SANATAN DHARM COLLEGE MUZAFFARNAGAR
(Affiliated to Maa Shakumbhari University, Saharanpur)

CELL BIOLOGY

THE CYTOSKELETON

Topic: Microfilaments (MFs)/Actin filaments

For

Subject Zoology

&

M.Sc. I Sem

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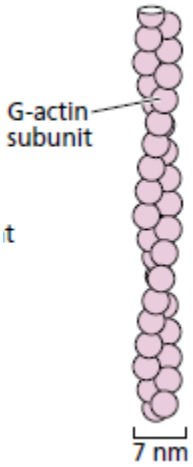
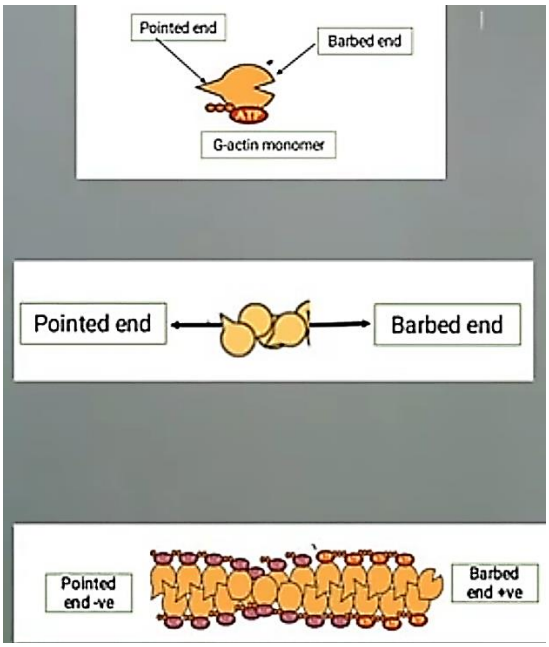
Actin filaments/Microfilaments

Microfilaments are solid and thinnest, with a diameter of **7 nm**; they are composed of a special form of the protein found in muscle: **globular actin, or G-actin**. Each **actin molecule** is composed of a single polypeptide with a molecular mass of approximately **42,000 daltons**. **A microfilament** consists of **two chains of polymerized actin subunits** that intertwine in a helical fashion (in following figure).





Sliding of actin filaments: Muscle contraction

- **Microfilaments are involved in cytoplasmic streaming and in tip growth.**
- **Cytoplasmic streaming** is the coordinated movement of particles and organelles through the cytosol in a helical path down one side of a cell and up the other side. The mechanism of cytoplasmic streaming involves bundles of microfilaments that are arranged parallel to the longitudinal direction of particle movement.
- The forces necessary for movement may be generated by an interaction of the microfilament protein actin with the **motor protein myosin** in a fashion comparable to that of the protein interaction that occurs during muscle contraction in animals.
- **Myosins** are proteins that have the ability to hydrolyze ATP to ADP and Pi when activated by binding to an **actin microfilament**. The energy released by ATP hydrolysis propels myosin molecules along the actin microfilament **from the minus end (pointed end) to the plus end (barbed end)**. Thus, **myosins** belong to the **general class** of **motor proteins** that drive **cytoplasmic streaming** and the **movements of organelles** such as chloroplasts within the cell.
- Examples of **other motor proteins** include the **kinesins** and **dyneins**, which drive movements of organelles and other cytoskeletal components along the surfaces of microtubules.
- **Actin microfilaments** also participate in the **growth of the pollen tube**. Upon germination, a pollen grain forms a tubular extension that grows down the style toward the embryo sac. As the tip of the pollen tube extends, new cell wall material is continually deposited to maintain the integrity of the wall.
- A network of microfilaments appears **to guide vesicles** containing **wall precursors** from their site of formation in the Golgi through the cytosol **to the site of new wall formation** at the tip. Fusion of these vesicles with the plasma membrane deposits wall precursors outside the cell, where they are assembled into wall material.
- Transport vesicles move through the cytoplasm in a directed manner, often pulled by motor proteins that operate on tracks formed by microtubules and microfilaments of the cytoskeleton (see Figure 9.1 *a*).
- In most animals, **phagocytosis** is a protective mechanism rather than a mode of feeding. Mammals possess a variety of **professional phagocytes**, including macrophages and

neutrophils that wander through the blood and tissues phagocytizing invading organisms, damaged and dead cells, and debris. These materials are recognized and bound by receptors on the surface of the phagocyte prior to uptake. Once inside the phagocyte, microorganisms may be **killed by lysosomal enzymes or by oxygen free radicals** generated within the lumen of the phagosome. The process of particle engulfment is pictured in **FIGURE 8.46 a**. The steps in the digestion of engulfed materials are illustrated in Figure 8.46 *b*. The engulfment of particulate material by phagocytosis is **driven by contractile activities of the actin-containing microfilaments** that underlie the plasma membrane.

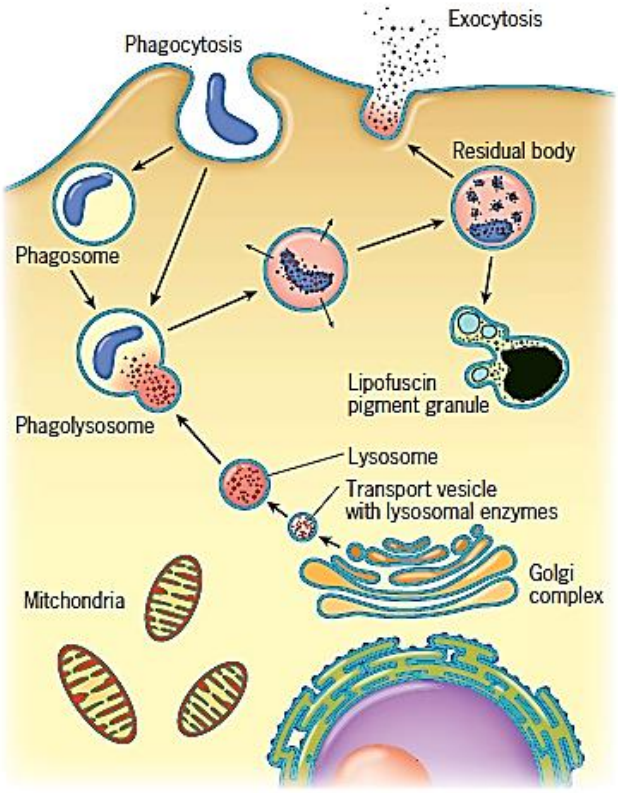


FIGURE 8.46 Phagocytosis.(a) The process of engulfment as illustrated by a polymorphonuclear leukocyte ingesting a yeast cell (lower left). (*b*) The steps that occur in the phagocytic pathway (see also page 292).

