


Figure 19–49 Organization of fibronectin into fibrils at the cell surface. This fluorescence micrograph shows the front end of a migrating mouse fibroblast. Extracellular fibronectin is stained *green* and intracellular actin filaments are stained *red*. The fibronectin is initially present as small dotlike aggregates near the leading edge of the cell. It accumulates at focal adhesions (sites of anchorage of actin filaments, discussed later) and becomes organized into fibrils parallel to the actin filaments. Integrin molecules spanning the cell membrane link the fibronectin outside the cell to the actin filaments inside it (see Figure 19–55). Tension exerted on the fibronectin molecules through this linkage is thought to stretch them, exposing binding sites that promote fibril formation. (Courtesy of Roumen Pankov and Kenneth Yamada.)

MBoC6 m19.74/19.50

Fibronectin instructs patterning in cells

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Restoration of Normal Morphology, Adhesion and Cytoskeleton in Transformed Cells by Addition of a Transformation-Sensitive Surface Protein

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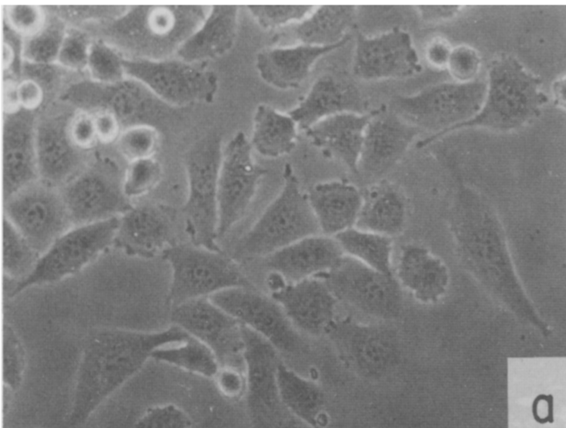
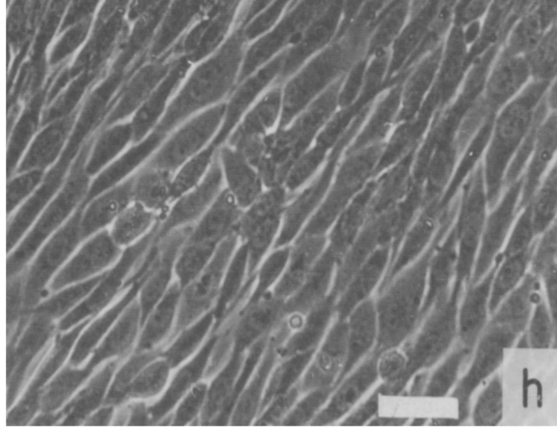
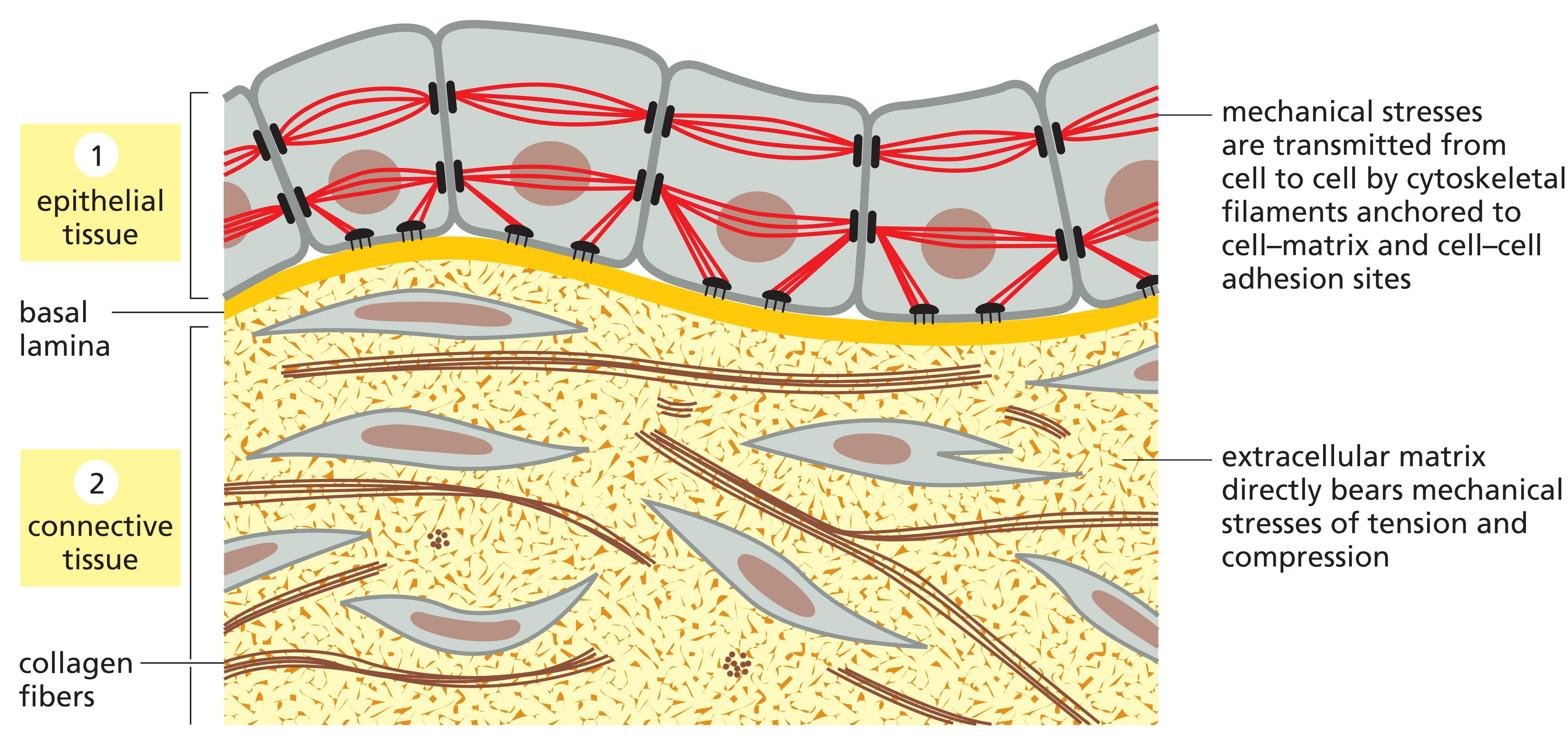




