CHAPTER 6

Actin and Membranes

Although actin is present throughout the cell, it is most highly concentrated in the region close to the plasma membrane. As a broad generalization (to which there are many exceptions), actin can be thought of as forming a peripheral layer around the cell, like an elastic stocking or a muscular skin. This layer, known as the *cell cortex*, gives the outer surface of the cell mechanical strength and enables it to move. Thanks to its actin cortex, an animal cell can produce extensions of its surface, crawl, engulf particles, and deform its external shape, as it does when it undergoes division. The invention of the cortex during cellular evolution, like the appearance of an opposable thumb in the evolution of primates, provided the necessary basis for the emergence of an entire range of properties. Indeed, all of the most distinctive features of higher animals, such as their complex internal anatomy, their immunity to infection and their complex nervous systems, depend on the actin cortex.

In this chapter we describe the principal molecular components of the cell cortex, and some of the specialized structures it forms. We will find that this part of the cytoskeleton is highly complicated in structural and biochemical terms and that there is still much to learn.

Filamin and α-actinin cross-link actin filaments

The largest and most clearly defined membrane cortices are seen in free-living amoebae and in those vertebrate cells that are 'free living' in the sense that they migrate independently through tissues. The cortex of a human macrophage, for example, is a layer some 0.2 to 0.5 μm thick beneath the plasma membrane that excludes most cytoplasmic organelles (Figure 6-1). It is made of a dense three-dimensional network of filaments tightly adherent to the plasma membrane that can be co-isolated with it

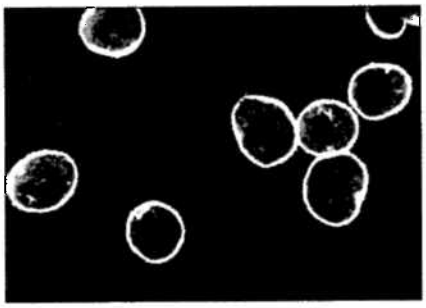


Figure 6-1 Actin cortex of rat liver cells. The cells were isolated from the liver and attached to a glass coverslip before being stained with rhodamine-phalloidin, a fluorescent dye that binds to actin filaments. The sharp plane of focus of the confocal microscope reveals the thick actin cortex beneath the plasma membrane as a peripheral ring of fluorescence. (Courtesy of I. Meijerman et al, *Biochem. Biophys. Res. Commun.* 240: 697–700, 1997. © Academic Press.)

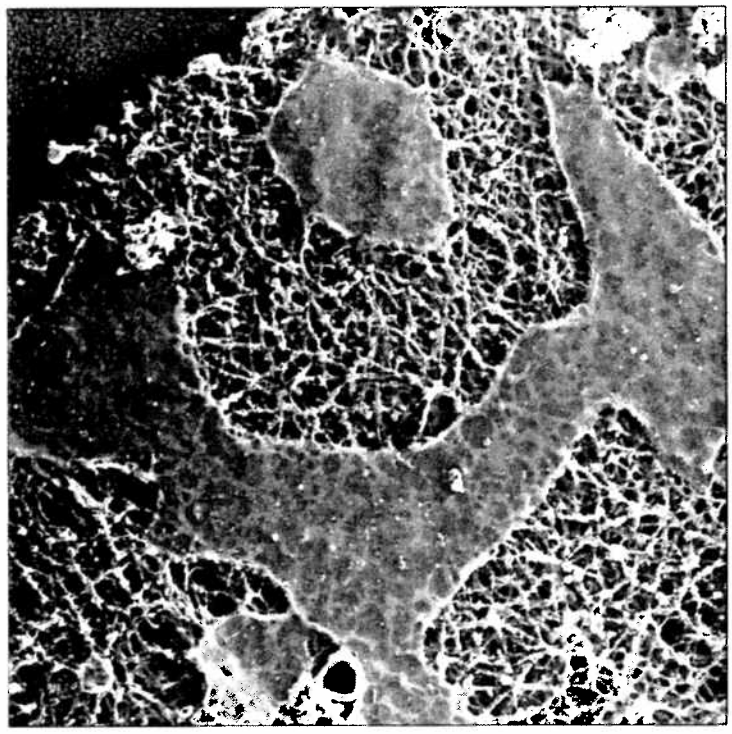
from disrupted cells (Figure 6-2). Within the cortex, actin filaments are linked together in a three-dimensional network with small pores (about 0.1 μm wide). Only small vesicles can pass through the network, thereby restricting the entry of larger organelles and granules, and this probably accounts for the hyaline (that is, 'glassy') appearance of the cell periphery in the light microscope.

The gel-like properties of the actin cortex are due to proteins that cross-link actin filaments, the most abundant of which, in vertebrate cells, are α-actinin and filamin. *α-Actinin* is composed of two polypeptide chains, each about 100 kDa in molecular weight. Each chain has three domains: an actin-binding domain at the N-terminus, a flexible central domain with similarities to spectrin (discussed below), and a calcium-binding domain. The two chains of α-actinin associate in an antiparallel fashion, bringing the actin-binding domain of one chain close to the calcium-binding site on the other chain. *Filamin*, also known as actin-binding protein (ABP-280), is similar to α-actinin, except that it is a larger protein and it forms a more open dimer, the subunits being attached mainly at their C-terminal ends (Figure 6-3). Perhaps because of its more open construction, filamin provides a flexible link between adjacent actin filaments.

Even small amounts of α-actinin or filamin have a dramatic effect on the physical properties of a solution of actin filaments, changing it from a viscous solution to a semisolid gel. This is especially true of the long, flexible molecule filamin. One filamin dimer per 200 molecules of actin monomers (about the ratio found in macrophages) is enough to create a large, continuous network whose pore size is further reduced by X-shaped entanglements between filaments. Synthetic actin gels made in this way have interesting mechanical properties, preserving their form when subjected to a sudden force but readily deforming under a low, steady pressure. This may be because the links have time to dissociate when the gel is subjected to a low, steady pressure.

α-Actinin and filamin are members of a large family of related actin cross-linking proteins. These all share a sequence of about 226 amino

Figure 6-2 Network of actin filaments in the cell cortex. Actin cortex of a cultured fibroblast exposed by partial extraction with a nonionic detergent and viewed by fast-freeze, deep-etch electron microscopy. Portions of the plasma membrane have been dissolved away revealing the underlying network of filaments. Filaments in the network are linked by proteins such as filamin and α-actinin into a three-dimensional network with the properties of a gel. (V. Small, *Electron Microscopy Rev.* 1: 165, © 1998 with permission from Elsevier Science.)



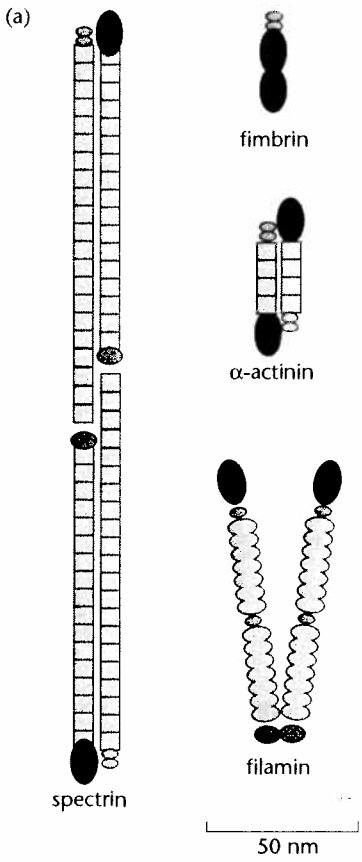
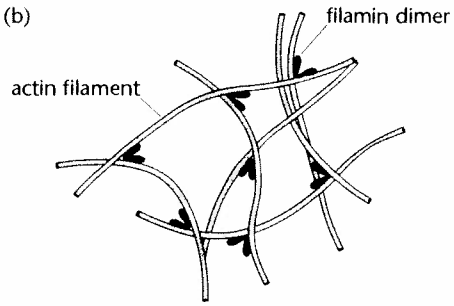


Figure 6-3 Actin cross-linking proteins. (a) Summary of the modular structures of four actin cross-linking proteins. Each of the proteins shown has two actin-binding sites (black) that are related in sequence. Fimbrin has two directly adjacent actin-binding sites, so that it holds its two actin filaments very close together (14 nm apart), aligned with the same polarity. The two actin-binding sites in α-actinin are more widely separated and are linked by a somewhat flexible spacer 30 nm long, so that it forms actin filament bundles with a greater separation between the filaments (40 nm apart) than does fimbrin. Filamin has two actin-binding sites that are very widely spaced, with a V-shaped linkage between them. Spectrin is a tetramer of two α and two β subunits, and the tetramer has two actin-binding sites spaced about 200 nm apart. The spacer regions of these various proteins are built in a modular fashion from repeating units that include α-helical motifs, β-sheet motifs, and Ca^{2+}-binding domains. (b) How a few filamin molecules can link actin filaments together, changing a solution of actin filaments into a gel.

acids that forms their actin-binding domain, but differ in the way these domains are linked together (see Figure 6-3). Other members of the family include spectrin and dystrophin, highly flexible actin cross-linking proteins found closely associated with the plasma membrane, and fimbrin, which is less flexible and is found mainly in parallel bundles of actin filaments. In cortexillin, a novel member of this family recently found in *Dictyostelium discoideum*, the two actin-binding domains are linked by a two-stranded coiled-coil domain.

