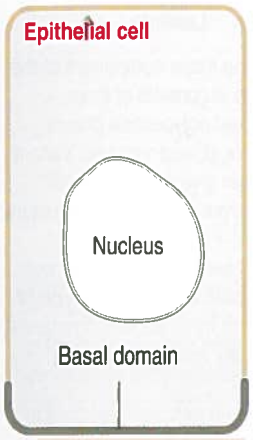
Figure 1-21. Basement membrane



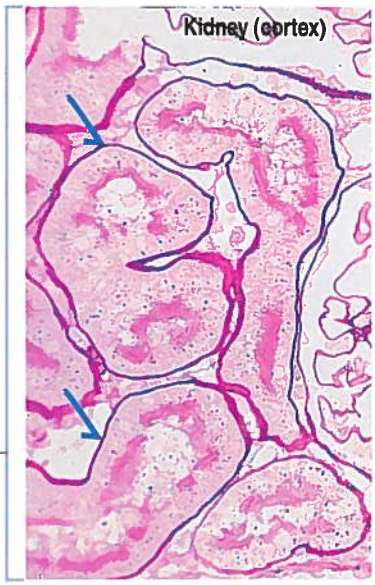
The **basement membrane**, an extracellular component in direct contact with the basal domain of epithelial cells, is visible under the **light microscope** after staining with the periodic acid–Schiff (PAS) reagent technique.

At the **electron microscopic level**, the basement membrane is defined by two layers or laminae:

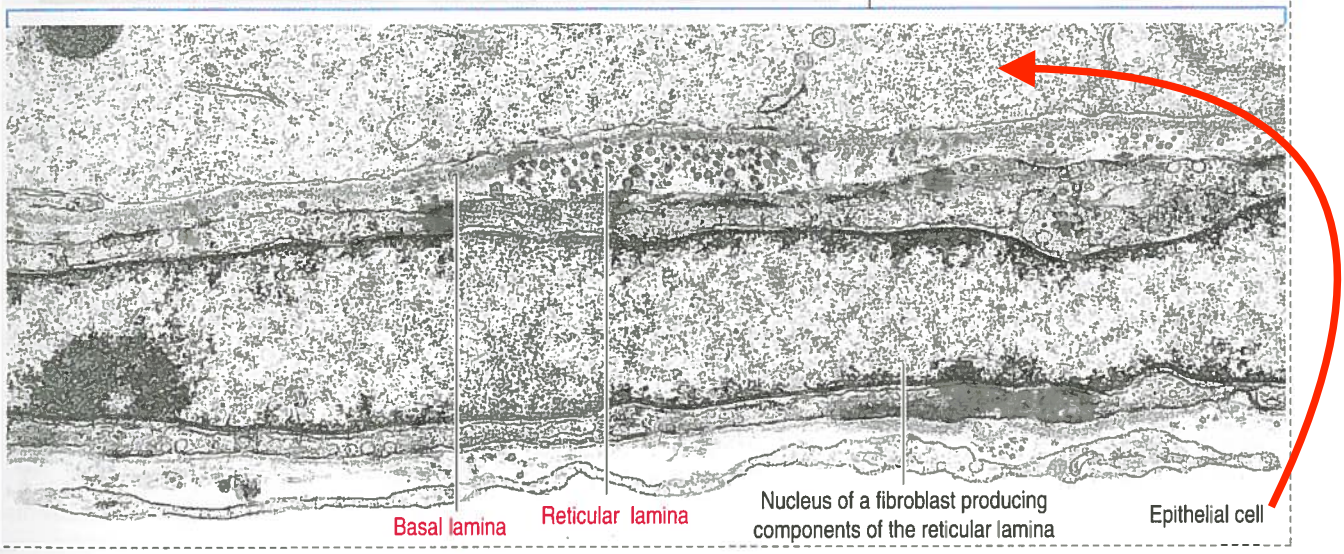
1. A **basal lamina**, which contains laminin, fibronectin, type IV collagen, heparan sulfate proteoglycans, and nidogen (also called entactin).
2. A **reticular lamina**, which contains type III collagen (also called reticular fibers)

The components of these two laminae are glycoproteins. They are **PAS positive**.