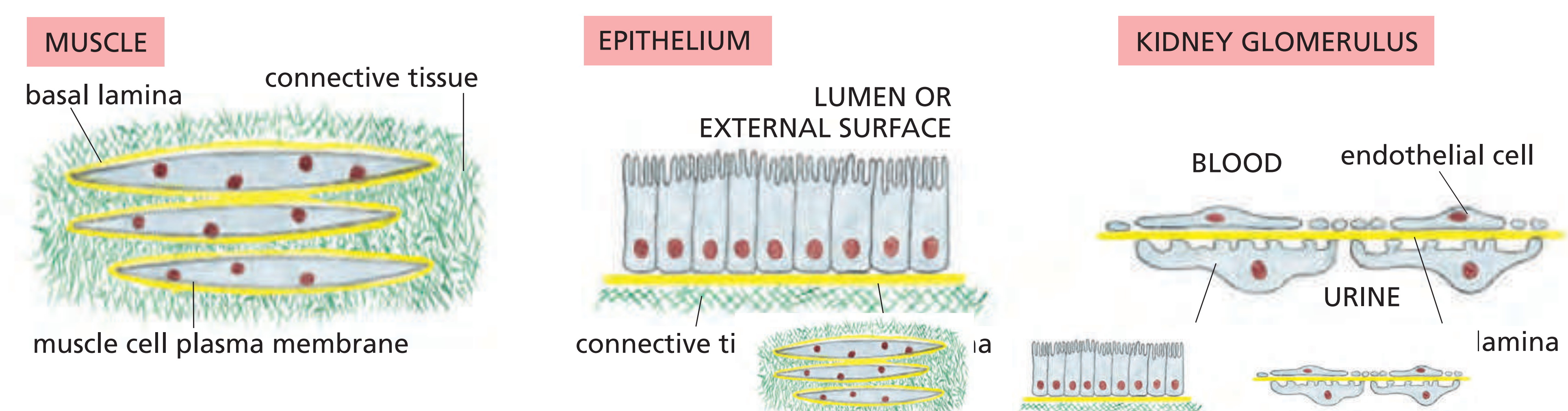
Summary

Transformed cells lack a large, external, transformation-sensitive (LETS) glycoprotein which is a major surface component of their normal counterparts. Addition of LETS glycoprotein isolated from normal cells to transformed cells restores certain morphological features and adhesive properties characteristic of normal cells. LETS protein is detected on the cell surface both by iodination using lactoperoxidase and by immunofluorescent staining. The surface distribution pattern detected by immunofluorescence is strikingly similar to that of normal cells. After addition of LETS protein, transformed cells also exhibit well defined actin cables which are not seen in untreated, transformed cells. All these alterations can be blocked by treating LETS protein with specific antisera or by subjecting it to mild trypsinization prior to addition to transformed cells. The effects are rapidly reversible by mild trypsinization, which removes the added LETS protein. The high rate of uptake of 2-deoxyglucose, characteristic of transformed cells, is not affected by LETS protein. These results suggest that LETS protein may have a role in cell attachment and spreading, and affect the organization of cytoskeleton.

The basal lamina has a special compostion



Different organizations of basal lamina



Also differences in composition of basal lamina between tissues

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Figure 19–50 **Three ways in which basal laminae are organized.** Basal laminae *(yellow)* surround certain cells (such as skeletal muscle cells), underlie epithelia, and are interposed between two cell sheets (as in the kidney glomerulus). Note that, in the kidney glomerulus, both cell sheets have gaps in them, and the basal lamina has a filtering as well as a supportive function, helping to determine which molecules will pass into the urine from the blood. The filtration also depends on other protein-based structures, called *slit diaphragms*, that span the intercellular gaps in the epithelial sheet.

Basal lamina

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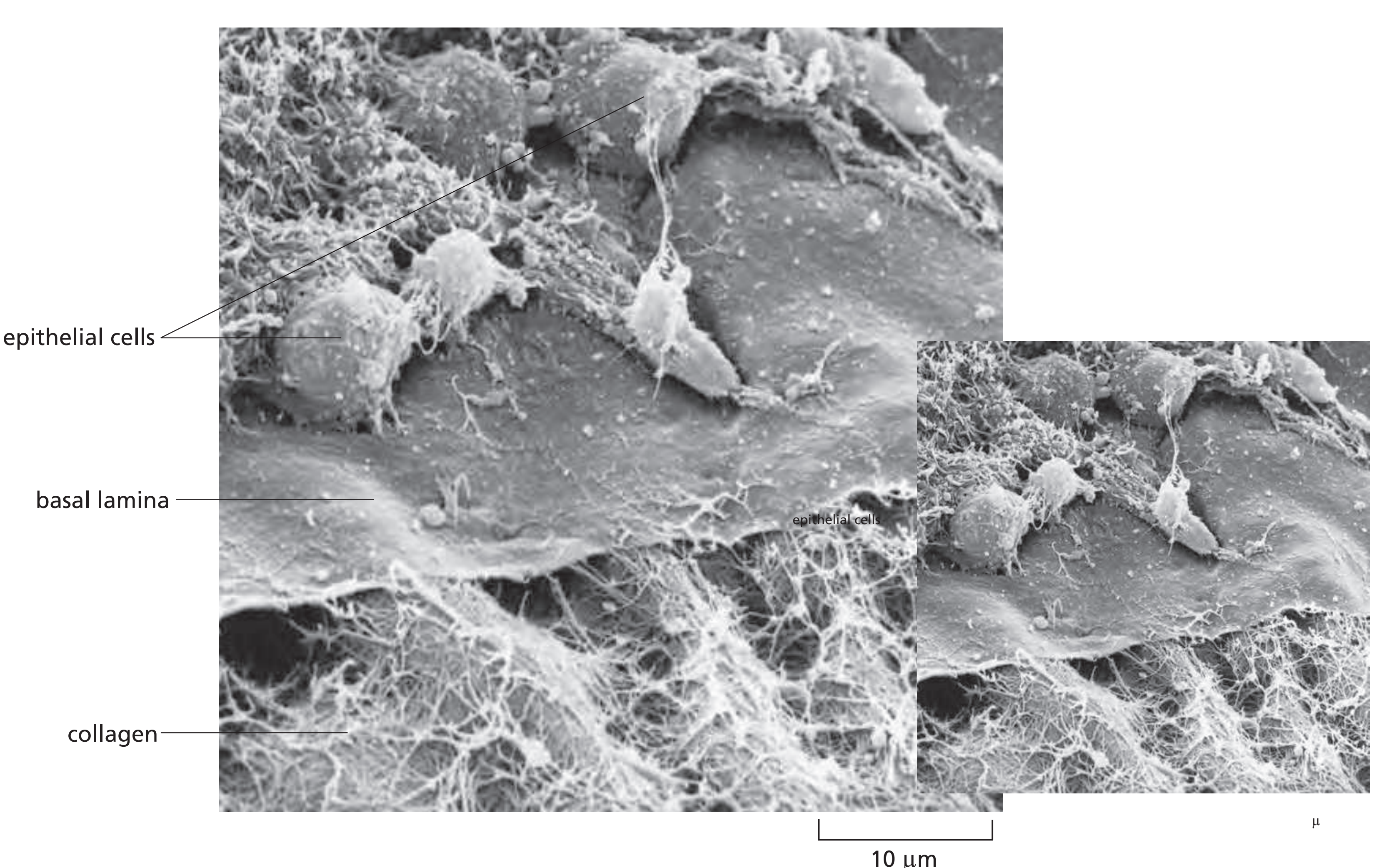

Figure 19–51 **The basal lamina in the cornea of a chick embryo.** In this scanning electron micrograph, some of the epithelial cells have been removed to expose the upper surface of the matlike basal lamina. A network of collagen fibrils in the underlying connective tissue interacts with the lower face of the lamina. (Courtesy of Robert Trelstad.)

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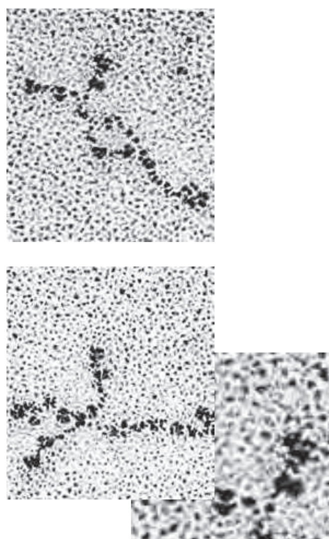
Laminin, basic structure