Actin-fragmenting proteins rapidly reorganize the cortex

If a dense suspension of white blood cells is homogenized in cold buffer lacking calcium ions, centrifuged to remove nuclei and large pieces of debris, and the fluid extract then warmed, it can be seen to set to a gel. Gels prepared in this way, which are composed largely of actin, have interesting physical properties: they can be induced to dissolve, contract, or produce streaming movements by changing the temperature and the concentration of ions and small molecules (especially calcium ions and ATP). These transformations mimic changes that take place in the cortex as cells move or change their shape. Such changes are produced by the action of multiple actin-binding proteins (in addition to α-actinin and filamin already mentioned). They include myosin—a motor protein described in detail in the following chapter that generates movement in combination with actin filaments—and gelsolin, one of a family of actin-fragmenting proteins.



Gelsolin is a compact protein of about 80,000 Da (780 amino acids) found in most vertebrate cells. Purified gelsolin has three distinct effects on actin: (1) it binds to actin monomers to promote actin nucleation; (2) it caps the fast-growing barbed end of actin filaments, preventing addition of further monomers to that end; (3) it severs actin filaments by breaking bonds between adjacent actin monomers. The effects of the protein are regulated by Ca^{2+} ions and by phosphoinositides, especially PIP_2. A transient rise in calcium ions promotes binding of gelsolin to actin and severing of filaments, whereas PIP_2 detaches gelsolin from the ends of actin filaments. Repeated cycles of this kind fuel the generation of short actin filaments, for example in the leading lamellipodium of a migrating cell (Chapter 8).

Like many proteins associated with the cytoskeleton, gelsolin is built on a modular plan, composed of a series of independently functioning domains. It has six similar domains, all of which have some affinity for actin. Most of the severing activity resides within the first two domains. Truncated versions lacking these two domains exhibit

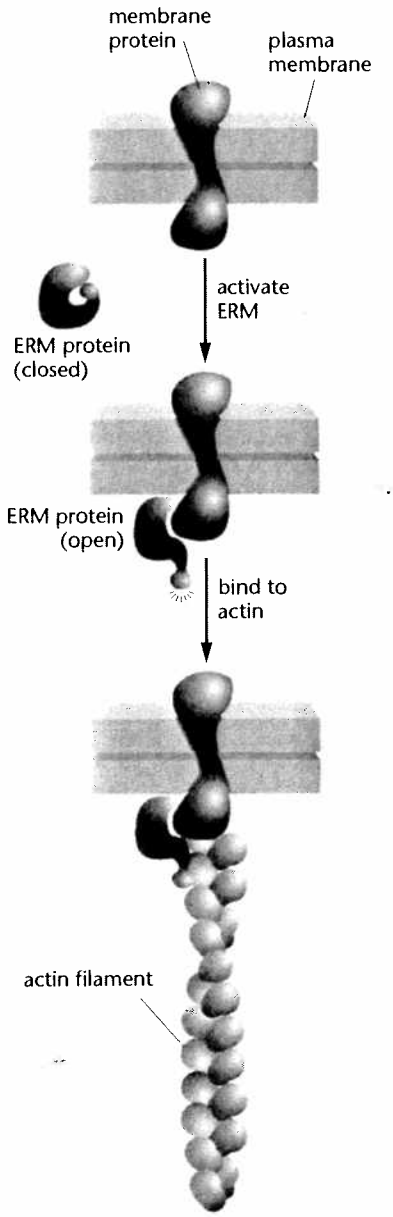


Figure 6-4 ERM proteins link actin filaments to membranes. ERM proteins have a globular head domain that interacts with integral membrane proteins and a tail domain that binds to actin filaments. In the inactive state, the molecule is folded into compact conformation in which the head and the tail domains inhibit each other. In the active, open state both membrane-binding and actin-binding domains are exposed. The closed-to-open conformational change is triggered by phosphorylation of the ERM protein.

calcium-independent severing, and injection of this molecule into living cells causes a rapid, calcium-insensitive destruction of actin filaments.[1] A number of other related proteins are known that also have actin severing activity, including severin and fragmin from protozoa, and villin, a major protein of intestinal microvilli described below.

Actin filaments attach, usually indirectly, to the plasma membrane

Actin can be linked to the plasma membrane in a variety of ways. The simplest mode of attachment is by direct binding to an integral membrane protein (that is, a protein so firmly embedded in the plasma membrane that it can be removed only by dissolving the lipid bilayer). In the slime mold *Dictyostelium*, for example, a 17 kDa integral membrane protein called *ponticulin* binds directly to actin filaments. A second protein, *cortexillin*, is able both to bundle actin filaments and to link them to the plasma membrane. Vertebrate cells also contain proteins, such as *calpactin* and *lipocortin*, that associate both with actin and phospholipids and might therefore mediate the direct association of actin to membranes.

However, the more usual interaction is indirect, and many proteins are known to act as linkers, or adapters, between actin filaments and membrane proteins. Both α-actinin and filamin can act in this way, as well as numerous other specific proteins in red blood cells, in cell junctions, and in actin-rich extensions of the cell surface. Each membrane linker is adapted to function in its particular location but all must have at least two distinct domains, one that binds to actin and another that binds to an integral membrane protein.

A good example is seen in the group of proteins known collectively as *ERM proteins* (for *e*zrin, *r*adixin, and *m*oesin, the principal members of the group). These proteins are widespread in vertebrate cells, found in cleavage furrows, microvilli, ruffling membranes, and other locations. ERM proteins are short molecules with a globular N-terminal domain and a rodlike, α-helical tail (Figure 6-4). The globular head interacts with integral membrane proteins whereas the C-terminal tail binds to actin. Interestingly, head and tail domains of ERM proteins are mutually inhibitory so that the protein can switch itself off by folding into a hairpin conformation—a switch-like feature shared by other cytoskeletal proteins such as vinculin and myosin. The ERM switch is probably activated by phosphorylation of the protein by a kinase that is under the control of the small G protein Rho described below.

The red blood cell membrane is supported by a network of spectrin and actin

One of the best-characterized associations of actin with the plasma membrane is seen in the thin membrane cortex of red blood cells. Mammalian red blood cells (erythrocytes) are highly specialized cells that lack internal organelles, microtubules, and intermediate filaments.[2] Their membrane is

[1] Gelsolin is a major substrate for caspase, one of the proteases responsible for programmed cell death (apoptosis). Proteolysis with caspase releases a gelsolin fragment that severs actin filaments in an uncontrolled manner perhaps causing dying cells to round up and detach from the substratum.

[2] The red blood cells of birds and amphibians have a more complicated structure. They retain their nucleus and their shape is influenced by a bundle of microtubules that runs around their perimeter (described in Chapter 19).

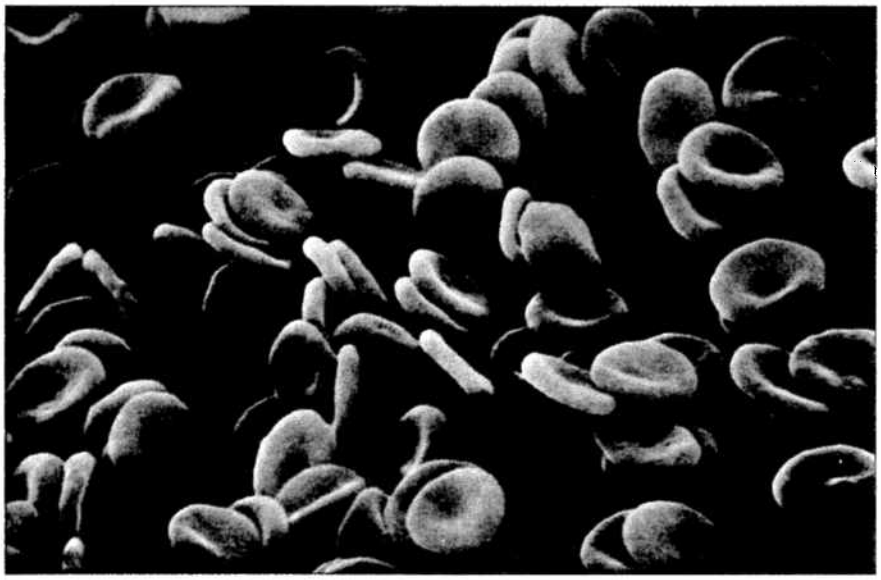


Figure 6-5 Scanning electron microscopy of human erythrocytes. (Courtesy of Bernadette Chailley.)

therefore the only means these bags of hemoglobin have to maintain their distinctive biconcave shape (Figure 6-5). The cell 'skin,' about 20 nm thick, comprises the plasma membrane together with a layer of associated protein. Although it is very flexible, this skin must also be tough: during its lifetime of 120 days, a human erythrocyte travels approximately 300 miles through narrow and crowded blood vessels.