Under **light microscopy**, both laminae are resolved as a single **basement membrane** after staining with the PAS technique.



Each lamina can be resolved as a separate entity by **electron microscopy**.



The basal and reticular laminae can be distinguished by electron microscopy. Under the light microscope, the combined basal and reticular laminae receive the name of basement membrane, which can be recognized by the **periodic acid–Schiff (PAS)** stain (see Figure 1-21; see Box 1-D).

The basal lamina has specific functions in different tissues. The double basal lamina of the renal corpuscle constitutes the most important element of the **glomerular filtration barrier** during the initial step in the formation of urine (see Chapter 14, Urinary System).

In skeletal muscle, the basal lamina maintains the integrity of the tissue, and its disruption gives rise to **muscular dystrophies** (see Chapter 7, Muscle Tissue).

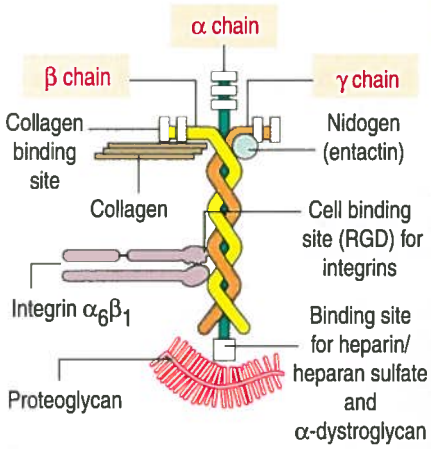
During the migration of primordial germinal cells, basal lamina components guide the migrating cells toward the gonadal ridge in preparation for the development of the gonads. The basal lamina not only provides support to epithelia, but also participates in other non–epithelial cell functions.

Laminin (Figure 1-22) is a cross-shaped protein consisting of three chains: the **α chain**, the **β chain**, and the **γ chain**. Laminin molecules can associate with each other to form a meshlike polymer. Laminin and **type IV collagen** are the major components of the basal lamina, and both are synthesized by epithelial

Box 1-D | Periodic acid–Schiff (PAS) reaction

- PAS is a widely used histochemical technique to show 1,2-glycol or 1,2-aminoalcohol groups, such as those present in glycogen, mucus, and glycoproteins.
- **Periodic acid**, an oxidant, converts these groups to **aldehydes**. The **Schiff reagent**, a colorless fuchsin, reacts with the aldehydes to form a characteristic **red-purple (magenta)** product.
- Some important PAS-positive structures are the **basement membrane**, **glycocalyx**, **mucus** produced by goblet cells, stored **glycoprotein hormones** in cells of the pituitary gland, and **collagens**.

Figure 1-22. Laminin and fibronectin

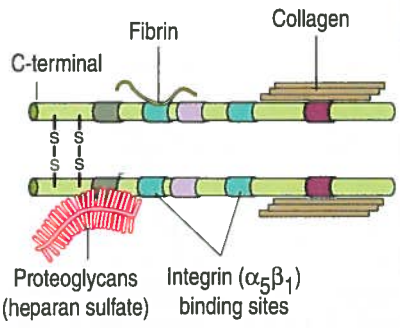


Laminin

Laminin is the major component of the basal lamina. It consists of three disulfide-linked polypeptide chains designated α, β, and γ chains. Variants for each chain give rise to several laminin isoforms with different structure and function.

Laminins have binding sites for cell surface receptors (**integrins**), **type IV collagen**, and other adhesion proteins (e.g., **nidogen**, also known as **entactin**).

Laminin monomers self-associate to form a network that is part of the **basal lamina**.



Fibronectin

Fibronectin is a glycoprotein formed by two identical chains joined by disulfide linkages close to the C-terminal.

There are two forms of fibronectin:

1. **Plasma fibronectin**, produced by **hepatocytes** and secreted into the bloodstream.
2. **Cellular fibronectin**, produced by **fibroblasts**, forms part of the extracellular matrix.

Fibronectin has binding sites for **integrins**, **collagen**, **heparan sulfate**, and **fibrin**.

cells resting on the lamina.