Role in microvilli formation:

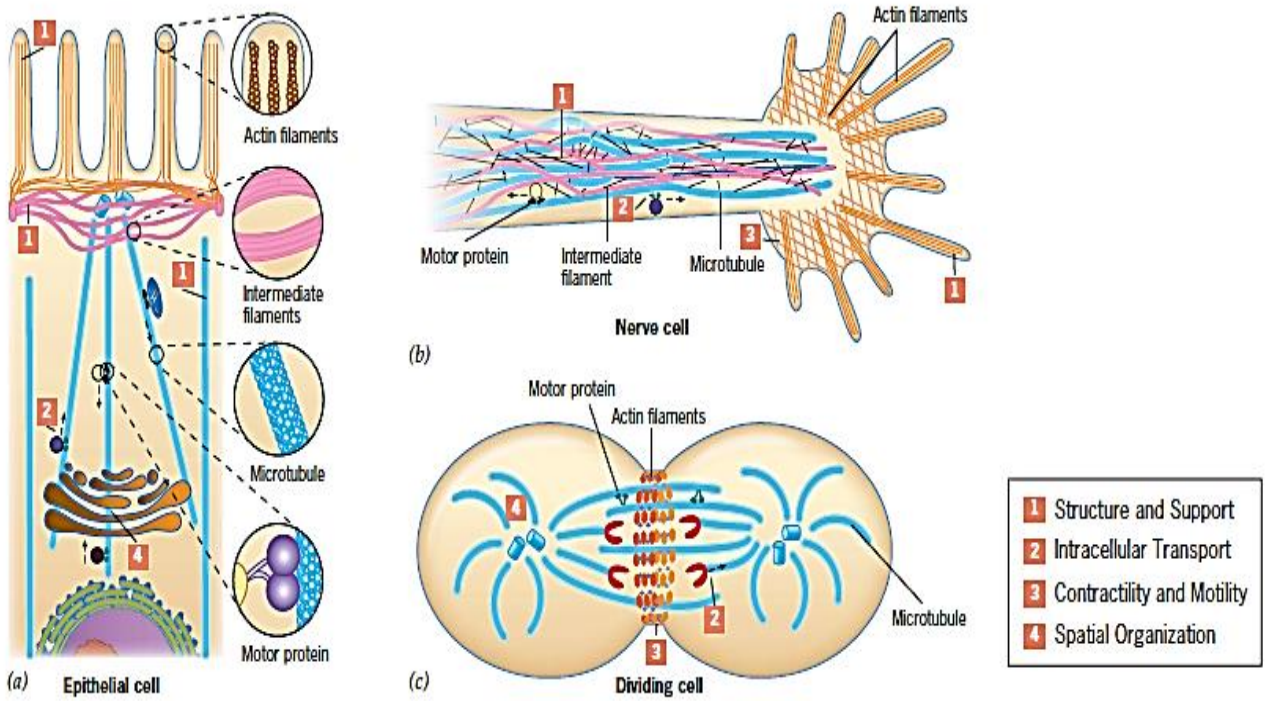


FIGURE 9.1 An overview of the structure and functions of the cytoskeleton. Schematic drawings of (*a*) an epithelial cell, (*b*) a neuron, and (*c*) a dividing cell. The microtubules of the epithelial and neurons function primarily in support and organelle transport, whereas the microtubules of the dividing cell form the mitotic spindle required for chromosome segregation. Intermediate filaments provide structural support for both the epithelial cell and neuron. Microfilaments support the microvilli of the epithelial cell and are an integral part of the motile machinery involved in neuronal elongation and cell division.

- The rate of assembly and disassembly of actin filaments in the cell can be influenced by a number of **different accessory proteins**. Changes in the local conditions in a particular part of the cell can push events there toward either the assembly or the disassembly of microfilaments. By controlling this dynamic behavior, the **cell can reorganize its actin** cytoskeleton.
- Such reorganization is required for dynamic processes such as **cell locomotion**, changes in cell shape, phagocytosis, and cytokinesis.
- As noted above, actin filaments play a role in nearly all of a cell's motile processes.

❖ **Intracellular transport by microfilament-based motor protein: (Movements of pigment granules)**

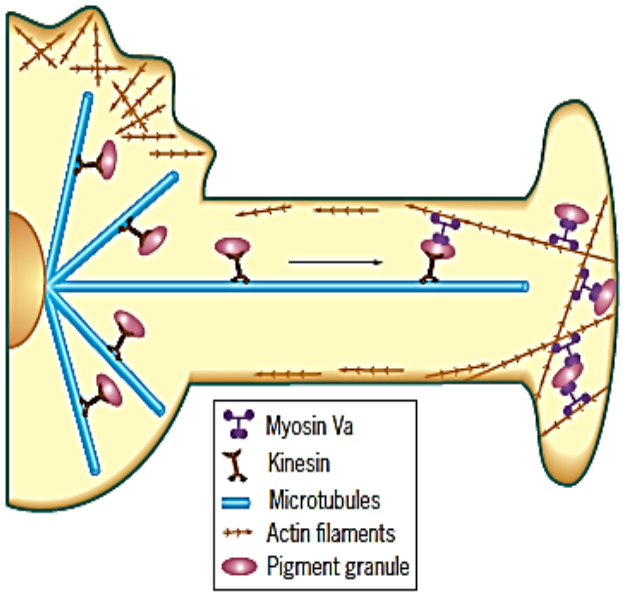


FIGURE 9.45 The contrasting roles of microtubule- and microfilament-based motors in intracellular transport. Most vesicle transport is thought to be mediated by members of the kinesin and dynein families, which carry their cargo over relatively long distances. It is thought that some vesicles also carry myosin motors, such as myosin Va, which transport their cargo over microfilaments, including those present in the peripheral (cortical) regions of the cell. The two types of motors may act in a cooperative manner, as illustrated here in the case of a frog pigment cell in which pigment granules move back and forth within extended cellular processes.

SOURCE: After X, Wu, et al., Copyright 1998, Rockefeller University Press. Originally published in the Journal of Cell Biology 143:1915.

Myosin: The Molecular Motor of Actin

- Myosins move toward the **barbed end of an actin filament**. Myosin was first isolated from mammalian skeletal muscle tissue and has subsequently been found in virtually all eukaryotic cells.
- All myosins share a characteristic motor (head) domain. The head contains a site that binds an actin filament and a site that binds and hydrolyzes ATP to drive the myosin motor. Whereas the **head domains of various myosins are similar**, the tail domains are highly divergent.
- Myosins also contain a variety of low-molecular weight (light) chains. Myosins are generally divided into two following broad groups:
- **The conventional** (or **type II) myosins**, which were first identified in muscle tissue.
- **The unconventional** myosins are subdivided on the basis of amino acid sequence into at least 17 different classes (**type I and types III–XVIII**).
- Some of these classes are expressed widely among eukaryotes, whereas others are restricted.
- **Myosin X**, for example, is found only in **vertebrates**.
- **Myosins VIII and XI** are present only in **plants**.
- Humans contain about **40 different myosins** from at least 12 classes, each presumed to have its own specialized function(s). Of the various myosins, those in the conventional (type II) class are best understood.
- Among their **non-muscle activities, type II myosins** are required for **splitting a cell** in two during **cell division**, generating **tension at focal adhesions**, **cell migration**, and the turning behavior of growth cones (see Figure 9.67).
- An electron micrograph of a pair of myosin II molecules is shown in **FIGURE 9.41 a**. Each myosin II molecule is composed of six polypeptide chains— one pair of heavy chains and two pairs of light chains—organized in such a way as to produce a highly asymmetric protein (Figure 9.41 *a*). Examination of the molecule in Figure 9.41 *b* shows it to consist of (1) a pair of globular heads that contain the catalytic site of the molecule; (2) a pair of necks, each consisting of a single, uninterrupted α helix and two associated light chains; and (3) a single, long, rod-shaped tail formed by the intertwining of long α-helical sections of the two heavy chains. Isolated myosin heads (S1 fragments of Figure 9.41 *b*) that have

been immobilized on the surface of a glass coverslip are capable of sliding attached actin filaments in an in vitro assay such as that shown in **FIGURE 9.42**.