The erythrocyte cortex can be obtained by lysing the cell by osmotic shock and collecting the empty sacs by centrifugation. Known as *erythrocyte ghosts* because of their transparency, these flexible sacs reseal under appropriate conditions and can regain their original shape if ATP is present. The membrane skeleton evidently plays an important part in this shape recovery since, if the membrane skeleton is detached from the inner face of the plasma membrane by mild proteolysis, the ghost cell does not return to its original biconcave disk form.

If the lipid component of the membrane is extracted by detergent, it leaves behind a shell of protein composed of a two-dimensional network of interconnected threads (Figure 6-6; Table 6-1). A major component of this network is a large dimeric protein, called *spectrin* for its ghostly origin. Spectrin is made of two large polypeptides, α-spectrin (280 kDa) and β-spectrin (246 kDa), each a flexible beads-on-a-string chain of repeating units containing about 106 amino acids. The repeating units consist of three α-helical strands wrapped together in a short coiled-coil (similar

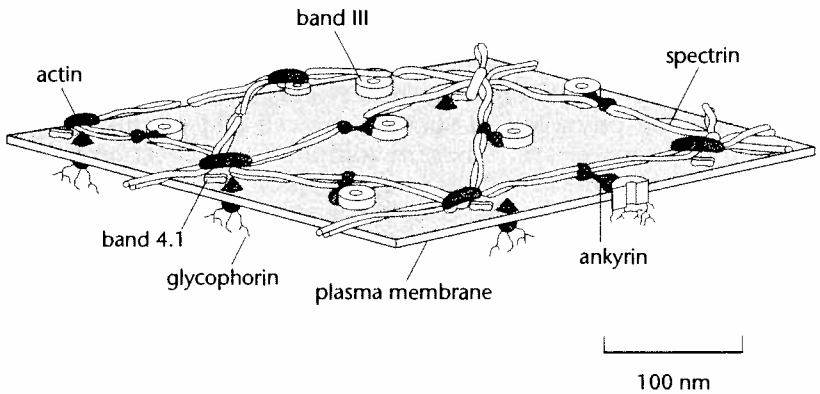


Figure 6-6 Schematic diagram of the spectrin-actin network on the inner face of the red blood cell membrane. Two regions of attachment to the plasma membrane are indicated, one mediated by ankyrin and band III, and the other by band 4.1 and glycophorin. The actual network is far more compact and complex than shown here.

Table 6-1 Proteins of the red blood cell cortex

Protein	Size (kDa)	Copies per cell	Relative number	Number per μm^2
INTEGRAL MEMBRANE PROTEINS				
band III	105	1,200,000	12	8000
glycophorin dimer	50	1,000,000	10	6600
CORTICAL PROTEINS				
spectrin, dimer	280/246	200,000	2	1300
ankyrin	206	100,000	1	700
actin, monomer	43	500,000	5	3300
tropomyosin	29/27	80,000	0.8	530
band 4.1	78	200,000	2	1300
adducin α/β	103/97	30,000	0.3	300

Calculations based on a surface area for human erythrocytes of 150 μm^2. The human erythrocyte membrane skeleton also contains calmodulin, CapZ, tropomodulin, caldesmon, dematin, and other proteins.

units are also found in α-actinin). Dimers of spectrin associate into a tetramer with a length of 200 nm, which is the form found in the erythrocyte cortex. The ends of spectrin tetramers in the red blood cell cortex are linked to short lengths of actin filament (each containing about 12 actin monomers, which is enough to form one half-turn of the filament helix).

The importance of spectrin is revealed in a number of genetic disorders that affect the shape and stability of red blood cells. For example, hereditary anemia in both mice and humans is caused by a deficiency in the synthesis of spectrin. Humans with this condition may have as little as 5% of the normal amount of spectrin; their red blood cells are spherical in shape and extremely fragile.

Spectrin was originally thought to be unique to erythrocytes but is now known to belong to a family of closely related spectrin genes expressed in cells as diverse as neurons and amoebae. It is usually associated with membranes but is often enriched in specific locations, such as regions of cell–cell contact and in regions at which exocytosis occurs. Different forms of spectrin are present in the axon compared to the dendrites and cell bodies of mature nerve cells, in skeletal muscle, and (in birds) in the intestinal brush border.[3]

Ankyrin and band 4.1 link spectrin to the plasma membrane

Spectrin is attached to the erythocyte plasma membrane through ankyrin and band 4.1 (see Figure 6-6). *Ankyrin* links spectrin to the anion transporter, also known as band III protein, which is held firmly in the lipid bilayer by its hydrophobic interactions. *Band 4.1*, related to the ERM family of proteins mentioned above, binds to the C-terminus of spectrin chains, to actin, and also to the cytoplasmic tail of *glycophorin*—a heavily glycosylated protein with a single transmembrane domain.

Significantly, band 4.1 attaches to glycophorin only when the signaling phospholipid PIP_2 is present. The concentration of this lipid changes rapidly with the physiological state of the cell and produces multiple effects on the cytoskeleton. Thus, in addition to an effect on band 4.1, PIP_2 also influences the activity of α-actinin, profilin, and gelsolin.

Just as spectrin has close relatives that are found in diverse cell types, so proteins similar to erythrocyte ankyrin and band 4.1 are widely distributed in vertebrate tissues. For example, synapsin-1, a prominent component of the cytoskeleton of neurons, is related to band 4.1. The binding of this protein to membrane-associated actin may play a part in the release of transmitter at synapses.

[3] Much of the diversity of mammalian spectrins arises from alternative mRNA splicing, in which the same gene produces multiple proteins. Variants of band 4.1 and many proteins of skeletal muscles are also produced in this manner (Chapter 10).

How does the cell regulate assembly of its spectrin network?

It is interesting to ask how the cortical layer of spectrin, ankyrin, and other proteins is built by the cell. Indeed, this is a general problem, since the cytoskeleton contains many complex assemblies of proteins each of which is built to precise specifications, with constituent proteins held in the correct numbers and locations. In the case of the spectrin network and others that have been analyzed in detail, it seems that posttranslational modifications and the selective degradation of unwanted molecules are both employed extensively.

As red blood cells form in the bone marrow, their membrane skeleton slowly matures from an undifferentiated and relatively labile network to a highly specific stable network of proteins. In the course of this refinement, crucial proteins are synthesized at different times, each contributing to the final fully stable network. The short segments of actin filaments associated with spectrin, for example, probably arise from the progressive depolymerization of longer filaments in a more open and irregular network. In a mature erythrocyte, these short actin rods are capped at either end by specific proteins (adducin and tropomodulin) which are also involved in the binding interactions that hold the network together.

Spectrin, the major component of the membrane skeleton, changes during erythropoiesis from the $\alpha\gamma$ isoform (also known as fodrin) common to many cell types, to the $\alpha\beta$ isoform unique to red blood cells. The changeover is a gradual process characterized by a progressive accumulation of erythrocyte spectrin and the downregulation and eventual disappearance of fodrin. Throughout the transitional period, both spectrin isoforms continue to be made, to assemble onto the plasma membrane, and to be degraded by endogenous proteases (perhaps calpain)—the α chain being at all times made in large excess.

The reason for this wasteful mode of assembly apparently lies in the high α-helical content of the spectrin molecule. All spectrin molecules have multiple repeating stretches of triple α-helices, 106 amino acids long, which interact with a heterologous spectrin to form large, antiparallel coiled-coil molecules. Since dimerization is substantially more rapid than synthesis, large quantities of unwanted oligomers such as $\alpha\alpha$- and $\beta\beta$-spectrin are initially formed, possibly assembling on the polysomes. With the formation of such homodimers, spectrin molecules are effectively trapped because of the high energy of activation required to unwrap the coiled-coil interactions. The only way to remove them may be by proteolysis.

A major factor in the stabilization of spectrin dimers is their anchorage to the plasma membrane. At an early stage of differentiation, the primary source of anchorage is ankyrin, which is able to interact directly with the phospholipid bilayer through the posttranslational addition of a fatty acid residue. Later, band III provides the principal anchorage for ankyrin and its attached spectrin. Other components such as band 4.1 and the membrane protein glycophorin all contribute to the final stable network in a complicated sequence of interactions.

Adherens junctions provide a stable anchorage for actin filaments

One of the most conspicuous sites of interaction between actin and the plasma membrane is an *adherens junction*. This is a location in an animal tissue cell where actin filaments come into close contact with the membrane and are linked, through integral membrane proteins, either to another cell or to the extracellular matrix.

In cell–cell adherens junctions the integral membrane proteins are *cadherins*, a family of Ca^{2+}-dependent cell adhesion proteins able to form

homophilic (that is, self–self) associations at their extracellular domain. Opposing faces of the junction are held together by the extracellular domains of cadherins, which bind each other. On the inside of the membrane, the cytoplasmic domains of cadherin molecules insert into a cluster of other proteins that itself is attached to actin filaments. In this way the adherens junctions forms a mechanically strong linkage between one cell and the next, holding them together in a tissue.

Adherens junctions are found between heart cells, at synapses in the central nervous system, and between folds of a myelin sheath. In epithelial cells they form a continuous adhesion belt (or zonula adherens) around each of the interacting epithelial cells between the apical and basal domains of the cell (Figure 6-7). A belt of actin filaments on the cytoplasmic side of the adhesion belt is potentially contractile. We will see in the final two chapters that it confers on the epithelial sheet the capacity to develop and change in remarkable ways, binding or rolling up into a tube or developing into a cup-shaped cavity, and so on.