Laminin has binding sites for **nidogen** (also called entactin), **proteoglycans** (in particular, heparan sulfate **perlecan**), **α-dystroglycan** (see Chapter 7, Muscle Tissue), and **integrins**.

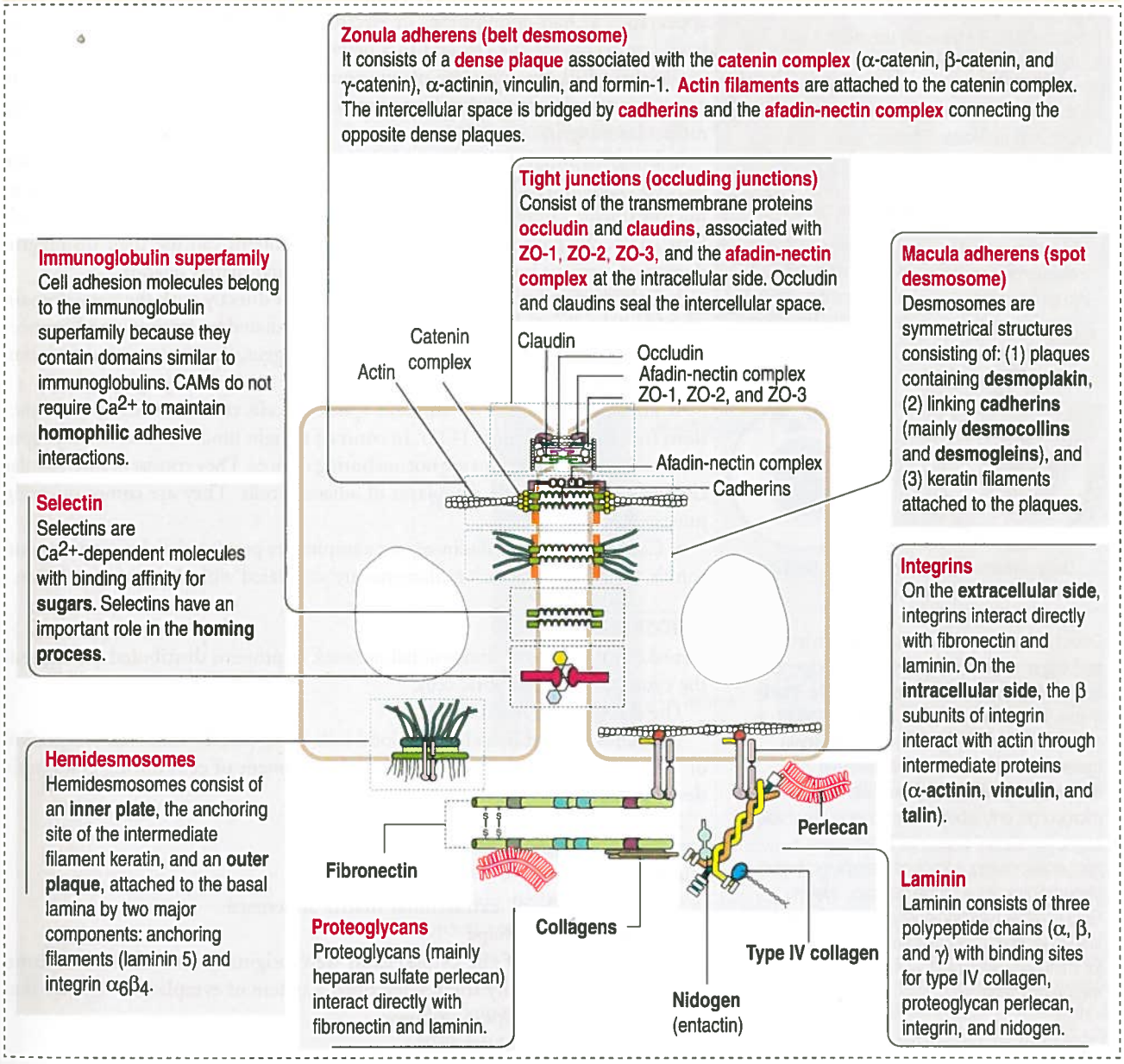
Fibronectin (see Figure 1-22) consists of two protein chains cross-linked by disulfide bonds. Fibronectin is the main adhesion molecule of the extracellular matrix of the connective tissue and is produced by fibroblasts. Fibronectin has binding sites for **heparin** present in proteoglycans, several types of **collagens** (types I, II, III, and V), and **fibrin** (derived from fibrinogen during blood coagulation).