- Thus, all of the machinery required for motor activity is contained in a single head. The mechanism of action of the myosin head, and the key role played by the neck, are discussed in Figure 9.52.
- The fibrous tail portion of a myosin II molecule plays a structural role, allowing the protein to form filaments. Myosin II molecules assemble so that the ends of the tails point toward the center of the filament and the globular heads point away from the center.

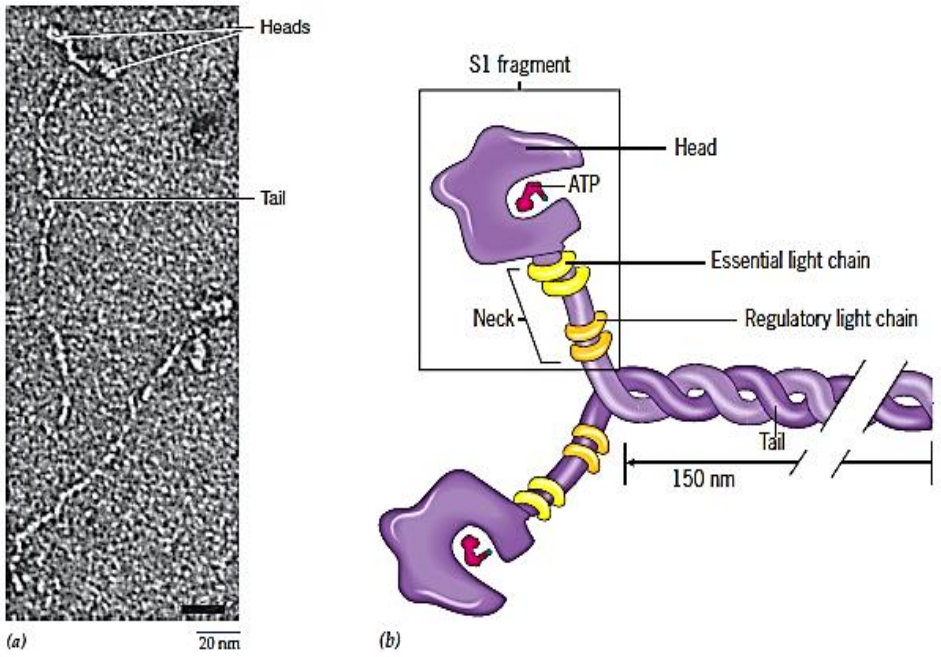


FIGURE 9.41 Structure of a myosin II molecule. (*a*) Electron micrograph of negatively stained myosin molecules. The two heads and tail of each molecule are clearly visible. (*b*) A highly schematic drawing of a myosin II molecule (molecular mass of 520,000 daltons). The molecule consists of one pair of heavy chains (purple) and two pairs of light chains, which are named as indicated. The paired heavy chains consist of a rod-shaped tail in which portions of the two polypeptide chains wrap around one another to form a coiled coil and a pair of globular heads. When treated with a protease under mild conditions, the molecule is cleaved at the junction between the neck and tail, which generates the S1 fragment.

SOURCE: (*a*) From S. A. Burgess, M. L. Walker, H. D. White, and J. Trinick, J. Cell Biol. 139:676,1997, Fig 1. Reproduced with permission of the Rockefeller University Press.

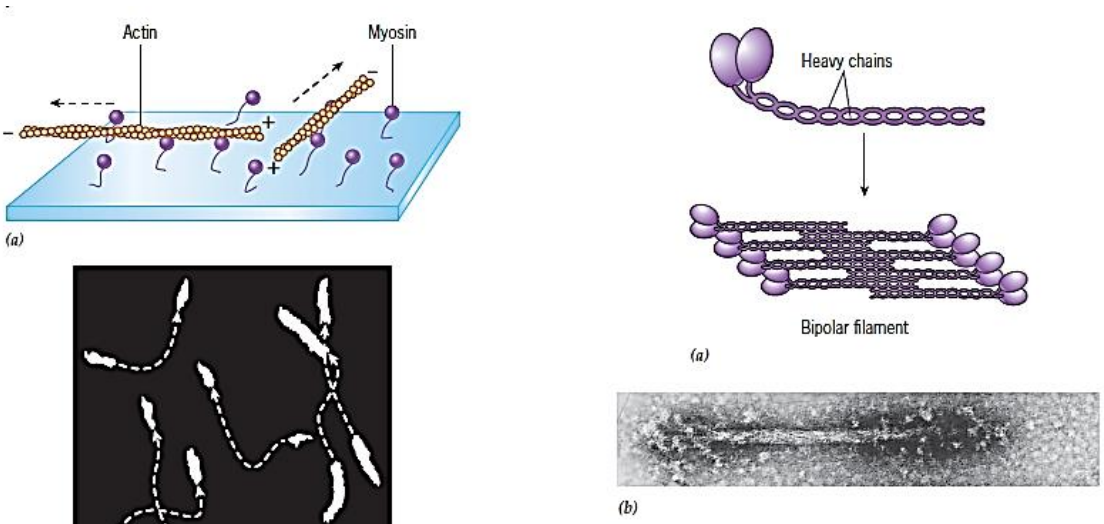


FIGURE 9.43 Structure of a bipolar myosin II filament. (a) Schematic diagram of the staggered arrangement of the individual myosin molecules in a myosin II filament. (b) Electron micrograph of a bipolar myosin filament formed in vitro. The heads of the filament are seen at both ends, leaving a smooth section in the center of the filament.