Adherens junctions have a complicated structure that is still under investigation. There are, to begin with, multiple different subtypes of cadherins, each characteristic of the particular cell that makes them. Different subtypes of cadherins combine with different affinities, and this confers much of the specificity in cell–cell contacts. Proteins that attach to the cytoplasmic domains of cadherins are also highly specialized, the list including ERM proteins and α-actinin, as well as specialized proteins such as catenins and plakoglobins (Table 6-2).

Why should a 'simple' mechanical attachment contain so many different proteins and have such a complicated structure? One reason is that mechanical properties are actually not simple. The strength, elasticity, shear strength, and viscoelasticity of different parts of a cell are crucially important for their functioning and must be closely controlled in a dynamic way. The second reason is that, as we will discuss shortly in the context of focal adhesions, cell adhesions have not only a mechanical role but also a signaling one. Because of their privileged position immediately subjacent to the plasma membrane, junctional proteins relay

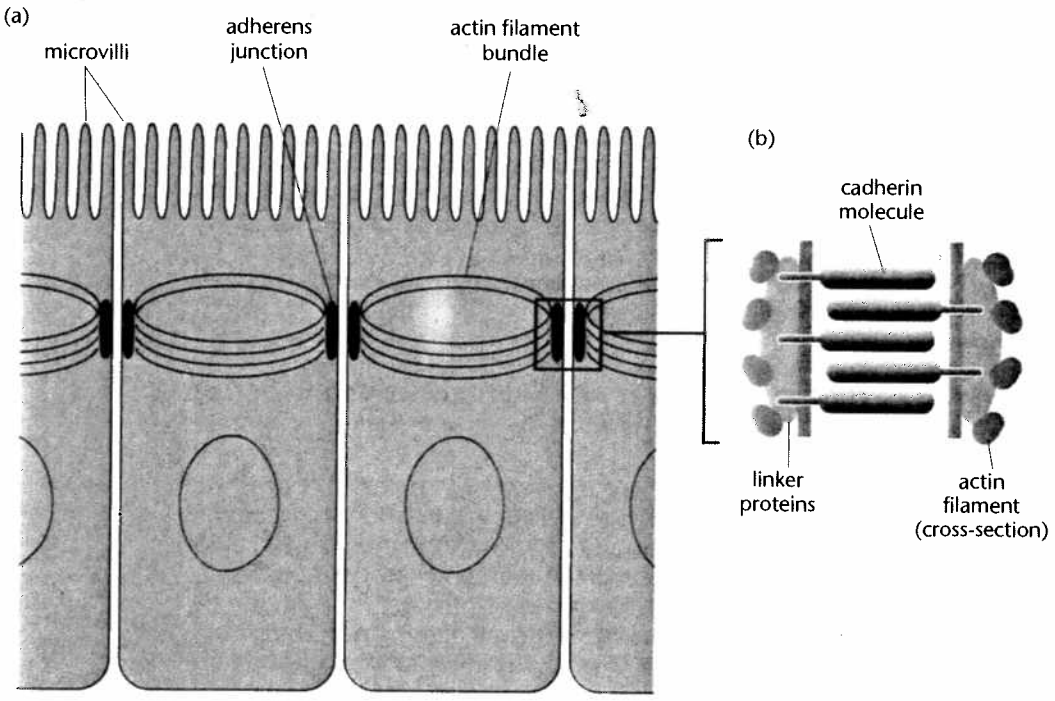


Figure 6-7 Adhesion belts between epithelial cells in the small intestine. (a) This beltlike anchoring junction encircles each of the interacting cells. Its most obvious feature is a contractile bundle of actin filaments running along the cytoplasmic surface of the junctional plasma membrane. (b) Actin filaments at an adherens belt are joined to those in a neighboring cell by transmembrane linker proteins (cadherins), which bind to identical cadherin molecules on the adjacent cell.

Table 6-2 Major proteins of cell–cell adherens junctions

Protein	Size (kDa)	Function
actin, monomer	43	major structural component
cadherin	~ 80	homophilic binding/integral membrane proteins
catenin	88/113	link cadherins to the cytoskeleton, β-catenin activates gene transcription
plakoglobin	82	binds cadherins and α-catenin
tensin	186	caps and cross-links actin filaments
vinculin	117	binds tensin and α-actinin
α-actinin	103	cross-links actin filaments, binds vinculin
zyxin	82	binds α-actinin
radixin	82	caps actin filaments
Src (pp60 $^{c\text{-}src}$)	43	protein tyrosine kinase
ena/VASP	46	nucleates actin filaments

information on the mechanical and chemical properties on the outside of the cell to its interior. We will see in Chapter 20 that the formation of cell-to-cell junctions is a vital stage in the formation of an embryonic tissue, which triggers many specific downstream events.

Cultured cells attach to their substratum at focal adhesions

A different type of adherens junction enables cells to attach to the extracellular matrix. Cultured fibroblasts migrating on a surface coated with extracellular matrix molecules, for example, form specialized *focal adhesions* where the plasma membrane is held approximately 15 nm from the substratum. Each focal adhesion is the site of attachment of a bundle of actin filaments, termed a *stress fiber* that terminates in a cluster of proteins attached to the plasma membrane. An important difference between these cell–substratum attachments and the cell–cell adherens junctions mentioned above is that the anchorage is mediated by integrins rather than cadherins. We will see below that integrins have binding sites for components of the extracellular matrix.

Focal adhesions may be visualized by reflection interference microscopy in which visible light is directed onto the 'underbelly' of cells growing on a thin glass coverslip (Figure 6-8). The junctions then appear as dark oval patches, typically 1–2 μm long but often longer, which form at the leading margin of the cell, remain fixed in position as the cell passes overhead, and detach farther back on the cell. Fibroblasts torn from the culture surface with a microelectrode or with a jet of fluid leave their focal adhesions behind, demonstrating that they are not only points of close contact but also sites of mechanical anchorage.

Formation of focal adhesions depends on the cell substratum and is typically promoted by extracellular matrix proteins such as fibronectin, laminin, and collagen. These multidomain proteins adhere to the glass or plastic surface of the tissue culture dish and present specific sequences for recognition by cell-surface receptors. The short sequence Arg-Gly-Asp (or 'RGD' using single-letter notation) found in the cell-binding domain of both fibronectin and laminin is especially important.

As just mentioned, focal adhesions are built around *integrins*: a large and diverse family of transmembrane proteins. Each integrin molecule is

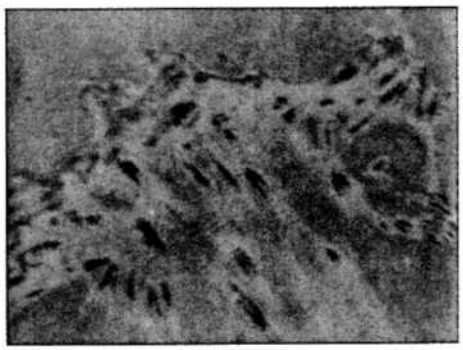


Figure 6-8 Focal adhesions. View of the underneath of a fibroblast using reflection interference microscope. The dark oval patches are focal adhesions. (Courtesy of Grenham Ireland.)

a noncovalently associated heterodimer of two distinct, high-molecular-weight polypeptides called α- and β-integrin, which act as transmembrane linkers in a variety of cells. Many subtypes of both α- and β-integrin exist, and these can combine to produce a huge variety of heterodimers, each with a selective affinity for a particular extracellular matrix molecule. Integrins are also found on blood platelets, where they are involved in blood clotting, and in lymphocytes and macrophages, where they play a role in the crucially important matrix interactions of these cells.

One domain of the integrin molecule is exposed on the cell surface and binds to molecules such as fibronectin or vitronectin in the extracellular space. At the other end of the integrin molecule, its cytoplasmic domain associates with a complex cluster of proteins. Some of these are similar to those found in cell–cell adherens junctions (α-actinin, vinculin, tensin), while others, such as talin, are found only in focal adhesions. Actin filaments from the cell cortex insert into this protein plaque, thereby becoming mechanically linked to the extracellular matrix on the other side of the plasma membrane (Figure 6-9).

Focal adhesions send and receive signals

Cell junctions are not simply sites of mechanical anchorage of the cell. They also function in cell communication. A cell that has formed focal adhesions knows that it has attached firmly to a suitable substratum, so it can now grow or differentiate. In reciprocal fashion, focal adhesions are less likely to be produced if a cell is actively dividing, or in the process of changing its shape. There is also evidence that the strength of attachment of focal adhesions to the extracellular matrix can be modulated by the cell, providing a clutchlike mechanism by which it can regulate its migratory speed (Chapter 8).