self-

self-
assembly

NH_2

β chain

coiled-coil domain

integrins

α chain

H_2N

dystroglycan

integrins

nidogen

perlecan

γ chain

20 nm

NH_2

self-
assembly

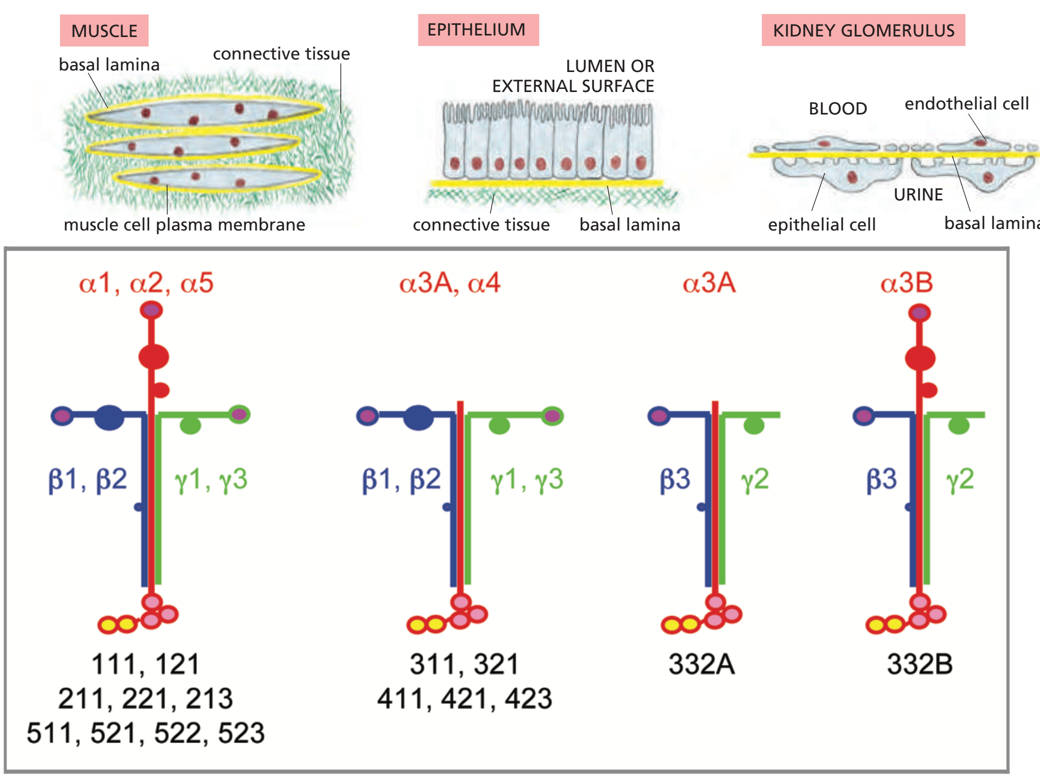
MBoC6 m19.42/19.53

(A)

(B) 100 nm

Figure 19–52 The structure of laminin. (A) The best-understood family member is laminin-111, shown here with some of its binding sites for other molecules *(yellow boxes)*. Laminins are multidomain glycoproteins composed of three polypeptides (α, β, and γ) that are disulfide-bonded into an asymmetric crosslike structure. Each of the polypeptide chains is more than 1500 amino acids long. Five types of α chains, four types of β chains, and three types of γ chains are known, and various combinations of these subunits can assemble to form a large variety of different laminins, which are named according to numbers assigned to each of their three subunits: laminin-111, for example, contains α1, β1, and γ1 subunits. Each isoform tends to have a specific tissue distribution: laminin-332 is found in skin, laminin-211 in muscle, and laminin-411 in endothelial cells of blood vessels. Through their binding sites for other proteins, laminin molecules play a central part in organizing basal laminae and anchoring them to cells. (B) Electron micrographs of laminin molecules shadowed with platinum. (B, from J. Engel et al., *J. Mol. Biol.* 150:97–120, 1981. With permission from Academic Press.)

Laminin, subtypes and heterogeneity in basement membrane



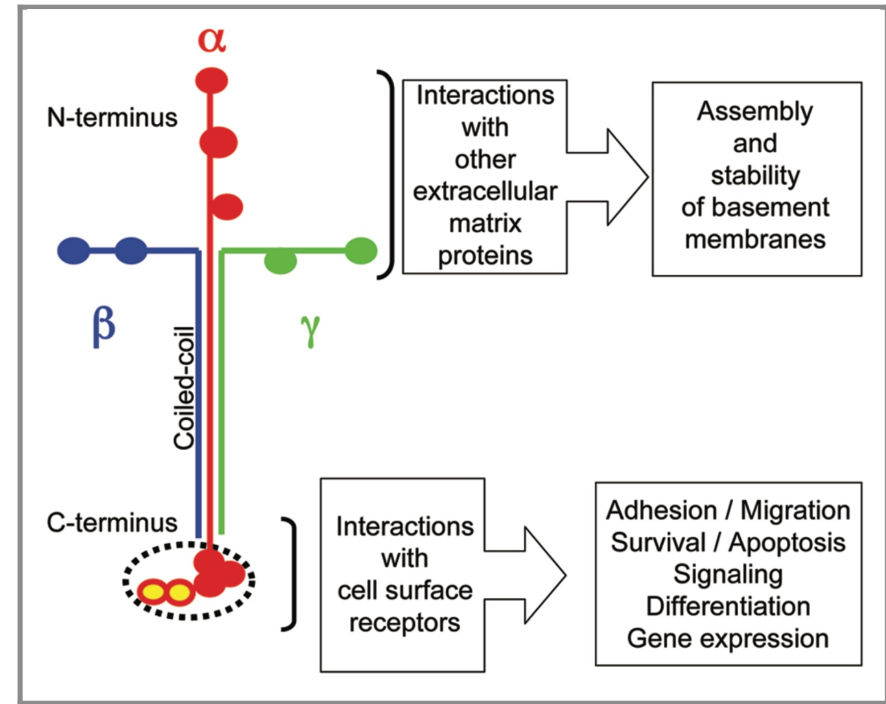


Figure 3. Mapping of the major functions of laminins. The laminin short arms (N-terminus) are involved in architectural function within the basement membrane, while the end of the long arm (C-terminus) is typically involved in cellular interactions.

Laminin 111 ubiquitous in the embryo, restricted to a small subset of basement membranes in the adult.

Laminins 511 and 521, are the most ubiquitous isoforms in the adult organism

Laminins 211 and 221 are present in basement membranes of skeletal and cardiac muscles

Laminins 411 and 421 are abundant in endothelial basement membranes

Laminin 332 is specific for the basal lamina underlying epithelial cells

The basal lamina is a complex network

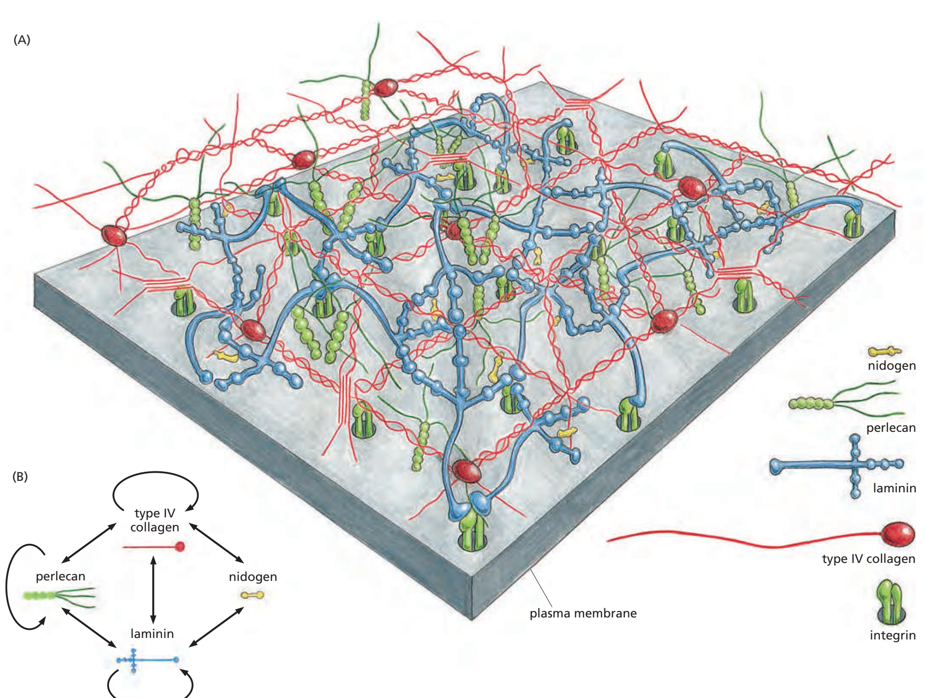
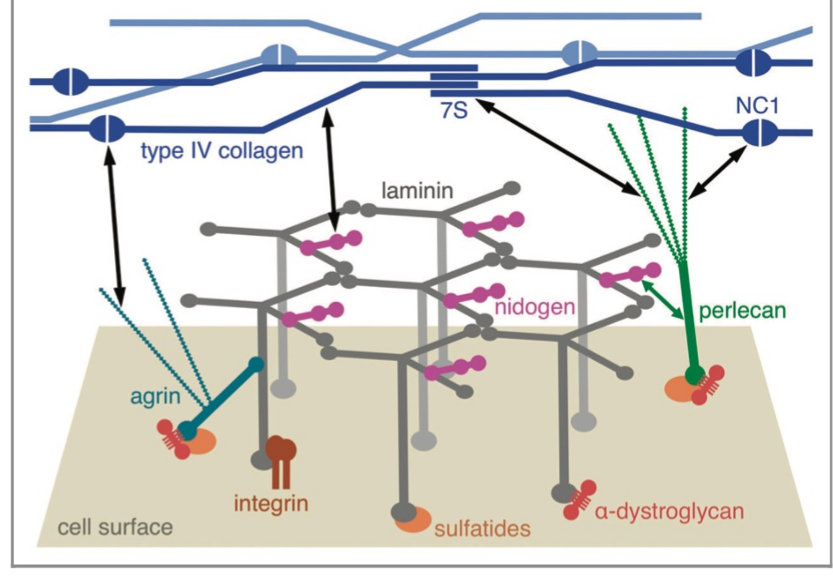