Fibronectin circulating in blood is synthesized in the liver by hepatocytes. It differs from fibronectin produced by fibroblasts in that it lacks one or two repeats (designated EDA and EDB for extra domain A and extra domain B) as a result of alternative mRNA splicing. Circulating fibronectin binds to fibrin, a component of the blood clot formed at the site of blood vessel damage. The RGD domain of immobilized fibronectin binds to integrin expressed on the surface of activated platelets, and the blood clot enlarges. We return to the topic of blood coagulation or hemostasis in Chapter 6, Blood and Hematopoiesis.

How cells interact with one another and with the basal lamina

Figure 1-23 summarizes the highlights of cell adhesion molecules and cell junctions. An epithelium is a continuous sheet of polarized cells supported by a basement membrane. The polarized nature of an epithelium depends on the tight junctions that separate the polarized cells into apical and basolateral regions. Tight junctions control the paracellular pathway of solutes, ions, and water. Tight junctions form a belt around the circumference of each cell.

Figure 1-23. Summary of cell junctions and cell adhesion molecules



Endothelial cells, the constituents of a simple squamous epithelium, are linked by tight and spot desmosomes tightly regulated to maintain the integrity of the endothelium and protect the vessels from unregulated permeability, inflammation, and reactions leading to blood coagulation in the lumen (see Chapter 12, Cardiovascular System). Leukocytes reach the site of infection by attaching to endothelial cell surfaces and migrate across the endothelium into the underlying tissues by a mechanism called **diapedesis**. Leukocytes find their way through endothelial cell-cell junctions after docking to activated or resting endothelial cells by the endothelial cell adhesion molecules ICAM-1 and VCAM-1 (see Figure 1-10). ICAM-1 and VCAM-1 bind to β_2 and β_1 integrin subunits in leukocytes (see Figure 1-12).

The cohesive nature of the epithelium depends on three factors: cell junctions, cell adhesive molecules in general, and the interaction of integrins with the extracellular matrix, produced to a large extent by fibroblasts. The basal lamina is essential for the differentiation of epithelial cells during embryogenesis.

Note in Figure 1-23 that:

Figure 1-24. Immunocytochemistry

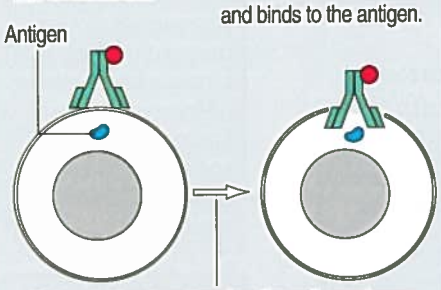
Pour en savoir plus!

Two techniques are generally used: direct and indirect immunocytochemistry.
Immunocytochemistry requires that cells under study are made permeable, usually with a detergent, so that antibody molecules (immunoglobulins) can enter a cell and bind to an antigen.

Direct immunofluorescence

The immunoglobulin molecule cannot enter into an intact cell.

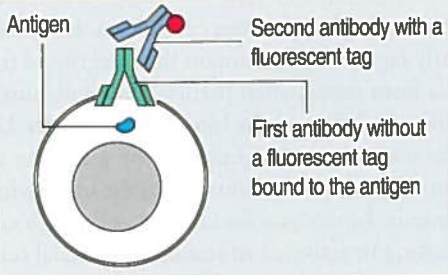
After detergent treatment, the immunoglobulin molecule enters the cell and binds to the antigen.



Detergent treatment makes the cell membrane permeable to the antibody.