SOURCE: (b) Courtesy Hugh Huxley

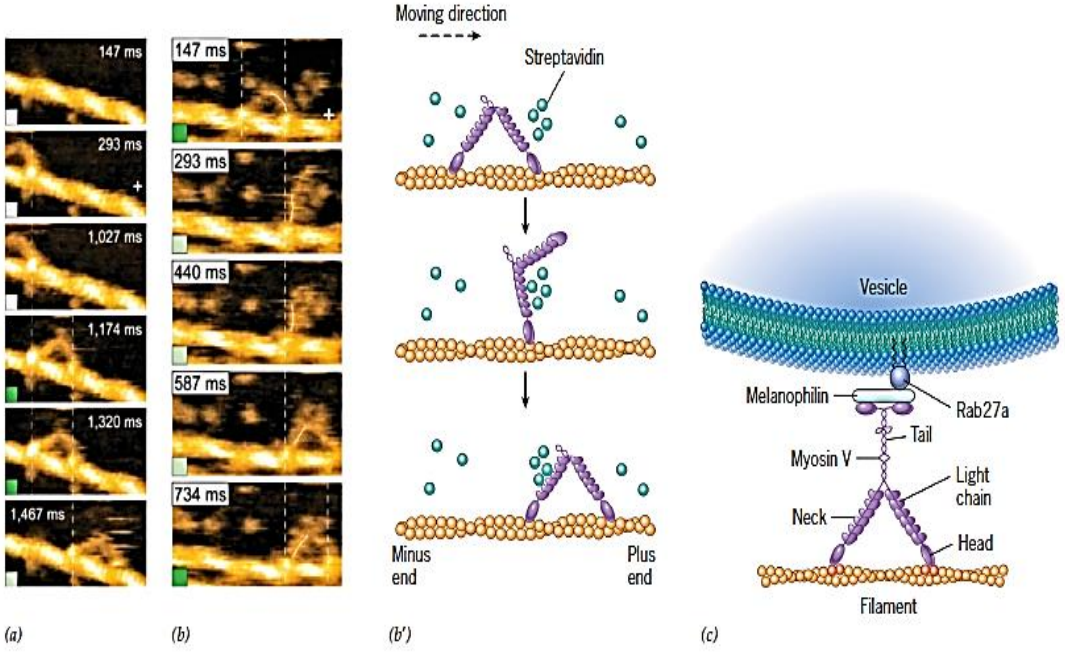


FIGURE 9.44 Myosin V—a two-headed unconventional myosin involved in organelle transport. (a) Direct visualization of a single myosin V molecule (one that is lacking its normal tail domain) as it moves along an actin filament in vitro in the presence of ATP. Using high-speed atomic force microscopy (HS-AFM), this series of images shows the movement of the molecule over a period of about one second. (b) Succesive HS-AFM images showing the hand-over-hand movement of a single myosin V molecule as it passes through a cluster of obstacles (consisting of streptavidin protein molecules). The swinging neck (or lever arm) is highlighted with a thin white line. Interpretive drawings (b′) depict the motor protein as seen in several of the corresponding HS-AFM images. A continuous movie of the protein's movement can be seen in the supplement to this article. (c) Schematic drawing of a complete dimeric myosin V molecule, including its numerous light chains, with both heads bound to an actin filament and its tail domain bound to a vesicle. Rab27a and melanophilin serve as adaptors that link the globular ends of the tail to the vesicle membrane. The long neck of myosin V binds 6 light chains.

SOURCE: From Noriyuki Kodera, et al., Nature, 468:74, 2010, © 2010, reprinted by permission from Macmillan Publsihers, Ltd. Courtesy of Toshio Ando.

Unconventional Myosins

- In 1973, Thomas Pollard and Edward Korn of the National Institutes of Health described a unique myosin-like protein that was extracted from the **protist *Acanthamoeba***. Unlike muscle myosin, this smaller unconventional myosin had only a single head and was unable to assemble into filaments in vitro; the protein became known as myosin I.
- Myosin I often serves as a cross-link between actin filaments of the cytoskeleton and the lipid bilayer of the plasma membrane. It has been suggested that myosin I can exert tension on the plasma membrane, which could play a role in processes that require movement or deformation of the membrane.
- Several unconventional myosins (including myosin I, V, and VI) are associated with various types of **cytoplasmic vesicles and organelles**. In some cases, these myosins may act primarily as organelle tethers and in other cases as organelle transporters. Some vesicles have been shown to contain microtubule-based motors (kinesins and/or cytoplasmic dynein) and **microfilament-based motors (unconventional myosins**), and, in fact, the two types of motors may be physically linked to one another.
- The movement of vesicles and other membranous carriers over long distances within animal cells occurs on microtubules, as previously described. However, once they approach the end of the microtubule, these membranous vesicles are often thought to switch over to microfilament tracks for the local movement through the actin-rich periphery of the cell, which is mediated by myosins (**FIGURE 9.45**).

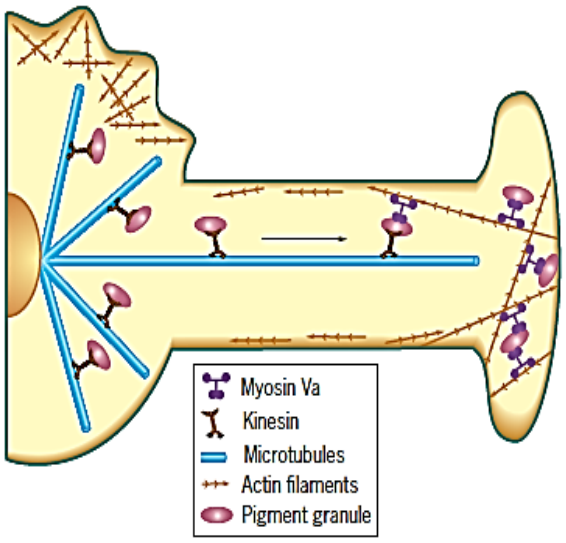


FIGURE 9.45 The contrasting roles of microtubule- and microfilament-based motors in intracellular transport. Most vesicle transport is thought to be mediated by members of the kinesin and dynein families, which carry their cargo over relatively long distances. It is thought that some vesicles also carry myosin motors, such as myosin Va, which transport their cargo over microfilaments, including those present in the peripheral (cortical) regions of the cell. The two types of motors may act in a cooperative manner, as illustrated here in the case of a frog pigment cell in which pigment granules move back and forth within extended cellular processes.
SOURCE: After X, Wu, et al., Copyright 1998, Rockefeller University Press. Originally published in the Journal of Cell Biology 143:1915.

- In mammals, **pigment granules (melanosomes) are transported** into fine peripheral processes of the pigment cell by one of the **myosin V** isoforms called Va. Melanosomes are then transferred to **hair follicles** where they become incorporated into a developing hair.
- Mice lacking **myosin Va** activity are unable to transfer melanosomes into hair follicles, causing their coat to have a much lighter color. Humans lacking a normal gene encoding myosin Va suffer from a rare disorder called **Griscelli syndrome**; these individuals exhibit partial albinism (lack of skin coloration) and suffer other symptoms thought to be related to defects in vesicle transport. In 2000 it was discovered that a subset of Griscelli patients had a normal myosin Va gene, but lacked a functional gene encoding a peripheral membrane protein called Rab27a.
- The **Rab family of proteins** was discussed on page 288 as molecules that regulate vesicle trafficking. Rabs are also involved in the attachment of myosin (and kinesin) motors to membrane surfaces (Figure 9.44 *c*).
- Inner ear hair cells, whose structure is shown in **FIGURE 9.46 a**, have been a particularly good system for studying the functions of unconventional myosins. Hair cells are named for the **bundle of stiff, hairlike stereocilia** that project from the apical surface of the cell into the fluid-filled cavity of the inner ear.
- Displacement of the **stereocilia** by mechanical stimuli leads to the opening of Ca2+ channels in the plasma membrane and the subsequent generation of nerve impulses that we perceive as sound. Stereocilia have no relation to the true cilia discussed earlier. Instead of containing microtubules, **each stereocilium is supported by a bundle of parallel actin filaments** (Figure 9.46 *b*) whose barbed ends are located at the outer tip of the projection and pointed ends at the base. Stereocilia have provided some of the most striking images of the dynamic nature of the actin cytoskeleton. While the stereocilia themselves are permanent structures, the actin bundles are in constant flux. Actin monomers continually bind to the barbed end of each filament, treadmill through the body of the filament, and dissociate from the pointed end. This process is captured in the fluorescence micrograph of Figure 9.46 *c*, which shows the incorporation of GFP-labeled actin subunits at the barbed end of each filament.

- Several unconventional myosins (I, III, V, VI, VII, and XV) are localized at various sites within the hair cells of the inner ear; two of these are shown in Figure 9.46 *d* and *e*. **Mutations in myosin VIIa** are the cause of **Usher 1B syndrome**, which is characterized by **both deafness and blindness**. The morphologic effects of mutations in the myosin VIIa gene on the hair cells of the inner ear of mice are shown in Figure 9.46 *f,g*. As in humans, mice that are homozygous for the mutant allele encoding this motor protein are deaf.
- Myosin VI, a processive organelle transporter in the cytoplasm of many cells, is distinguished by its movement in a "reverse direction,"that is, toward the pointed end of an actin filament. Myosin VI is located at the base of the stereocilia where it might connect the cytoskeleton to the overlying plasma membrane. In other types of cells, myosin VI is thought to be involved in the formation of clathrin-coated vesicles at the plasma membrane, the movement of uncoated vesicles to early endosomes, and the maintenance of Golgi morphology. Mutations in myosin VI are the cause of several inherited diseases. Now that we have described the basics of actin and myosin structure and function, we can see how these two proteins interact to mediate a complex cellular activity.