An interesting example of the cell's control over focal adhesions occurs during transformation by tumor viruses. Transformation typically changes the flattened form of a cell such as a fibroblast into a rounded

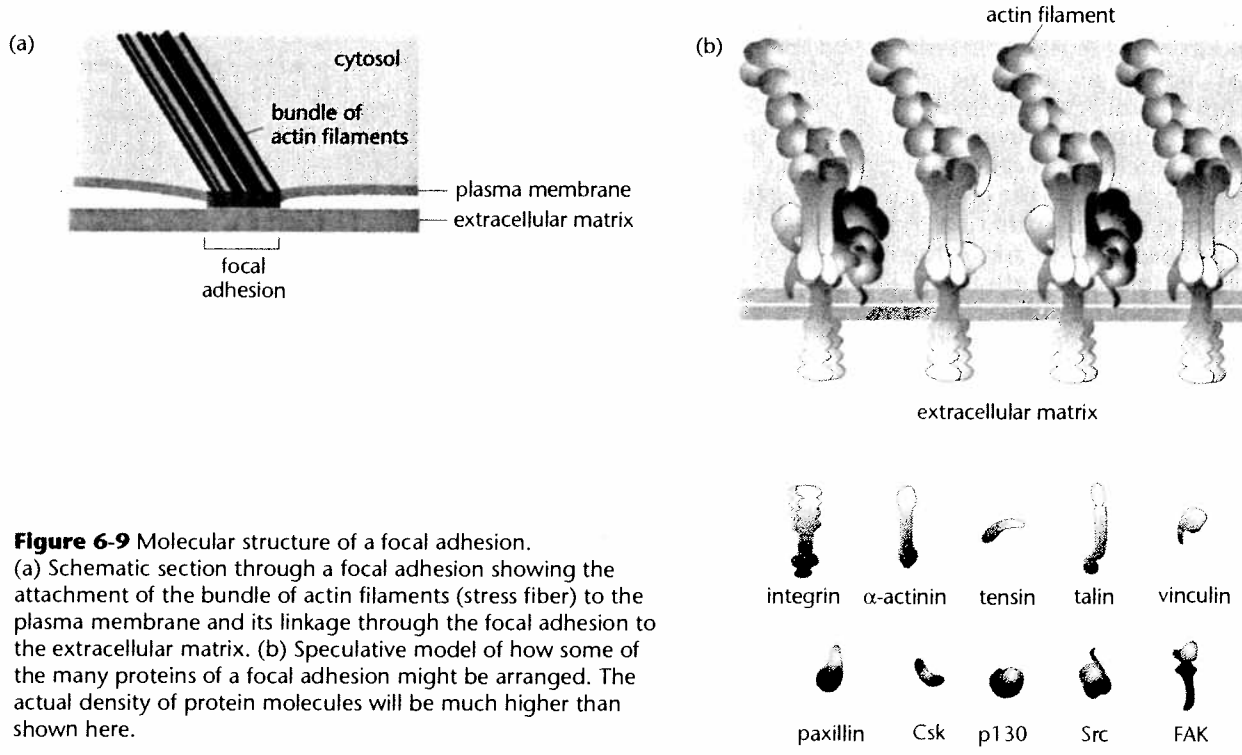


Figure 6-9 Molecular structure of a focal adhesion. (a) Schematic section through a focal adhesion showing the attachment of the bundle of actin filaments (stress fiber) to the plasma membrane and its linkage through the focal adhesion to the extracellular matrix. (b) Speculative model of how some of the many proteins of a focal adhesion might be arranged. The actual density of protein molecules will be much higher than shown here.

morphology, at the same time altering growth properties so that the cells are no longer responsive to contact inhibition by adjoining cells (and are therefore more likely to grow into a tumor). In the well-studied case of transformation of avian cells by the Rous sarcoma virus, changes are initiated by a specific tyrosine kinase (v-Src) produced by the virus. This kinase localizes to the focal adhesions and triggers their breakup and eventual degradation. In uninfected cells, a less active tyrosine kinase, known as c-Src helps to control the normal turnover of focal adhesions during cell motility.

Some proteins in a focal adhesion have a signaling rather than a mechanical or structural role (Table 6-3). The focal adhesion kinase, or FAK, for example, is an enzyme with a remarkably wide range of functions. It binds to structural components of the focal adhesion such as integrins as well as to the tyrosine kinase Src and the adapter protein Grb2. FAK phosphorylates tyrosines in various proteins in the focal adhesion, including itself, thereby causing other proteins with binding domains for phosphorylated tyrosines to cluster in this region. The complex both sends and receives signals passing between the focal adhesion and targets in the cytoplasm and nucleus.

We see that even though a focal adhesion is a large cytoskeletal-based structure, it also acts like a signaling complex. It receives multiple inputs from components of the extracellular matrix, mechanical tension, and phosphorylation signals, integrates them, and then produces multiple outputs that influence the growth and division of the cell as well as its state of differentiation.

Focal adhesions are triggered by both external and internal influences

How cells make focal adhesions is a topic of great interest. One of the earliest steps is thought to be the side-by-side association of integrin molecules to form a cluster in the membrane. In a fibroblast that has made contact with a suitable surface (one coated with vitronectin, for example), integrin molecules diffusing in the membrane become trapped when they bind to the extracellular matrix. A set of tethered integrin molecules is produced that under normal conditions then matures into a focal adhesion.

However, a suitable surface for the attachment of integrin molecules is not sufficient by itself. It is possible to prevent focal adhesions from forming even after integrin molecules have collected together. Thus, if cells are exposed to inhibitors of protein phosphorylation, or if certain signaling components are inactivated, then focal adhesions will not form. A widely used experimental protocol employs fibroblasts attached to

Table 6-3 Major proteins of focal adhesions

Protein	Size (kDa)	Function
actin	43	major structural component
integrin (α and β)	~ 100	integral membrane proteins, bind extracellular matrix molecules
talin	270	binds vinculin, actin, and integrins
FAK	125	protein tyrosine kinase, binds integrins
tensin	186	caps and cross-links actin filaments
vinculin	117	binds α-actinin and talin
α-actinin	103	cross-links actin filaments, binds vinculin
paxillin	68	binds vinculin and FAK
Src (pp60 $^{c\text{-}src}$)	43	protein tyrosine kinase
p130	130	substrate for Src, binds tensin

Focal adhesions also contain fimbrin (actin-bundling), VASP (actin-nucleating), PI_3-kinase (phospholipid signaling), calpain (proteolysis), and many other proteins.

surfaces in media lacking serum. Such cells lack focal adhesions (which can be detected by staining for vinculin) but make them rapidly following the injection of specific signaling components. The most important of the latter is the small GTP-containing protein Rho, which, as we will see shortly, has a central role in the control of many actin–membrane associations.

The final ingredient needed to make a large, mature focal adhesion is mechanical tension. If a fibroblastic cell in culture is stretched mechanically by the stroking action of an electrode on its upper surface, then focal adhesions on its lower surface become larger and oriented in the direction of the stretching. Evidently mechanical forces lead directly to the recruitment of more focal adhesion proteins and the insertion of more actin filaments. Functionally this is exactly what one would expect, since the purpose of a focal adhesion is to resist tensile forces experienced by the cell. But the synergism it reveals between biochemical and mechanical forces is impressive, and one that we will encounter repeatedly in the operation of the cytoskeleton. Mechanical aspects of the cytoskeleton are the subject of Chapter 18.

Rho GTPases control actin's association with the membrane

The protein *Rho* belongs to a large family of over 40 GTP-binding proteins (*G proteins*) known as the Ras superfamily, which relay intracellular signals and regulate many processes in cells. Many human cancers, for example, can be produced by oncogenic mutations in Ras genes. In general, these proteins function as self-inactivating molecular switches that exist in either an active state, when GTP is bound, or an inactive state, when GDP is bound ('active' in this context meaning that the molecule triggers downstream signaling events in the cell). GTP addition and loss of GTP are catalyzed by other proteins, which in this way, control the rate of switching of the G protein. Downstream of the G protein pathway, Rho and its relatives interact with kinases and other proteins, and thereby control a variety of processes, many of them associated with the actin cortex.

Exactly how Rho acts is not fully known at present but it is certain to be complex. A family of serine/threonine kinases has been found that is activated by Rho and these have multiple targets in the cytoskeleton. With regard to the linkage of actin to the plasma membrane, for example, one of the direct actions of Rho may be through the ERM family of proteins. Rho controls a protein kinase that phosphorylates, and thereby activates, ERM proteins. Activation of ERM proteins by phosphorylation (see Figure 6-4) may contribute to the assembly of a focal adhesion. Rho also affects the level of inositol phospholipid PIP_2 in the membrane, which in turn is known to act on several cytoskeletal proteins.

Possibly the most important link between Rho and focal adhesions, however, is the development of mechanical tension. Rho activation causes an increase in the phosphorylation of myosin light chains, which, as we will see in Chapter 7, causes bundles of myosin and actin to form and contract. We have already mentioned that tension development is essential for focal adhesion formation. Thus when actin filaments are attached to integrins, Rho-induced contractions can draw the integrins together into a tight patch. A cascade of phosphorylation and other reactions could be initiated, resulting in a mature focal adhesion.[4]

Two closely related G proteins are also intimately involved with the actin cortex. We have just seen that injection of quiescent, serum-starved, fibroblasts in culture with Rho leads to the rapid formation of both stress fibers and focal adhesions. Injecting a closely related G protein, called Rac, into serum-starved fibroblasts produces not stress fibers and focal

[4] Tension development at focal adhesions is also subject to negative regulation. Caldesmon, a protein abundant in smooth muscle and in focal adhesions, has the capacity to inhibit myosin, and microtubules in the vicinity of a focal adhesion may also oppose the development of tension.