Figure 19–53 A model of the molecular structure of a basal lamina. (A) The basal lamina is formed by specific interactions (B) between the proteins laminin, type IV collagen, and nidogen, and the proteoglycan perlecan. *Arrows* in (B) connect molecules that can bind directly to each other. There are various isoforms of type IV collagen and laminin, each with a distinctive tissue distribution. Transmembrane laminin receptors (integrins and dystroglycan) in the plasma membrane are thought to organize the assembly of the basal lamina; only the integrins are shown. (Based on H. Colognato and P.D. Yurchenco, *Dev. Dyn.* 218:213–234, 2000. With permission from Wiley-Liss.)



Intergrin ECM interaction shapes the cytoskeleton

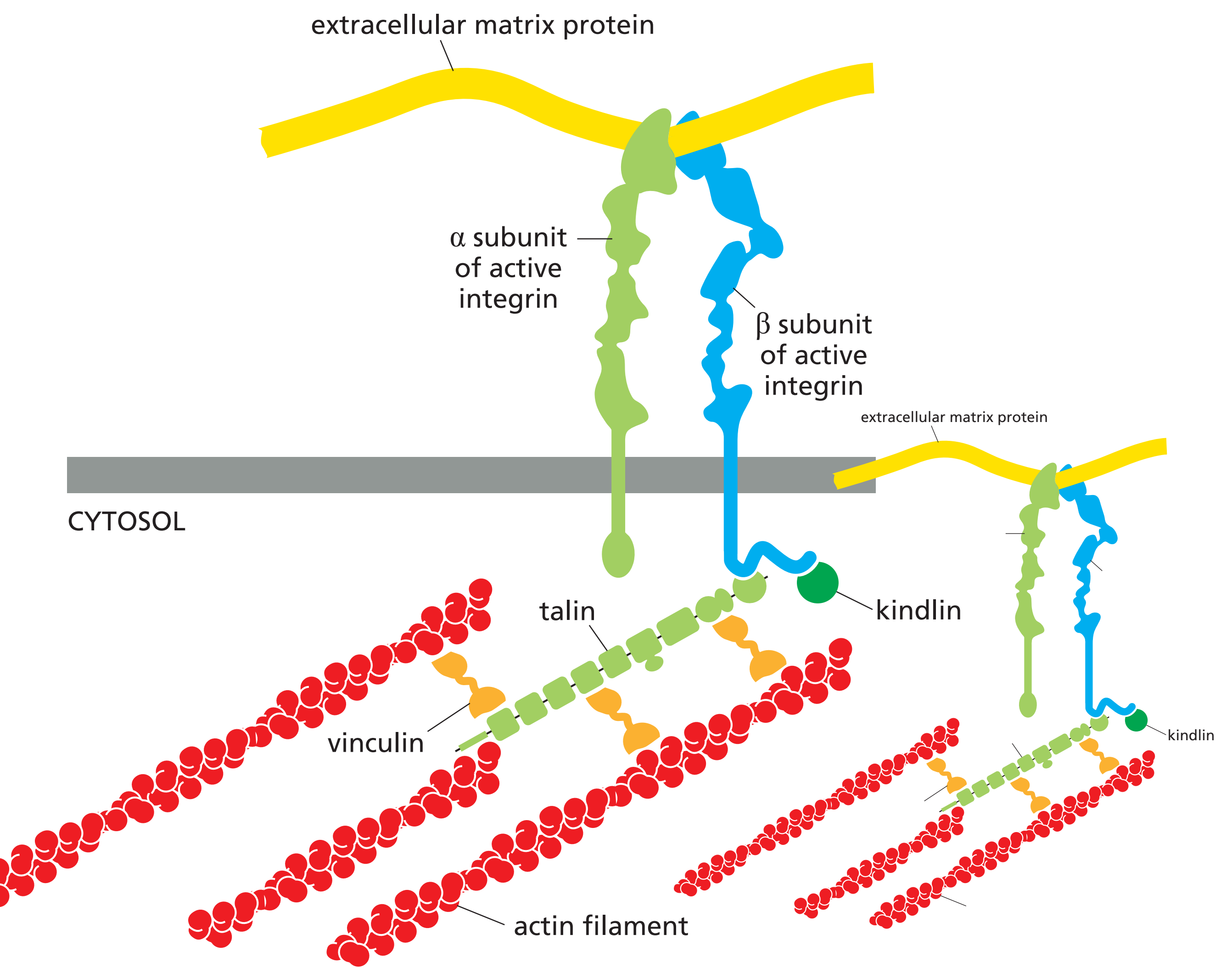


Figure 19–55 The subunit structure of an active integrin molecule, linking extracellular matrix to the actin cytoskeleton. The N-terminal heads of the integrin chains attach directly to an extracellular protein such as fibronectin; the C-terminal intracellular tail of the integrin β subunit binds to adaptor proteins that interact with filamentous actin. The best-understood adaptor is a giant protein called talin, which contains a string of multiple domains for binding actin and other proteins, such as vinculin, that help reinforce and regulate the linkage to actin filaments. One end of talin binds to a specific site on the integrin β subunit cytoplasmic tail; other regulatory proteins, such as kindlin, bind at another site on the tail.

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There are many types of integrins

TABLE 19–3 Some Types of Integrins

Integrin	Ligand*	Distribution	Phenotype when α subunit is mutated	Phenotype when β subunit is mutated
$\alpha_5\beta_1$	Fibronectin	Ubiquitous	Death of embryo; defects in blood vessels, somites, neural crest	Early death of embryo (at implantation)
$\alpha_6\beta_1$	Laminin	Ubiquitous	Severe skin blistering; defects in other epithelia also	Early death of embryo (at implantation)
$\alpha_7\beta_1$	Laminin	Muscle	Muscular dystrophy; defective myotendinous junctions	Early death of embryo (at implantation)
$\alpha_L\beta_2$ (LFA1)	Ig superfamily counterreceptors (ICAM1)	White blood cells	Impaired recruitment of leucocytes	Leukocyte adhesion deficiency (LAD); impaired inflammatory responses; recurrent life-threatening infections
$\alpha_{IIb}\beta_3$	Fibrinogen	Platelets	Bleeding; no platelet aggregation (Glanzmann's disease)	Bleeding; no platelet aggregation (Glanzmann's disease); mild osteopetrosis
$\alpha_6\beta_4$	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.