Drugs related to Actin filaments:

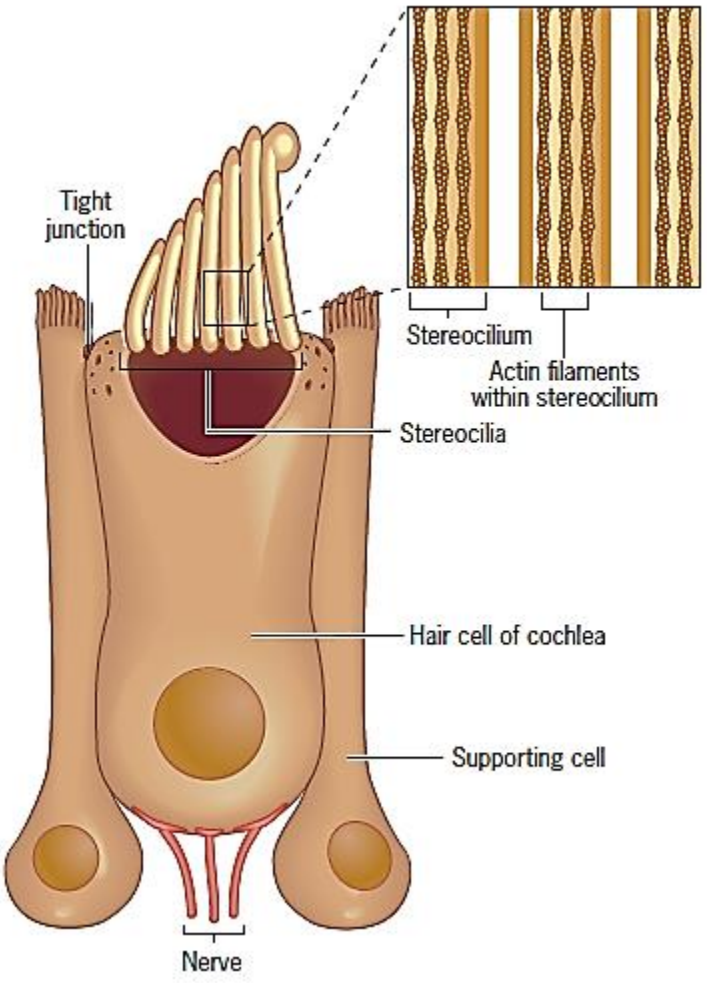
- The involvement of these filaments is most readily demonstrated by treating the cells with one of the following drugs that disrupt dynamic actin-based activities:
- **Cytochalasin**, derived from **a mold**, which blocks the barbed ends of actin filaments and allows depolymerization at the pointed end.
- **Phalloidin**, obtained from **a poisonous mushroom**, which binds to intact actin filaments and prevents their turnover.
- **Latrunculin**, obtained from **a sponge**, which binds to free monomers and blocks their incorporation into the polymer.
- Actin-mediated processes rapidly grind to a halt when cells contain one of abovesaid compounds.

ACTIN-SPECIFIC DRUGS

Phalloidin	binds and stabilizes filaments
Cytochalasin	caps filament plus ends
Swinholide	severs filaments
Latrunculin	binds subunits and prevents their polymerization

A hair cell of the cochlea:

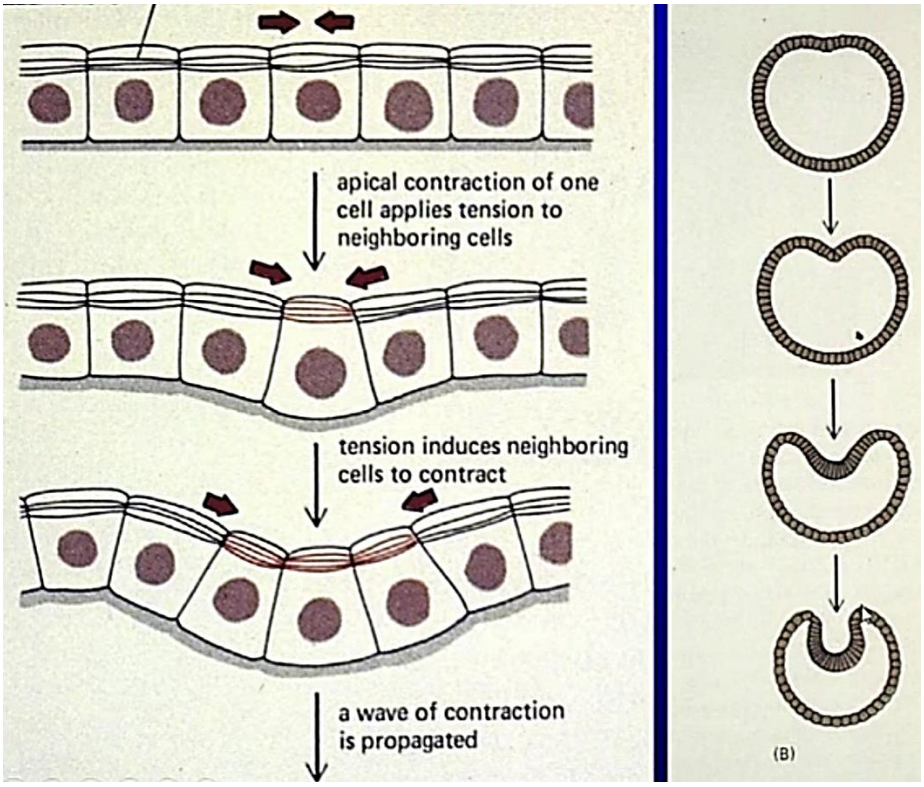
The inset shows a portion of several of the stereocilia, which are composed of a tightly grouped bundle of actin filaments.

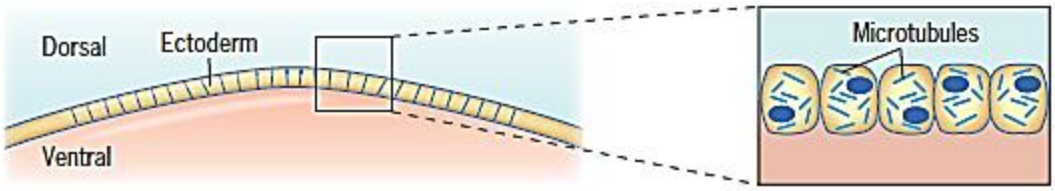


Variuos Functions:

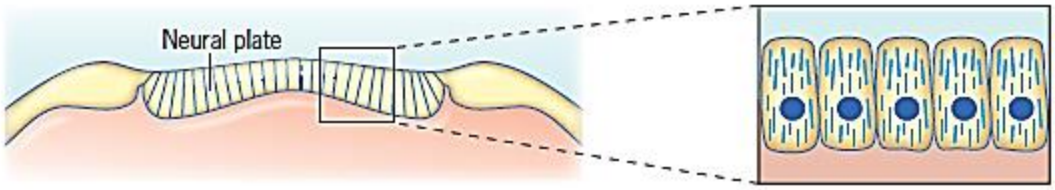
- Actin filaments, often working in conjunction with myosin motors and the actin-binding proteins described above, are responsible for a variety of dynamic activities in non-muscle cells, including cytokinesis, phagocytosis, cytoplasmic streaming (the directed bulk flow of cytoplasm that occurs in certain large plant cells), vesicle trafficking, blood platelet activation, lateral movements of integral proteins within membranes, cell–substratum interactions, cell locomotion, axonal outgrowth, and changes in cell shape. Cellular motility is illustrated by the following examples.
- Cell locomotion is required for many activities in higher vertebrates, including tissue and organ development, formation of blood vessels, development of axons, wound healing, and protection against infection. Cell locomotion also contributes to the spread of cancerous tumors.