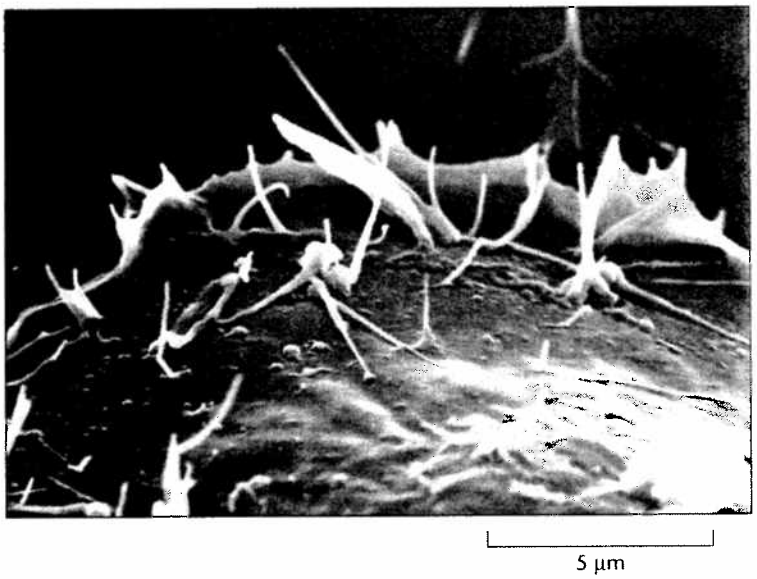


Figure 6-10 Filopodia on the cell surface. Scanning electron micrograph of a fibroblast in tissue culture. (Courtesy of Julian Heath.)

adhesions but multiple actin-rich lamellipodia or ruffles over the cell surface. A third G protein, Cdc42, under the same conditions, causes long thin filopodia rather than lamellipodia to be produced (Figure 6-10). Although each G protein is distinct in its action, some cross-talk occurs, so that Cdc42 activates Rac, and Rac (after a significant delay) activates Rho. Taken together, these observations suggest that members of the Rho GTPase family are part of a regulatory network controlling the association of surface receptors with the actin cytoskeleton (Figure 6-11).

The effects of Rac, Rho, and Cdc42 are not restricted to fibroblasts. These proteins also operate in nerve cells, epithelial cells, lymphocytes, and macrophages, and the detailed consequences of their activation are dependent on the particular cellular context.

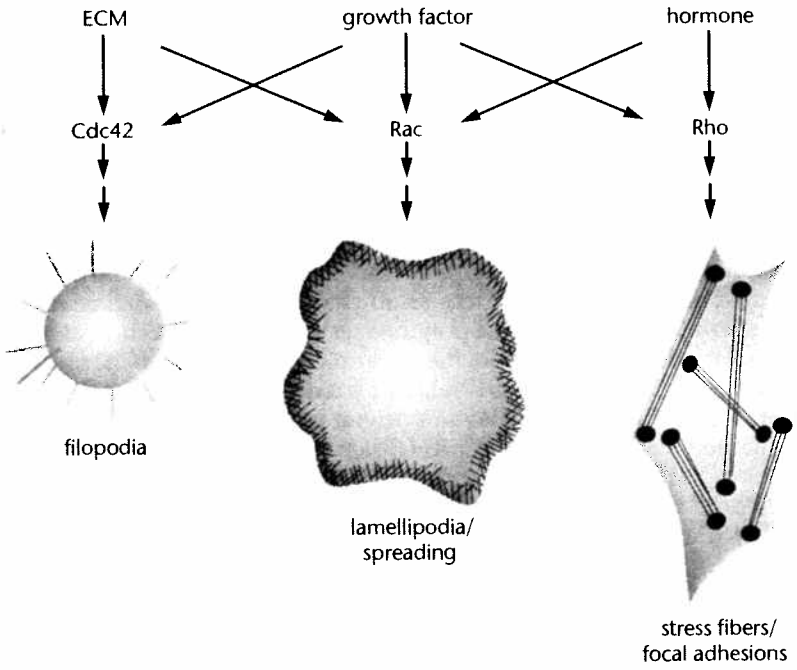


Figure 6-11 Rho proteins and the actin cortex. Injection of activated Cdc42, Rac, or Rho into quiescent, serum-starved fibroblasts causes changes in surface morphology and the production of the actin-based structures shown. Each G protein can also be selectively activated by specific hormones or growth factors acting on cell-surface receptors (ECM, extracellular matrix).

Lamellipodia, filopodia, and microvilli form in response to external stimuli

The surface structures generated in response to Rac and Cdc42 contain actin filaments and are part of the cortical cytoskeleton. In filopodia, actin filaments are collected into a loose bundle and cross-linked by bundling proteins such as fimbrin. In lamellipodia they form nets rather than bundles, terminating at the leading margin in a dendritic filigree of filaments. These structures occur widely in cells both in culture and in the animal and are often found on cells that are moving, or dividing or undergoing a sudden increase in growth. Thus, neuronal growth cones produce filopodia and lamellipodia as they crawl toward distinct synaptic targets, and neutrophils or amoeboid cells of *Dictyostelium* generate lamellipodia and filopodia on their surface when exposed to chemotactic agents. We discuss in Chapter 8 the relationship between these structures and the forward crawling of the cell as a whole.

Another role for surface ruffling is displayed during the *phagocytosis* performed by a macrophage coming into contact with a foreign organism or a dead cell. The macrophage develops a local accumulation of actin-rich cortical material at the site of contact that develops into pseudopodia (the nonspecific, generic term) rich in actin. These surround and eventually engulf the particle, which is then attacked by lysosomal enzymes and eventual digested. Perversely, some pathogenic bacteria such as *Salmonella* deliberately induce a similar surface response in intestinal epithelial cells, and use it to gain entrance to the cells they infect (Chapter 16).

A different kind of cortical response is shown by some oocytes following fertilization. In the large eggs of sea urchins, for example, contact with a sperm causes microvilli on the egg surface to change in length. Before fertilization, the surface of a sea urchin egg is covered with some 130,000 short microvilli. Upon fertilization these increase in average length from about 0.3 μm to 1.0 μm. In the region of the sperm head, a cluster of microvilli becomes even longer, extending up and around the head and thereby aiding its attachment and fusion. These rapid changes follow, and may be triggered by, a wave of release of calcium ions from intracellular stores. The Ca^{2+} wave, which can be displayed by injecting specific fluorescent dyes into the egg, starts at the point of fertilization and spreads around the large cell. As it progresses, the wave is accompanied by increasing tension of the surface and the changes in length of microvilli just mentioned.

Filopodia grow by controlled actin polymerization

The structural core of actin-rich extensions of the surface such as filopodia is a loose bundle of actin filaments. These are arranged with their fast-growing, barbed ends close to the plasma membrane, and when the protrusion grows on the cell surface it is because actin monomers are adding to the barbed ends of the filaments. Recall that in the acrosomal reaction of *Thyone* sperm, a pool of G-actin polymerizes explosively on contact with the egg and produces a long thin membrane-enclosed spike (Chapter 5). Recall also that as *Listeria* and related bacteria spread through and between the cells they infect, they are driven by the controlled assembly of actin filaments at their barbed ends (Chapter 5).

The force required to push the plasma membrane outward could in principle come from the free energy released during polymerization. It can be shown experimentally that if G-actin is enclosed in a large lipid vesicle and then induced to polymerize, it can distend the surface. However, actin does not operate in isolation and in the cell many different molecules and molecular processes collaborate to regulate polymerization. Monomeric actin itself is held in reserve, sequestered in complexes with proteins such as thymosin. There are also hints that, in at least some

cells, concentrated accumulations of unpolymerized actin may exist close to the membrane, like reserve depots.

The point of actual assembly of actin monomers—the barbed end of the nascent filament—is the location of clusters of actin-binding proteins. These might be created by gelsolin-mediated fragmentation of existing actin filaments or by the formation of nucleating complexes such as the Arp2/3 complex close to the plasma membrane in response to external signals. We will describe in Chapter 8 how the continual activation of nucleating complexes just beneath the leading margin of a migrating cell drives its lamellipodia forward, and a similar mechanism is likely to exist for filopodia. It is also likely that the characteristic exploratory function of filopodia has a molecular correlate and that specific proteins will be found at their tips.

Bundling proteins increase the rigidity of cell extensions

Once a filopodium or microvillus has grown out of the cell by actin polymerization, its strength and stiffness rely on proteins that hold actin filaments together. The strategy of cross-linking to increase stiffness, mentioned in Chapter 4, is widely used by cells, which employ for this purpose a variety of actin bundling and cross-linking proteins. Some of the most bizarre examples are found in invertebrate sperm, which produce long actin-containing spikes (acrosomal processes) when they make contact with the egg. In the rapidly made *Thyone* acrosomal process mentioned above, the filaments are only loosely cross-linked, probably by a form of spectrin. But the acrosomal process of *Limulus*, the horseshoe crab, is premade and much more tightly bundled. A bundle of actin filaments some 60 μm long, and cross-linked by a distinctive bundling protein called scruin, lies coiled up around the base of the head of the unreacted sperm. Contact with the egg causes the actin filaments in the bundle to change their relative packing, probably triggered by a calcium-induced change in conformation of scruin. The bundle uncoils and extends, causing the process literally to screw itself into the egg jelly.