Integrins in hemidesomoses

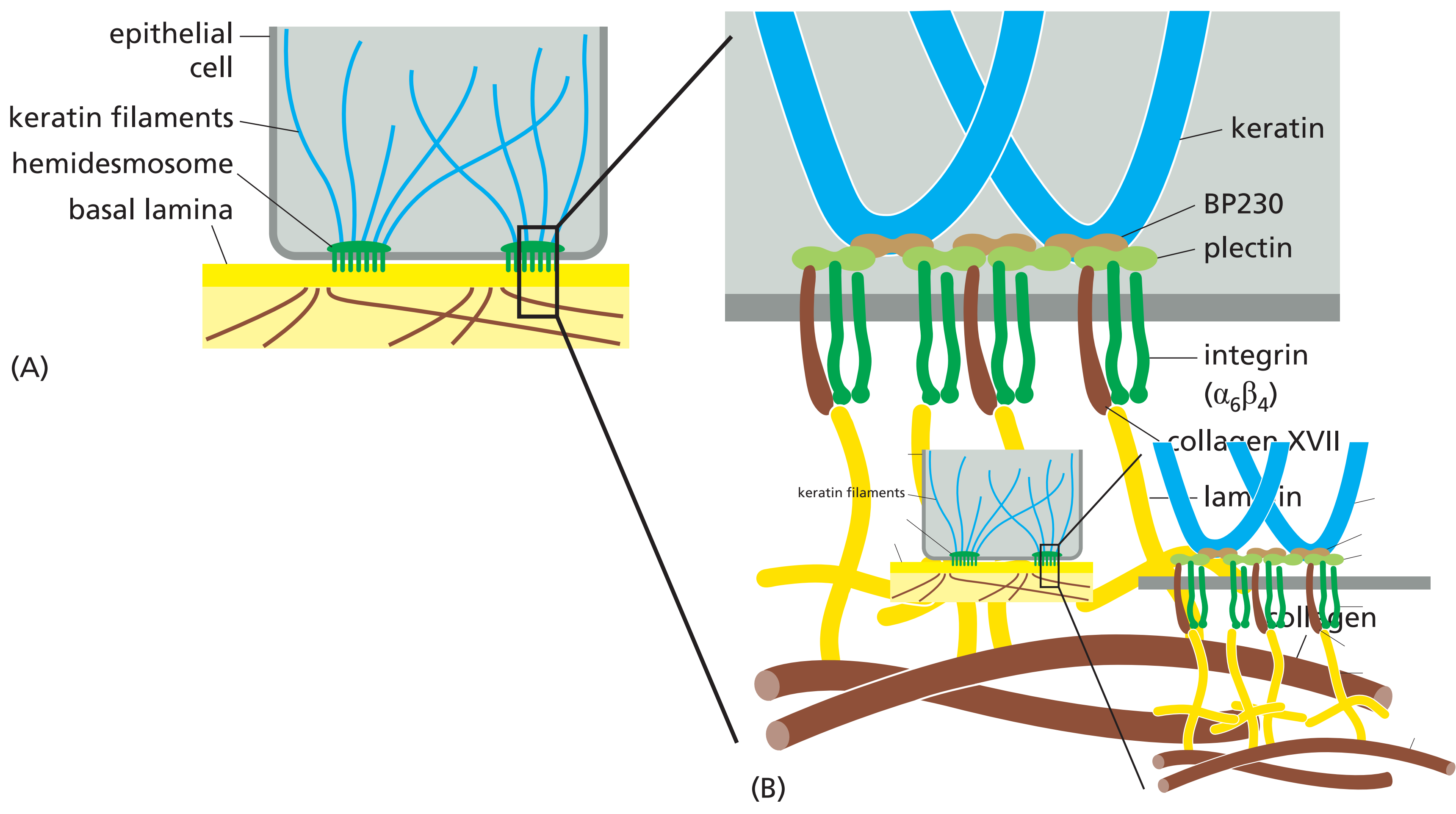


Figure 19–56 Hemidesmosomes. (A) Hemidesmosomes spot-weld epithelial cells to the basal lamina, linking laminin outside the cell to keratin filaments inside it. (B) Molecular components of a hemidesmosome. A specialized integrin ($\alpha_6\beta_4$ integrin) spans the membrane, attaching to keratin filaments intracellularly via adaptor proteins called plectin and BP230, and to laminin extracellularly. The adhesive complex also contains, in parallel with the integrin, an unusual collagen family member known as 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. One such disease, called *bullous pemphigoid*, is an autoimmune disease in which the immune system develops antibodies against collagen XVII or BP230.

Two conformations of integrins

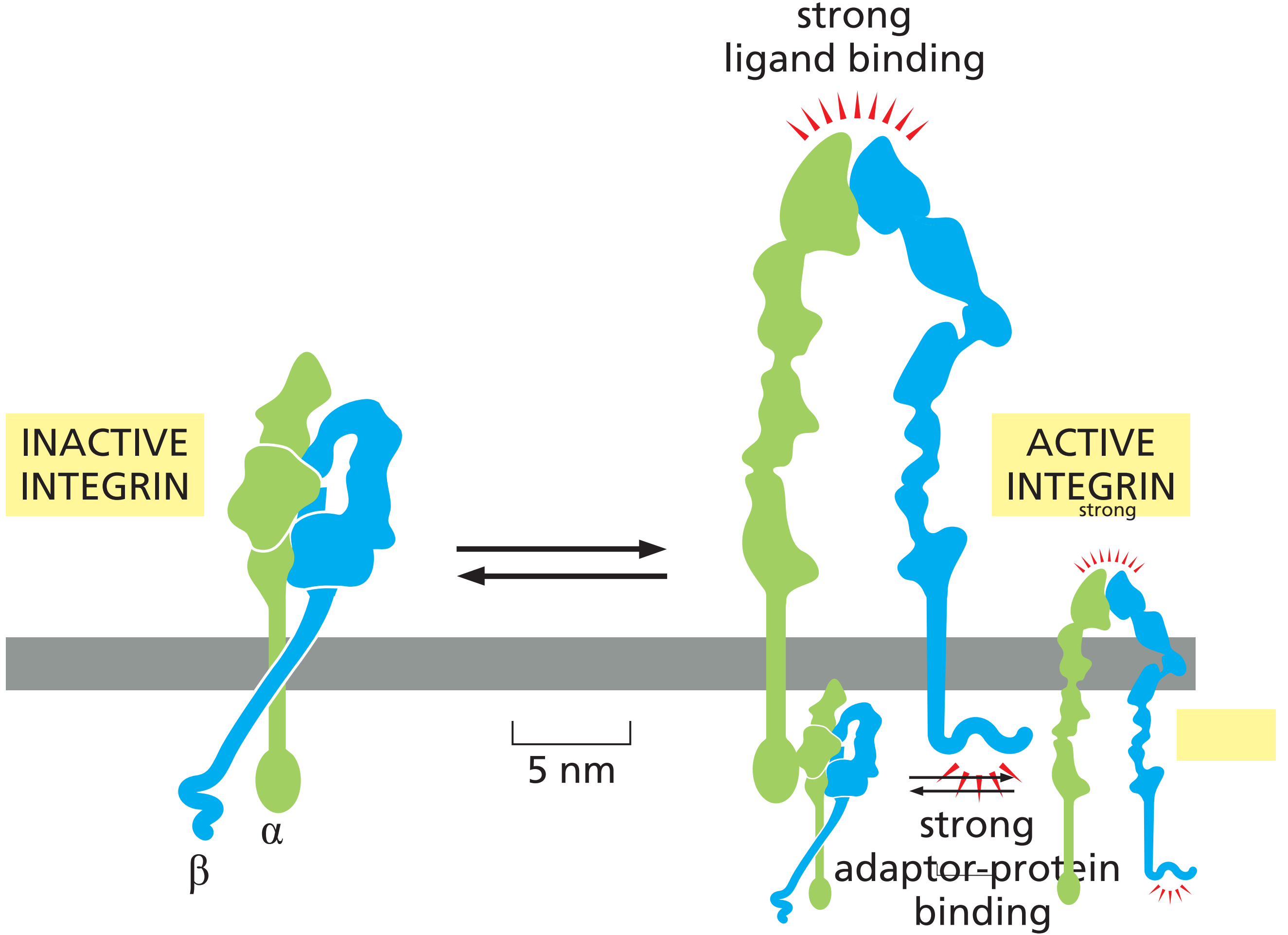


Figure 19–57 Integrins exist in two major activity states. Inactive (folded) and active (extended) structures of an integrin molecule, based on data from x-ray crystallography and other methods.