Direct immunocytochemistry involves a specific antibody or some agent with specific binding affinity to an antigen tagged with a visible marker. Visible markers attached to the immunoglobulin molecule can be a fluorescent dye such as **fluorescein** (green fluorescence) or **rhodamine** (red fluorescence). When examined with a fluorescence microscope, only labeled components are visible as bright, fluorescent structures. Direct immunofluorescence involves a single incubation step and provides a simple detection system. Gold particles (electron-dense) attached to immunoglobulin molecules are convenient markers for immunocytochemistry at the electron microscopic level.

Indirect immunofluorescence



Indirect immunocytochemistry involves a second antibody tagged with a visible marker. This second antibody binds to a nontagged first antibody specific for an antigen. The indirect method requires two separate incubations (one each for the first and second antibodies) and is more specific for the identification of antigens.

1. The basal domain of epithelial cells interacts with the basal lamina through hemidesmosomes and integrins. Hemidesmosomes, so called because of their appearance as half-desmosomes in electron micrographs, are anchored to the basal lamina ouside the cell and to a network of keratin intermediate filaments inside the cell through a plate-plaque complex. Mutations in hemidesmosome components cause severe skin blistering as a result of a rupture of the anchoring molecular integrity.

2. Integrins interact directly with laminin and fibronectin, in particular the RGD domain to which integrins bind. Inside the cell, integrins interact with actin microfilaments. Integrins connect the extracellular environment to the intracellular space. We have seen that some ADAM proteins can use their disintegrin domain to prevent integrin binding to extracellular matrix ligands.

3. Collagens and proteoglycans do not interact directly with the basal domain of epithelial cells. Instead, this interaction is mediated by laminin and fibronectin, which contain specific binding sites for collagens, the proteoglycan perlecan, and nidogen.

4. The lateral domains of adjacent epithelial cells communicate by gap junctions (not shown in Figure 1-23). In contrast to tight junctions and belt and spot desmosomes, gap junctions are not anchoring devices. They consist of intercellular channels connecting the cytoplasm of adjacent cells. They are communicating junctions.

5. Cadherins and the afadin-nectin complex are present in tight junctions and zonula adherens. Actin microfilaments are associated with these two junctions.

CYTOSKELETON

Cytoskeleton is a three-dimensional network of proteins distributed throughout the cytoplasm of eukaryotic cells.

The cytoskeleton has roles in:

1. **Cell movement** (crawling of blood cells along blood-vessel walls, migration of fibroblasts during wound healing, and movement of cells during embryonic development)
2. **Support and strength for the cell**
3. **Phagocytosis**
4. **Cytokinesis**
5. **Cell-cell and cell–extracellular matrix adherence**
6. **Changes in cell shape**

The components of the cytoskeleton were originally identified by **electron microscopy**. These early studies described a system of cytoplasmic "cables" that fell into three size groups, as follows:

1. **Microfilaments** (7 nm thick)
2. **Intermediate filaments** (10 nm thick)
3. **Microtubules** (25 nm in diameter)