Rolling of the plate into a tube is thought to be driven by contractile forces generated by actin filaments at the apical ends of the cells

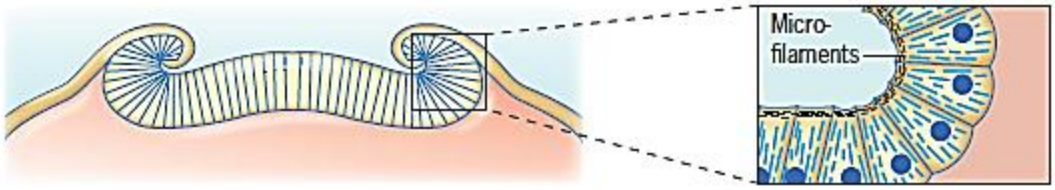



Dorsal
Ectoderm
Ventral
Microtubules

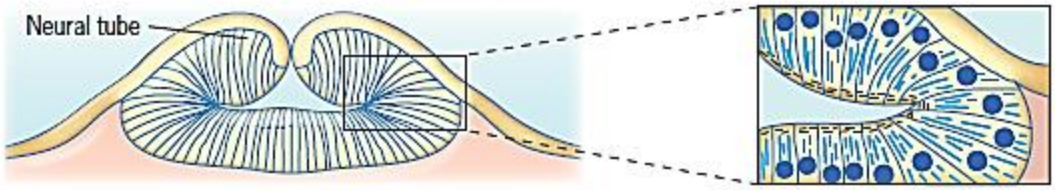
(a)


Neural plate

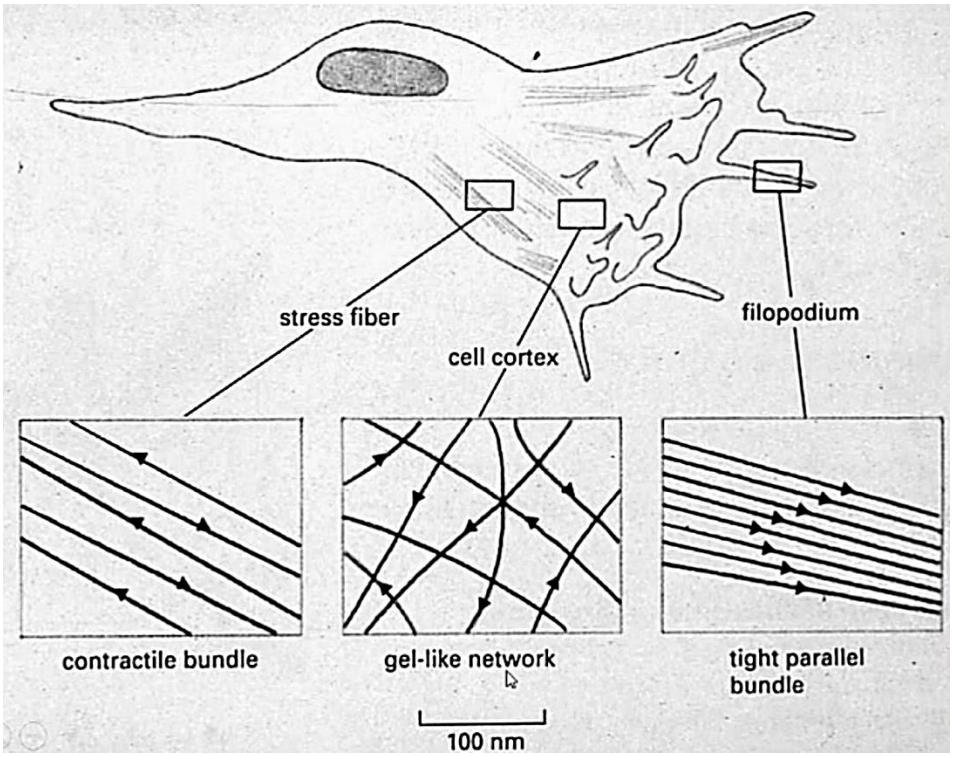
(b)

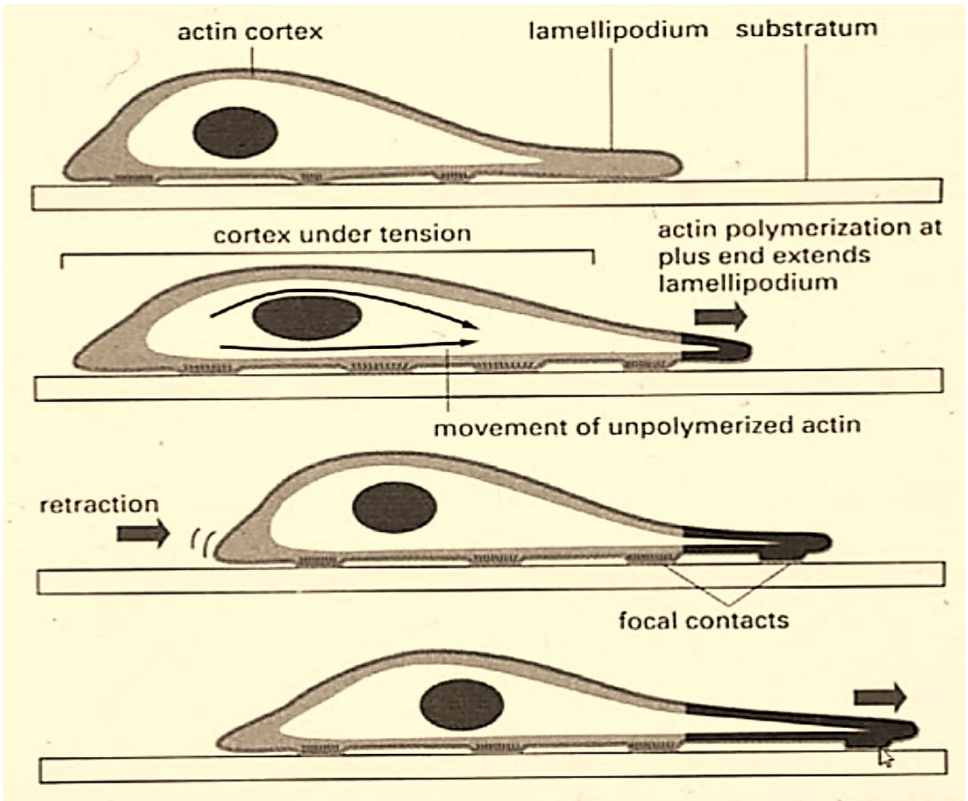

Micro-
filaments

(c)