Talin is an integrin interaction molecule

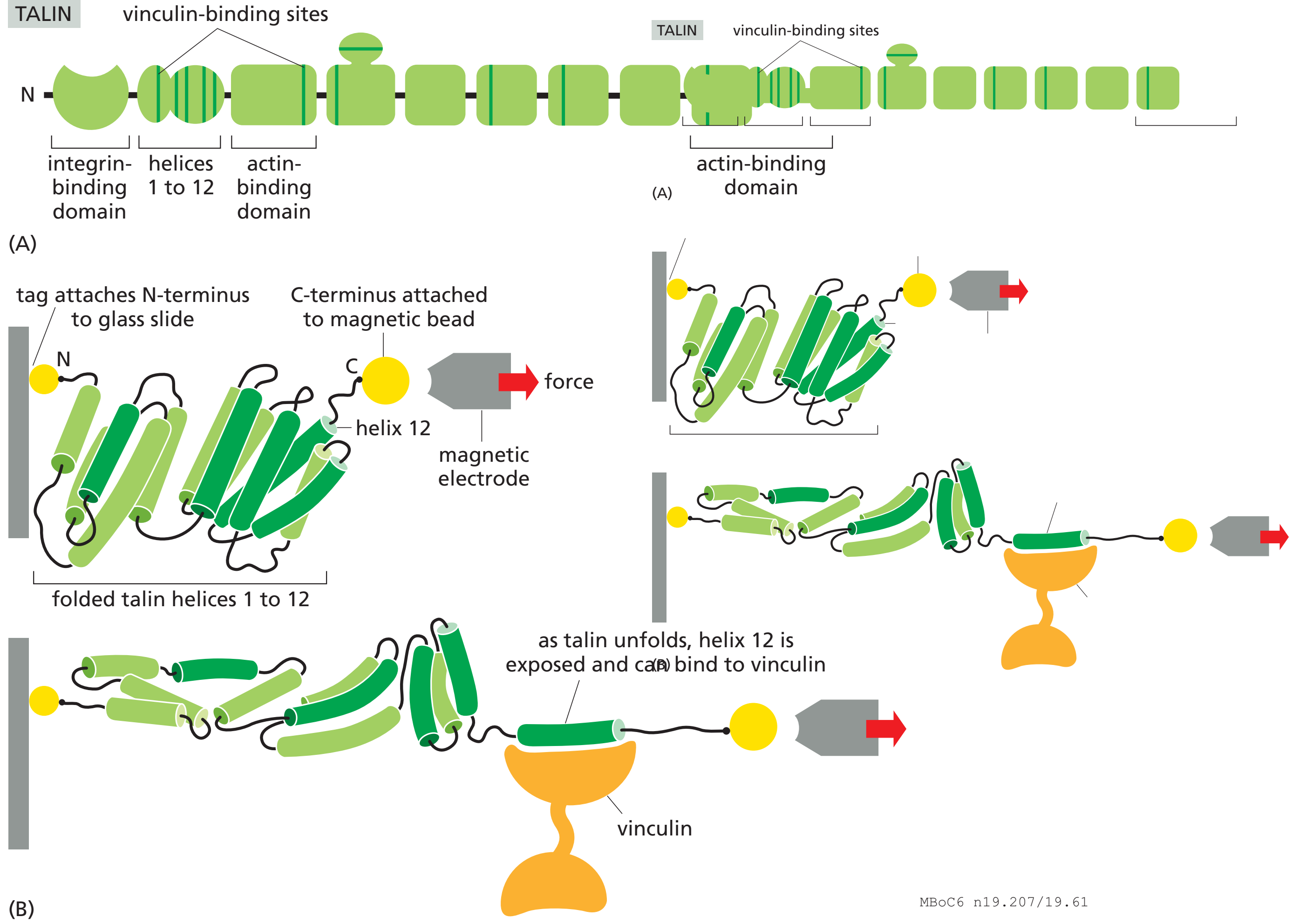
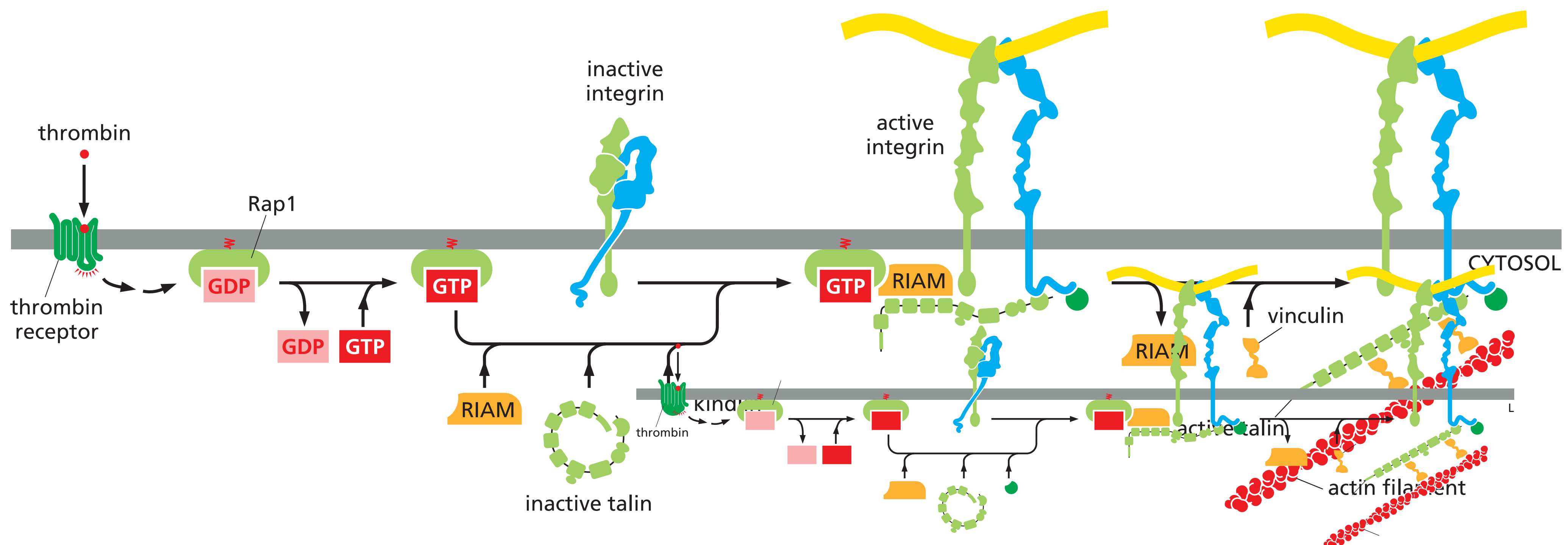


Figure 19–60 Talin is a tension sensor at cell–matrix junctions. Tension across cell–matrix junctions stimulates the local recruitment of vinculin and other actin-regulatory proteins, thereby strengthening the junction's attachment to the cytoskeleton. The experiments presented here tested the hypothesis that tension is sensed by the talin adaptor protein that links integrins to actin filaments (see Figure 19–55). (A) The long, flexible, C-terminal region of talin is divided into a series of folded domains, some of which contain vinculin-binding sites *(dark green lines)* that are thought to be hidden and therefore inaccessible. One domain near the N-terminus, for example, comprises a folded bundle of 12 α helices containing five vinculin-binding sites. (B) This experiment tested the hypothesis that tension stretches the 12-helix domain, thereby exposing vinculin-binding sites. A fragment of talin containing this domain was attached to an apparatus in which the domain could be stretched, as shown here. The fragment was labeled at its N-terminus with a tag that sticks to the surface of a glass slide on a microscope stage. The C-terminal end of the fragment was bound to a tiny magnetic bead, so the talin fragment could be stretched using a small magnetic electrode. The solution around the protein contained fluorescently tagged vinculin proteins. After the talin protein was stretched, excess vinculin solution was washed away, and the microscope was used to determine if any fluorescent vinculin proteins were bound to the talin protein. In the absence of stretching *(top)*, most talin molecules did not bind vinculin. When the protein was stretched *(bottom)*, two or three vinculin molecules were bound (only one is shown here for clarity). (Adapted from A. del Rio et al., *Science* 323:638–641, 2009.)

Activation of integrin signaling



Of note many other receptors can activate Rap1!