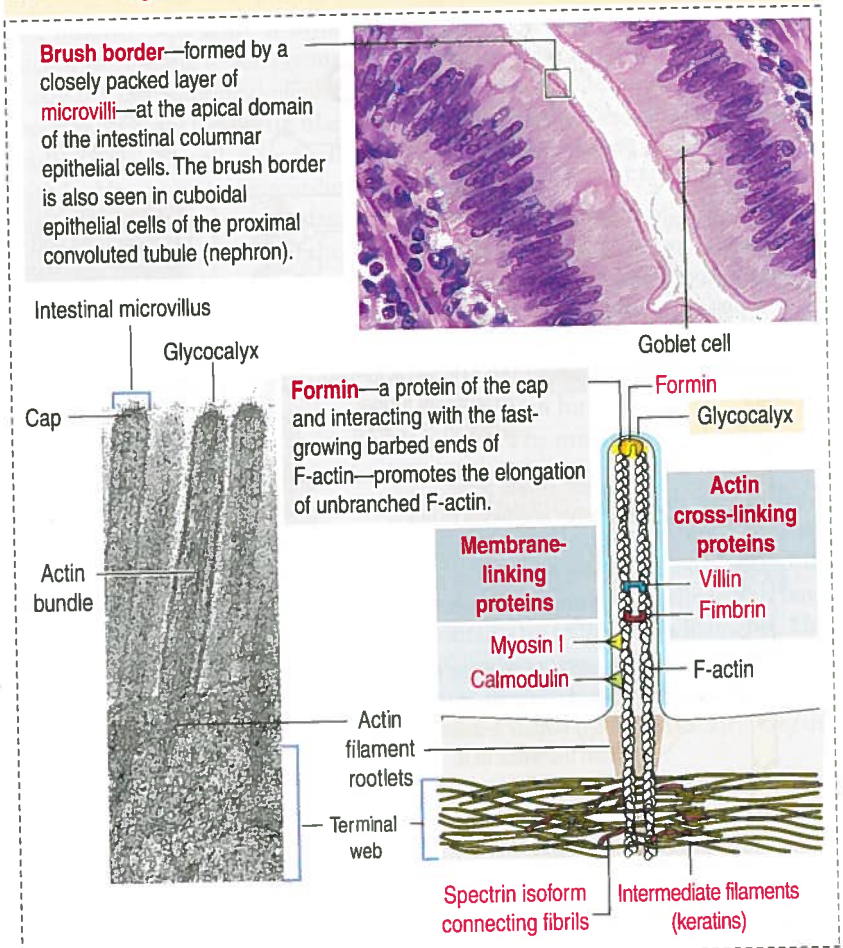
Biochemical studies, involving the extraction of cytoskeletal proteins from cells with detergents and salts and in vitro translation of specific mRNA, showed that each class of filaments has a unique protein organization. When cytoskeletal proteins were purified, they were used as antigens for the production of antibodies. Antibodies are used as tools for the localization of the various cytoskeletal proteins in the cell. The **immunocytochemical localization** of cytoskeletal proteins (Figure 1-24) and **cell treatment with various chemical agents** disrupting the normal organization of the cytoskeleton have been instrumental in understanding the organization and function of the cytoskeleton.

Microfilaments

The main component of microfilaments is **actin**. Actin filaments are composed of globular monomers (**G-actin**, 42 kd), which polymerize to form helical and asymmetrical filaments (**F-actin**).

Actin is a versatile and abundant cytoskeletal component forming static and

Figure 1-25. F-actin bundles form the core of intestinal microvilli



contractile bundles and filamentous networks specified by actin-binding proteins and their distinctive location and function in a cell. F-actin bundles are present in the microvilli of the intestinal (Figure 1-25) and renal epithelial cells (brush border) and the stereocilia from the hair cells of the inner ear.

We have already seen that the intracellular portion of the cell adhesion molecules cadherins and integrin β_1 interacts with F-actin through linker proteins (see Figures 1-8 and 1-11). As discussed in Chapter 6, Blood and Hematopoiesis, actin—together with spectrin—forms a filamentous network on the inner face of the red blood cell membrane that is crucial for maintaining the shape and integrity of red blood cells. **Spectrin** is a tetramer consisting of two distinct polypeptide chains (α and β).

Growth of actin filaments may occur at both ends; however, one end (the "**barbed end**" or **plus end**) grows faster than the other end (the "**pointed end**" or **minus end**). The names correspond to the arrowhead appearance of myosin head bound at an angle to actin. Actin filaments can branch in the **leading edge** (**lamellipodia**) of cells involved in either motility or interaction with other cell types. F-actin branching is initiated from the side of a preexisting actin filament by **Arp2/3** (for **actin-related protein**), an actin nucleating complex of seven proteins (Figure 1-26). **Formin** regulates the assembly of unbranched actin in cell protrusions such as the intestinal microvilli (see Figure 1-25).

Actin monomers have a binding site for adenosine triphosphate (ATP), which is hydrolyzed to adenosine diphosphate (ADP) as polymerization proceeds. **Actin polymerization is ATP-dependent** (see Box 1-E).

The kinetics of actin polymerization involves a mechanism known as **treadmilling**: **G-actin monomers assembled at one end of the filament concurrently**