Neural tube

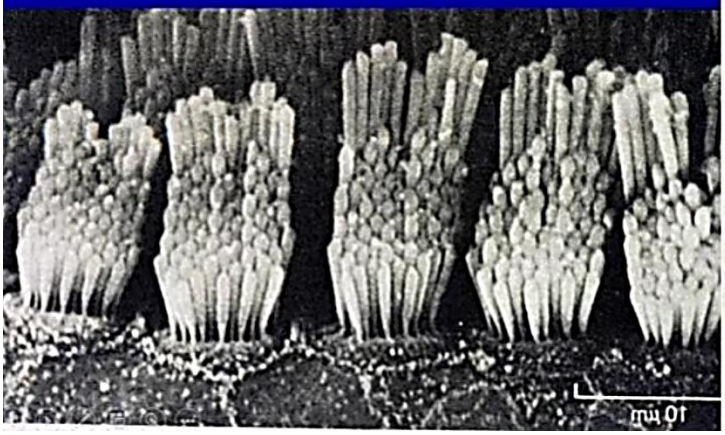
(d)

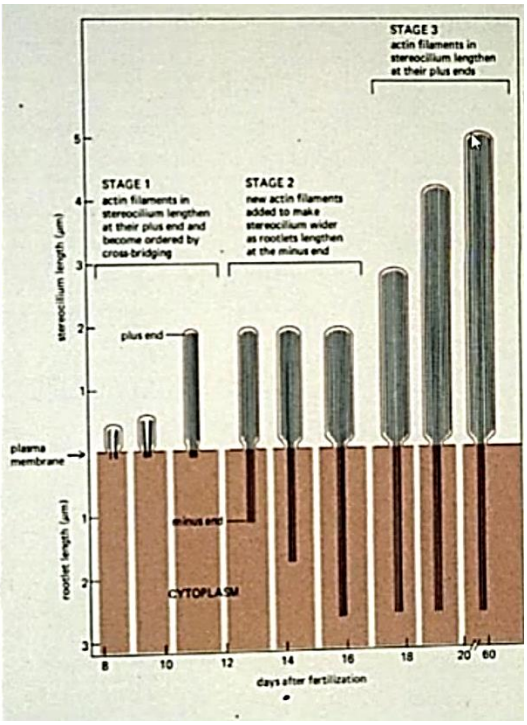

stress fiber
cell cortex
filopodium
contractile bundle
gel-like network
tight parallel bundle
100 nm


actin cortex
lamellipodium
substratum
cortex under tension
actin polymerization at plus end extends lamellipodium
movement of unpolymerized actin
retraction
focal contacts

Microfilaments - function

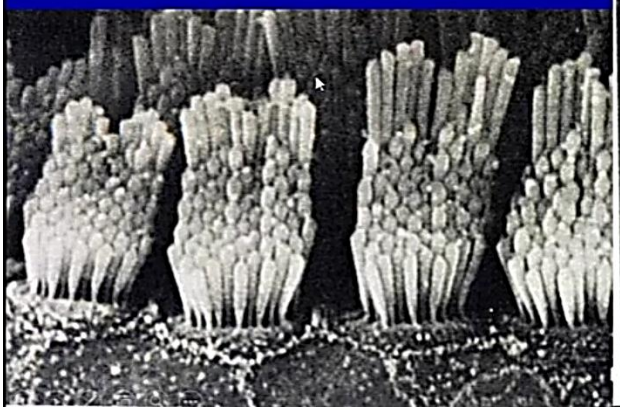
Structural support -
Stereocilia - extension