Figure 19–58 Activation of integrins by intracellular signaling. Signals received from outside the cell can act through various intracellular mechanisms to stimulate integrin activation. In platelets, as illustrated here, the extracellular signal protein thrombin activates a G-protein-coupled receptor on the cell surface, thereby initiating a signaling pathway that leads to activation of Rap1, a member of the monomeric GTPase family. Activated Rap1 interacts with the protein RIAM, which then recruits talin to the plasma membrane. Together with another protein called kindlin, talin interacts with the integrin β chain to trigger integrin activation. Talin then interacts with adaptor proteins such as vinculin, resulting in the formation of an actin linkage (see Figure 19–55).

Talin regulation depends in part on an interaction between its flexible C-terminal rod domain and the N-terminal head domain that contains the integrin-binding site. This interaction is thought to maintain talin in an inactive state when it is free in the cytoplasm. When talin is recruited by RIAM to the plasma membrane, the talin head domain interacts with a phosphoinositide called $PI(4,5)P_2$ (not shown here, but see Figure 15–28), resulting in dissociation of the rod domain. Talin unfolds to expose its binding sites for integrin and other proteins.

Integrins interact with the ECM

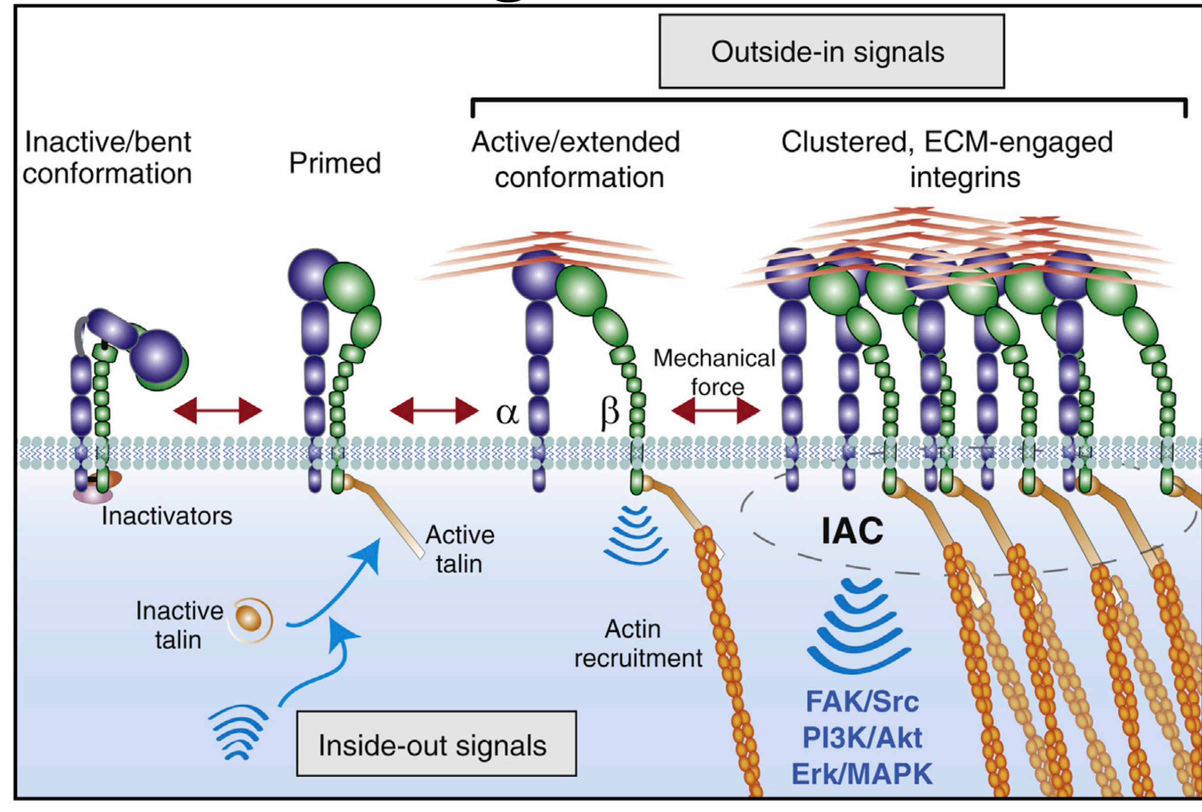


Figure 1. Integrin activation at the plasma membrane

Integrin activation at the plasma membrane involves the progression of αβ heterodimers from a bent to a primed conformation, then to an extended conformation upon both ligand binding and application of mechanical force, which promotes clustering and integrin adhesion complex (IAC) formation. This then leads to reinforcement of the link with the actin cytoskeleton and downstream signal transduction. Figure and legend are reproduced from Chastney, M.R., Curr Biol 31, R536-R542, with permission of the publishers.

Different integrins interact with different components!

Arginine – Glycine – Aspartic acid
RGD are the 3 amino acids in fibronectin interacting with the integrin a5b1

Focal adhesions can be visualized

Focal Adhension Kinase

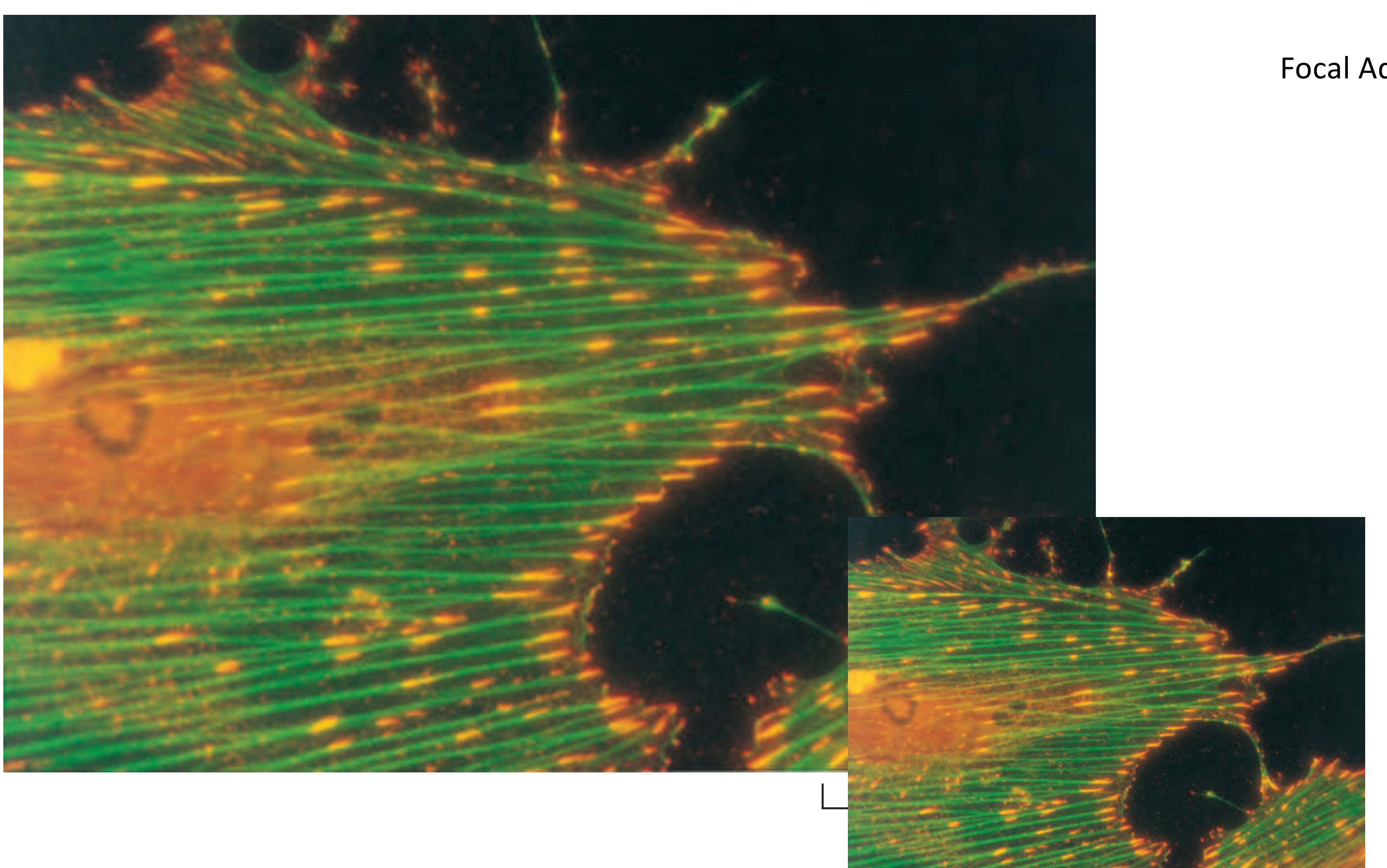


Figure 19–59 **Tyrosine phosphorylation at focal adhesions.** A fibroblast cultured on a fibronectin-coated substratum and stained with fluorescent antibodies: actin filaments are stained *green* and activated proteins that contain phosphotyrosine are *red,* giving *orange* where the two components overlap. 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. (Courtesy of Keith Burridge.)