Oocytes also show rearrangements of their actin cortex. Unfertilized sea urchin eggs, for example, contain numerous short microvilli that within an hour of fertilization rearrange to form filopodia 5–10 μm long over their entire surface. The actin filaments within these projections are linked together in regular hexagonal bundles by a protein called fascin, producing distinctive 12 nm traverse striations. Fascins are widespread in other tissues of both invertebrates and vertebrates and can be detected in both stress fibers and filopodia.

Actin filaments in tightly-packed structures such as microvilli, acrosomal processes, and stereocilia are almost always arranged in longitudinal alignment. In other words, the crossovers of adjacent helices lie in a series of planes at right angles to the axis of the bundle (Figure 6-12). This feature, which often results in cross-striations in electron micrographs, probably arises from the fact that bundling proteins are usually short dimeric

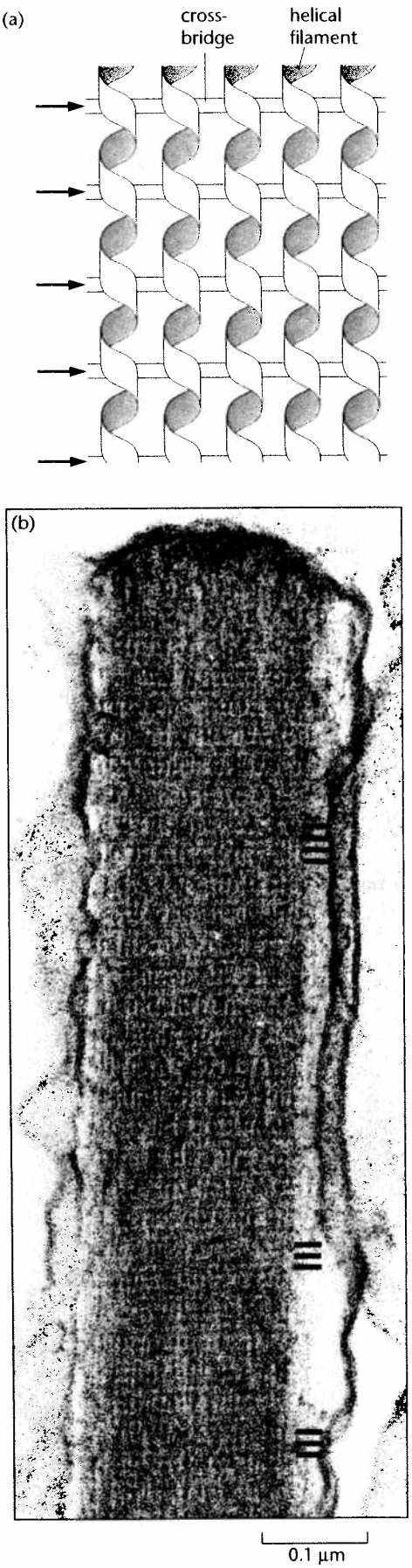


Figure 6-12 Packing of helical filaments into bundles. (a) Short cross-links between helical filaments tend to force them into longitudinal alignment, often generating regular striations across the bundle. The precise geometry of a bundle of actin filaments is more complicated because the filaments are not usually in a planar array and because each twist of an actin filament does not usually contain an integral number of subunits. (b) Cross-section of the bundle of actin filaments in a stereocilium showing regular striations about 12 nm apart. (Micrograph courtesy of Lewis Tilney.)

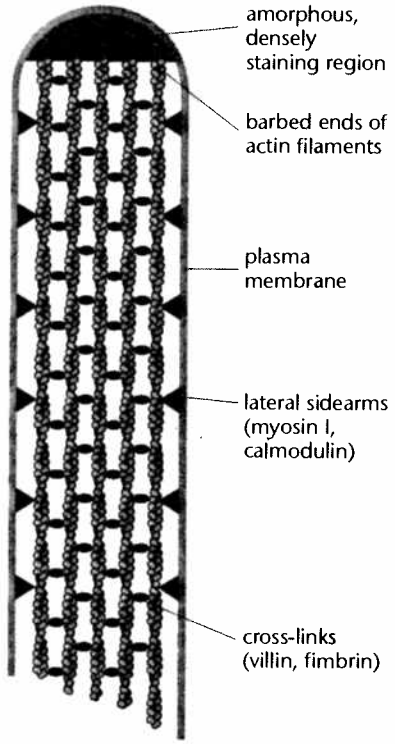


Figure 6-13 A microvillus. A bundle of parallel actin filaments held together by the actin-bundling proteins villin and fimbrin forms the core of a microvillus. Lateral arms (complexes of myosin I and the Ca^{2+}-binding protein calmodulin) connect the sides of the actin filament bundle to the overlying plasma membrane. The barbed ends of the actin filaments are embedded in an amorphous densely stained substance of unknown composition.

molecules, and tend to tie corresponding regions of actin filaments together. How the filaments pack in the other dimension (at right angles to the axis of the bundle) is a more difficult question to answer. The helically arranged subunits in a filament do not automatically fit into a regular geometrical lattice, so some flexibility is needed, either in the cross-links or in the twist of the helices themselves.

Intestinal microvilli are held together by fimbrin and villin

One of the best-characterized forms of stable actin filament bundle is seen in the intestinal brush border. Intestinal *microvilli* are cylindrical projections, 1–2 μm long and about 0.1 μm in diameter (see Figure 6-7). They are profuse on the apical surfaces of epithelial cells lining the intestine, forming a close-packed layer called a *brush border.* This has an absorptive function, increasing the area of the cell surface exposed to the intestine 10–20-fold. The microvillar membrane carries many specialized enzymes involved in the breakdown and membrane transport of food, including those responsible for hydrolysis of sucrose and other disaccharides, as well as proteins required for the transport of fatty acids from the intestinal cavity, and the Na^+ cotransport of sugars and amino acids.

Each microvillus contains a bundle of 30 or so actin filaments held together by cross-linking proteins, notably fimbrin and villin (Figure 6-13). *Fimbrin* is a widely occurring protein also present in filopodia and stereocilia that has the ability to bundle actin filaments into a tight, almost crystalline array. *Villin,* is an actin-fragmenting protein related to gelsolin. At low calcium concentrations (less than 0.1 μM Ca^{2+}) villin causes actin filaments to form bundles. However, if calcium rises above 5 μM, villin causes fragmentation of actin filaments, by a similar mechanism to that used by gelsolin. Villin-induced breakdown of the actin-rich core may be an essential step allowing large vesicles of membrane to be sloughed off and released into the intestine, where the digestive enzymes then continue their action.

As for any large cytoskeletal structure, microvilli are enormously complex in detailed molecular composition and structure (Table 6-4). Apart from actin filaments, fimbrin, and villin, there are also specific proteins at the tip of the structure (around the barbed ends of the actin filaments). A single-headed myosin (one of a large family of motor proteins discussed in the next chapter) with calmodulin light chains forms the side-arms that extend at regular intervals of 33 nm from the actin filaments to make contact with the plasma membrane. The presence of myosin is an indication that microvilli are not static structures. In fact, both the cells and

Table 6-4 Major proteins of intestinal microvilli

Protein	Size (kDa)	Function
actin, monomer	43	forms microvillus core
fimbrin	68	bundles actin filaments
villin	95	cross-links actin filaments, severs them in response to Ca^{2+}
myosin I	110	anchors membrane to actin core
calmodulin	17	associated with myosin I
spectrin (α and β)	240/235	anchors actin filaments to membrane at microvillus base
myosin II	200	cross-links actin filaments at base, generates cortical tension
paxillin	68	binds vinculin and FAK
Src (pp60 $^{c\text{-}src}$)	43	protein tyrosine kinase
p130	130	substrate of Src, binds tensin

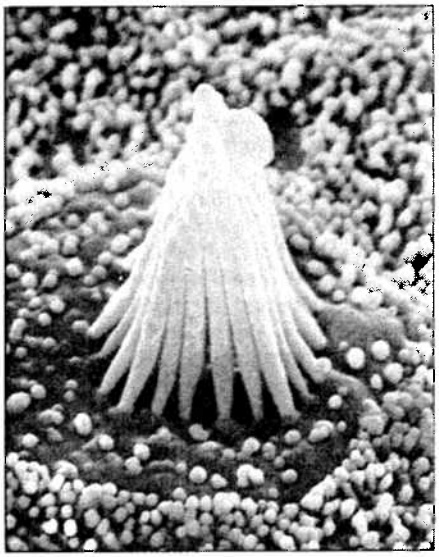


Figure 6-14 Stereocilia. Regular array of stereocilia on a hair cell in the inner ear of a bullfrog. The hair bundle comprises about 50 stereocilia and one true cilium (the kinocilium), which in this species has a bulbous swelling at its end. The longest stereocilia are 8.4 μm in length. Note the stubby microvilli on the surfaces of the surrounding cells. (Scanning electron micrograph courtesy of R.A. Jacobs and A.J. Hudspeth.)

their microvilli are in a state of continual change. Most of the proteins in a micovillus *turnover* (that is, they are degraded and replaced by newly synthesized molecules) within an hour or so, and membrane components move continually toward the microvillus tip as part of the continual sloughing of surface membrane.