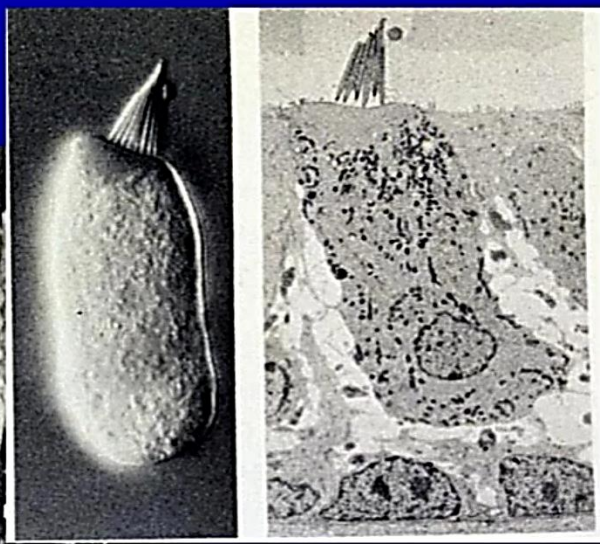




Microfilaments - function

Structural support -
Stereocilia - extension

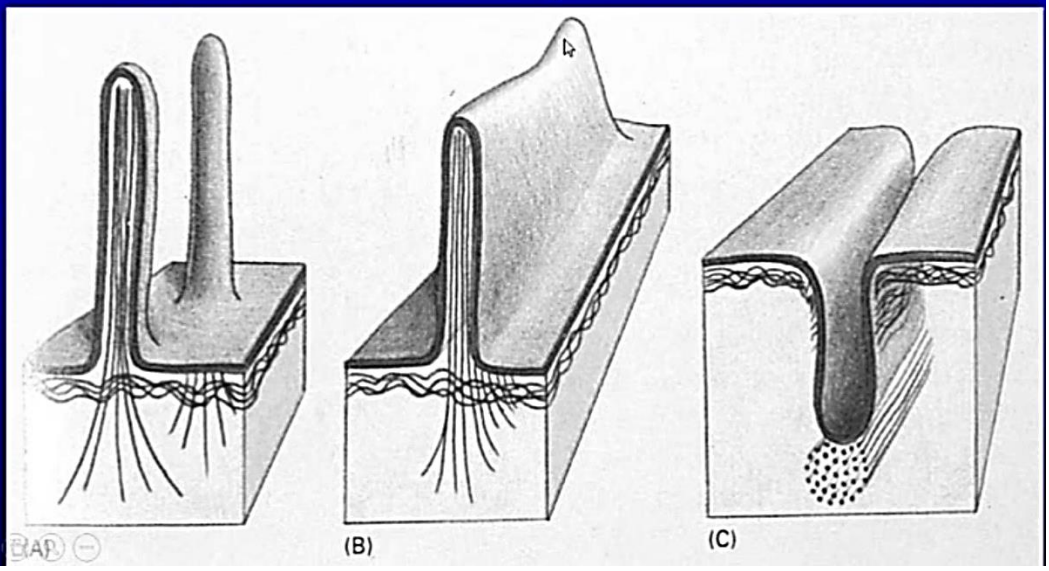




Microfilaments - function

Structural support -

– Microvilli - movement and shape

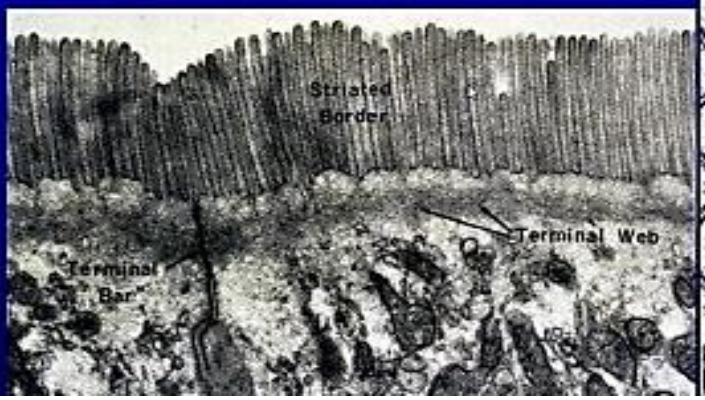


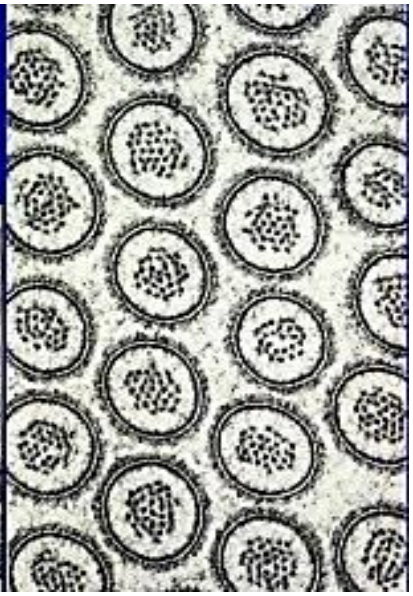
Microfilaments - function

Structural support -

– Microvilli movement and shape

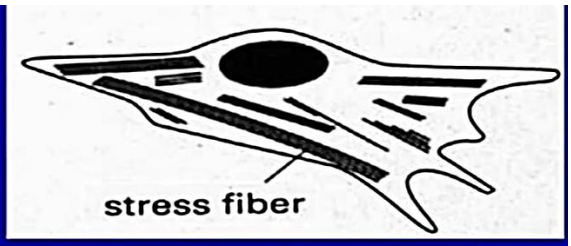
– Pushes membrane out from cell

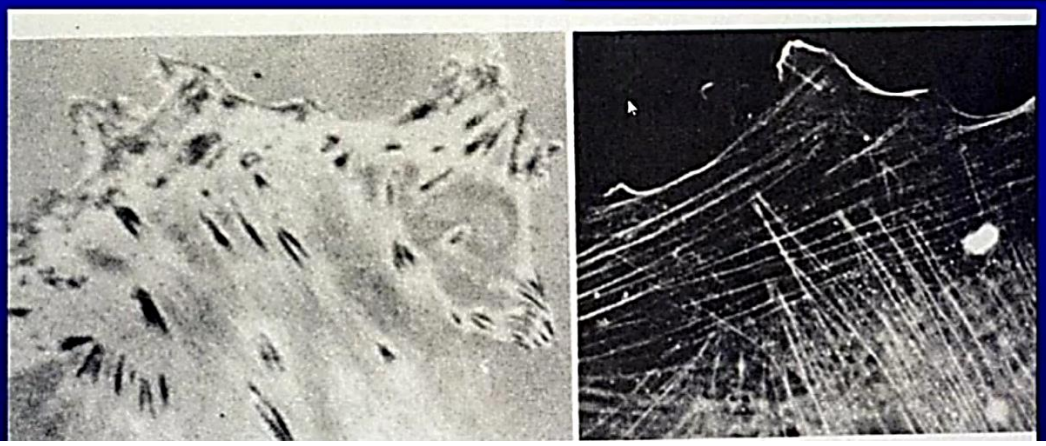




Microfilaments - function

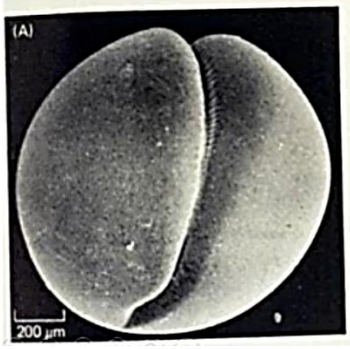
Structural support
– Stress fibers

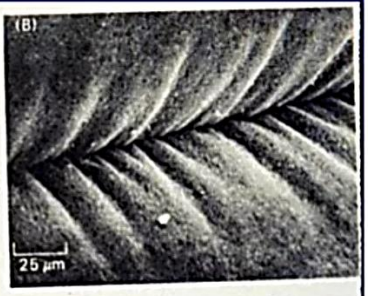


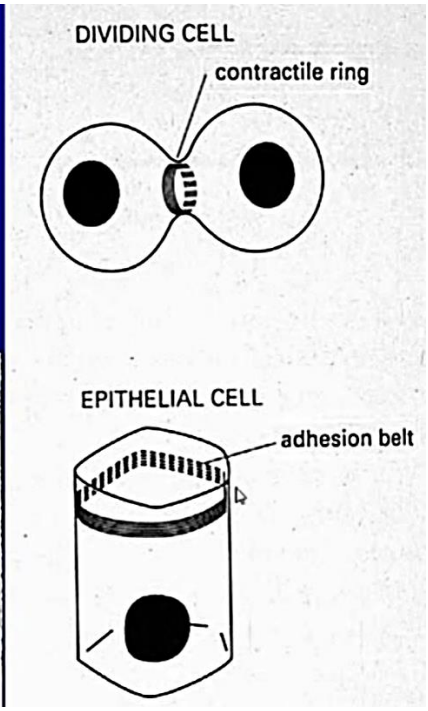


Microfilaments - function

Cytokinesis - division of cytoplasm

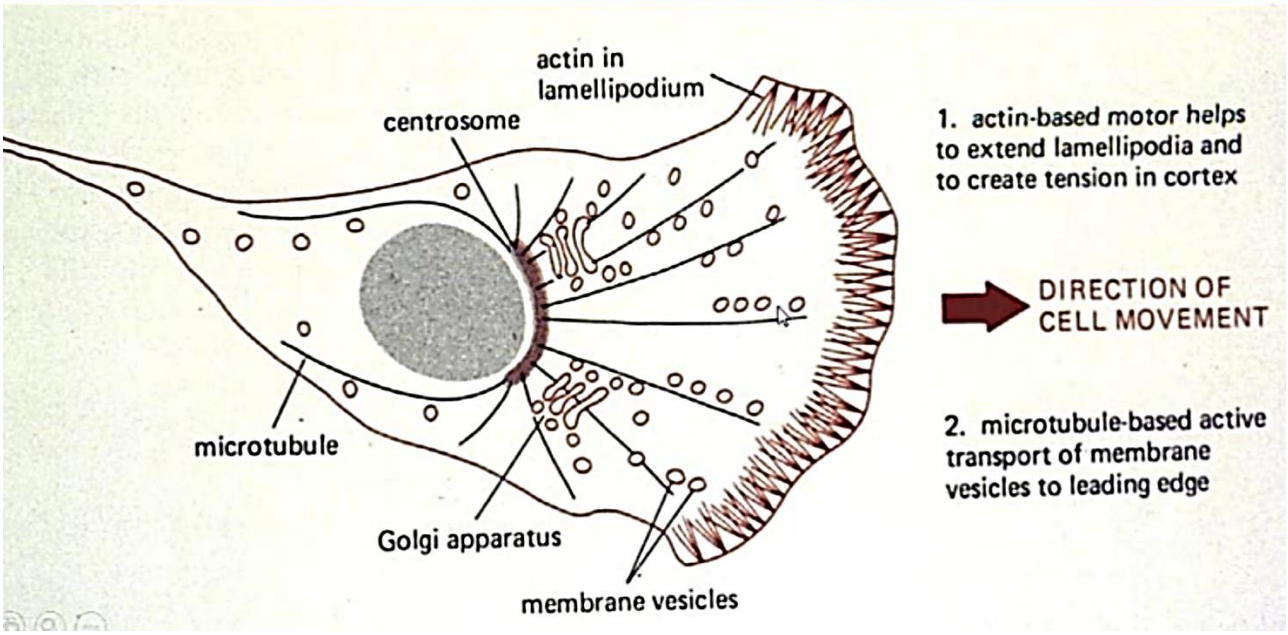






Microfilaments - function

- Cell motility - actin and myosin



Microfilaments - function

- Cell motility - actin and myosin