A model of how forces generated in the actin-rich cortex move a cell forward

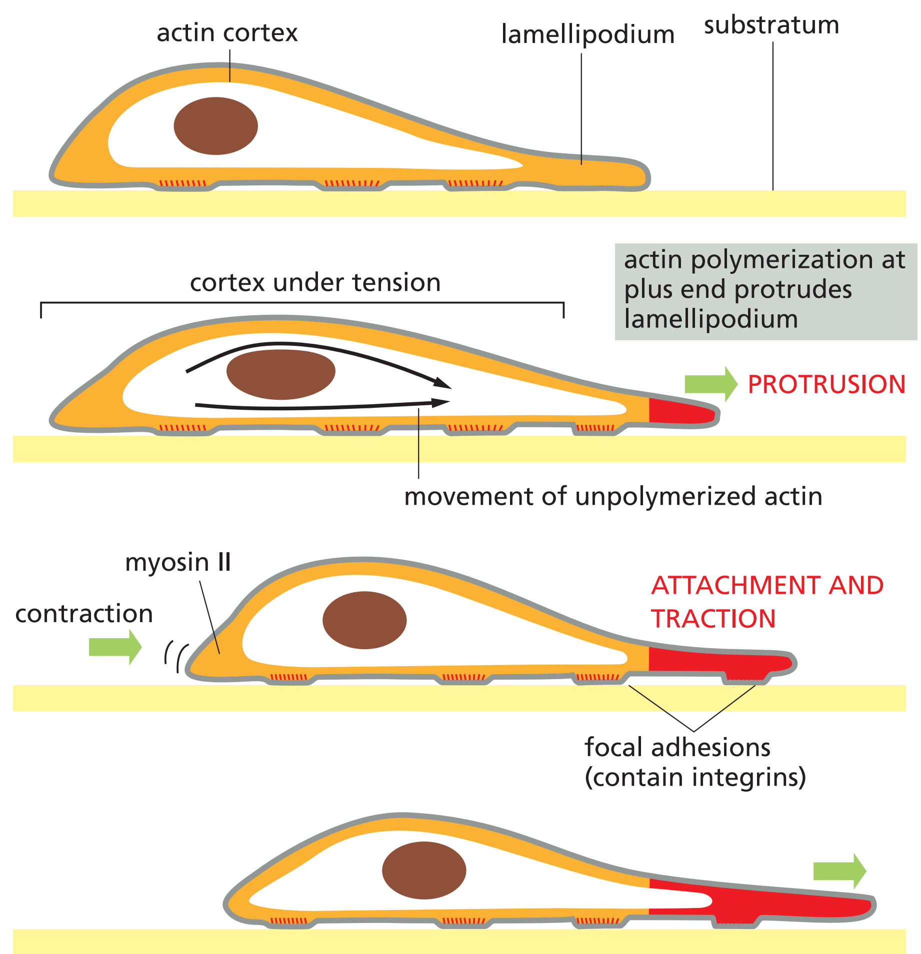


Figure 16–75 A model of how forces generated in the actin-rich cortex move a cell forward. The actin-polymerization- dependent protrusion and firm attachment of a lamellipodium at the leading edge of the cell move the edge forward (*green* arrows at front) and stretch the actin cortex. Contraction at the rear of the cell propels the body of the cell forward (*green* arrow at back) to relax some ofthe tension (traction). New focal contacts are made at the front, and old ones are disassembled at the back as the cell crawls forward. The same cycle can be repeated, moving the cell forward in a stepwise fashion. Alternatively, all steps can be tightly coordinated, moving the cell forward smoothly. The newly polymerized cortical actin is shown in *red*.

Integrins are involved in cell migration as well

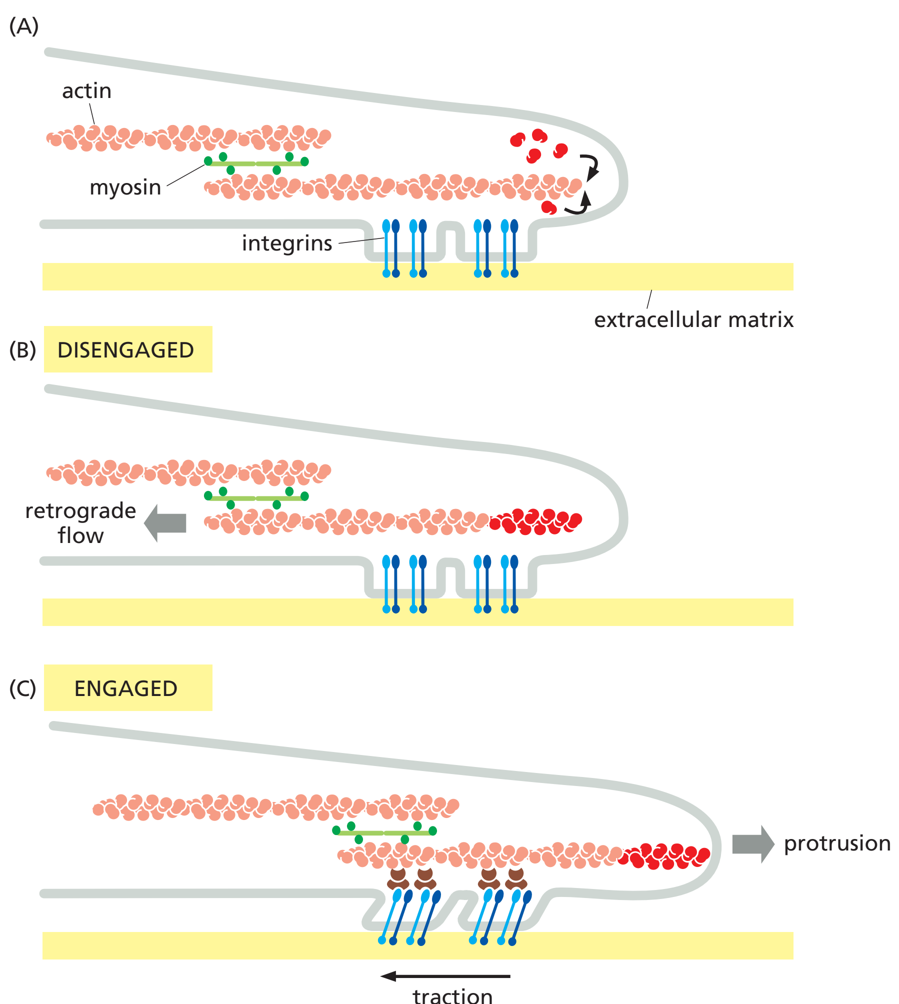


Figure 16–82 Control of cell– substratum adhesion at the leading edge of a migrating cell. (A) Actin monomers assemble on the barbed end of actin filaments at the leading edge. Transmembrane integrin proteins *(blue)* help form focal adhesions that link the cell membrane to the substrate. (B) If there is no interaction between the actin filaments and focal adhesions, the actin filament is driven rearward by newly assembled actin. Myosin motors *(green)* also contribute to filament movement. (C) Interactions between actin-binding adaptor proteins *(brown)* and integrins link the actin cytoskeleton to the substratum. Myosin-mediated contractile forces are then transmitted through the focal adhesion to generate traction on the extracellular matrix, and new actin polymerization drives the leading edge forward in a protrusion.