Stereocilia are specialized microvilli that perform a sensory function

Microvilli, like chocolates, come in many shapes and flavors. All are made in basically the same way, but they differ in the length and number of actin filaments they contain, how these are bundled, and how the filaments are attached to the membrane. Possibly the most elaborate structures of this kind, at least in mammals, are the *stereocilia* present in the hair cells of the ear (Figure 6-14).

Hair cells are found in the cochlea of the vertebrate ear where they are primary detectors of sound vibrations, and in the vestibular apparatus where they respond to the position and movements of the head. In fish, hair cells are present in the lateral line organs along the flank, which monitor the progress of the animal through the water. Stereocilia have even been found on the surface of some species of protozoa, acting as delicate triggers for the discharge of poisoned darts known as nematocysts.

Stereocilia can be up to 30 μm long and 1 μm wide. They are cylindrical and taper at their base where they meet the surface of the hair cell, like pencils resting on their points. In a typical stereocilium there may be several thousand actin filaments in parallel array tied to each other through proteins such as fimbrin (see Figure 6-12).[5] Actin filaments in the center of the core bundle are longer than the others, and extend into the base of the stereocilium. Here a cuticular plate of dense filaments extends laterally to make contact with adjacent supporting cells.

Hair cells are amazingly sensitive to mechanical displacement. The mammalian ear, for example, reliably measures mechanical stimuli whose average amplitude at threshold is about the diameter of a hydrogen atom. This performance is believed to be due to thin threads that extend from the membrane at the tip of one stereocilium to that of an adjacent stereocilium, where they are physically attached to ion channels. The rigid structure of the stereocilium might thus enable minute movements to be amplified into a change in electric potential.

Cells control the number, length, and position of actin filaments in their cortex

The length and position of individual stereocilia are precisely determined, often to an amazing degree. In the cochlea of the chick, for example, each hair cell has a bank of stereocilia arranged in hexagonal pattern in which sequential rows increase stepwise in height. The array as a whole has a definite orientation on the hair cell and detailed measurements of the number, size, and arrangement of stereocilia show that these vary systematically from one region of the cochlea to another. Variations are so

[5] In cross-section the filaments in a stereocilium appear disordered, like a liquid crystal. In other types of actin bundle, the arrangement can be more regular, however, showing hexagonal or square lattices.

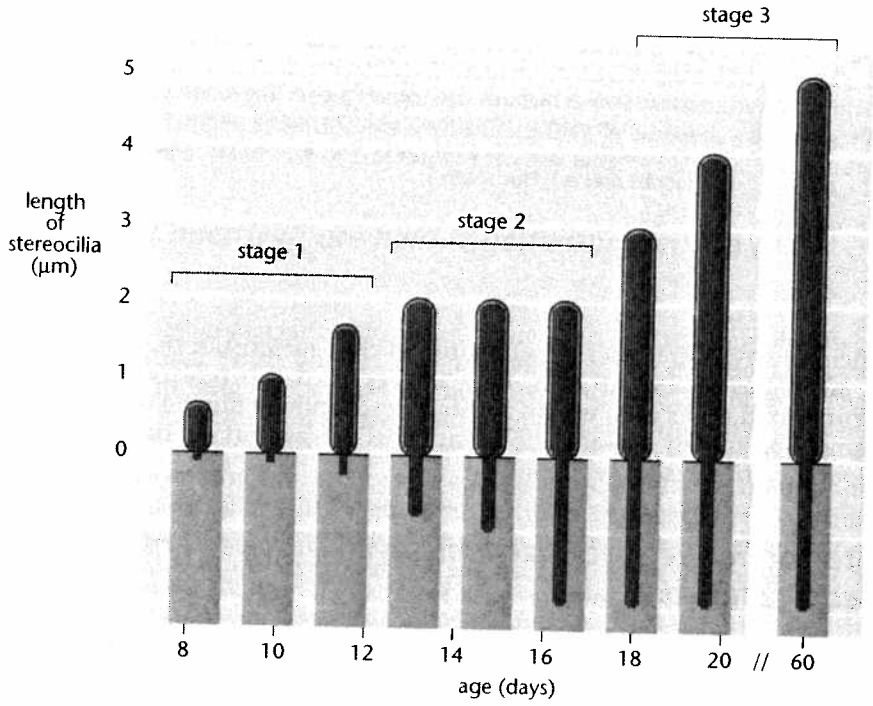


Figure 6-15 Phases of actin growth in stereocilia. In *stage 1*, actin filaments in stereocilia grow at their barbed ends and become more ordered by cross-linking. In *stage 2*, new actin filaments are added to make the stereocilium wider, and subunits are added to the pointed end of core actin filaments to lengthen the rootlets. In *stage 3*, actin filaments in the stereocilium again elongate at their barbed ends. All stereocilia go through the same three stages, but the time of initiation and duration of growth vary both among the stereocilia on a single hair cell and from one hair cell to another. In this way a remarkably precise gradation of filament lengths is produced.

precise and reproducible from animal to animal that one could tell the position of a cell by the number, length, and orientation of the stereocilia it carries.

Formation of the cochlea presents a remarkable demonstration of the ability of cells to control the length, number, and position of actin filaments in their cortex (Figure 6-15). Three stages of growth of stereocilia have been distinguished. (1) Stereocilia begin to grow on each hair cell, emerging in a staggered sequence that generates a truncated version of the final organ-pipe array. Growth during this phase is believed to be at the barbed end of actin filaments. (2) New actin filaments accumulate on the outside of the core bundle to make the stereocilia thicker while the central filaments elongate, apparently at their pointed ends, to form rootlets. (3) Actin filaments elongate at their barbed ends and grow to an extent that depends on their position in the cochlea.

Outstanding Questions

What is the composition of the actin cortex? How does it vary from one location of the cell to another? How are these differences created and what is their function? Which molecules are unique to filopodia and how do they contribute to the exploratory action of these structures? What determines the biconcave shape of a red blood cell? Is it the spectrin network—if so, how? What is the composition and spatial arrangement of proteins in adherens junctions? How is their growth controlled by mechanical tension? What tells a cell that it has made an effective adhesion to another cell or to the substratum? Do conformational changes propagate across the membrane, through cadherin or integrin molecules? How do Rho GTPases interact with adherens junctions and focal adhesions? What is the network of signal reactions controlling the actin cortex? How does the Limulus *sperm acrosomal process elongate? Is it by propagated conformational changes through the structure? Do intestinal microvilli move? If so how, and why? How does a cell control the position and lengths of stereocilia on its surface? How important are the mechanical properties of stereocilia? Are they purely passive structures?*

Essential Concepts

- All animal cells possess a cortical layer of actin filaments on the inner face of the plasma membrane. This layer supports the flimsy lipid bilayer mechanically and is responsible for many cell surface movements.
- The cortex is made of actin filaments attached at their barbed (plus) ends to the membrane and cross-linked into a three-dimensional web, or matrix, by proteins such as α-actinin and filamin.
- Dynamic restructuring of the cortex occurs in response to calcium changes, mediated by severing proteins such as gelsolin.
- Actin filaments are usually anchored to the plasma membrane by proteins with binding sites for both actin (or an actin-binding protein) and an integral membrane protein.
- In the membrane skeleton of vertebrate red blood cells, short lengths of actin filament cross-linked by flexible spectrin molecules are attached to the plasma membrane by specific proteins such as ankyrin and band 4.1.
- Adherens junctions between cells comprise clusters of cadherin molecules, which provide anchorage between one cell and the next, and bundles of actin filaments attached to their cytoplasmic face.
- Focal adhesions on the lower surface of cells in culture form in response to extracellular matrix molecules such as fibronectin. Integrin molecules bind to matrix proteins on the outside of the cell and to talin, vinculin, α-actinin, and other proteins on the inside.
- In addition to a simple anchoring function, adherens junctions and focal adhesions also have a signaling role and contain a number of protein kinases and their substrates.
- Cortical actin also assembles in specific regions in response to external cues. Examples include the region of contact between a phagocytic cell and a suitable particle, where the accumulation of actin gives rise to pseudopodia that extend and engulf the particle.
- Small G proteins, Rho, Rac, and Cdc42, have an important controlling influence over the association of actin with the plasma membrane, as in the formation of focal adhesions and filopodia.
- Rapid formation and elongation of surface extensions occurs in many cells in response to surface signals, for example following fertilization of an egg or chemotactic stimulation of neutrophils or in the activation of blood platelets.
- Actin filaments that form during these changes are assembled locally from actin monomers sequestered in the cytoplasm. Protein complexes near the membrane, formed in response to external cues, nucleate actin polymerization at specific locations.
- Once formed, surface extensions can be stabilized by extensive cross-linking of their actin core and the linking of the latter to the plasma membrane, a well-characterized example being intestinal microvilli.
- The packing of actin filaments in such stable extensions can be extremely regular. Hair cell stereocilia, present in amazingly precise arrays in the cochlea, illustrate the fine control a cell exerts over its cortical actin structures.

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