Genetics
& Cell biology

◉ Sheet ○ Slides

Number

8

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Introduction

In this sheet, we will continue the discussing the final organelle, the nucleus, after that we will start discussing the cytoskeleton and cell movement.

Recall

In the last lecture, we talked about the nuclear bodies; which are physiological compartments with special functions in the nucleus. Notice that such compartments are not physically separated; the nucleolus for example does not have special membrane that encloses it.

The Nucleolus

The nucleolus is the most prominent of the nuclear bodies. And although it may seem a prominent part of the nucleus when examined with the microscope, it is continuous with the nucleus, and it does not have a surrounding membrane.

Functions of the Nucleolus

The nucleolus performs many important functions; such as:

1. rRNA synthesis.
2. Ribosome production.
3. RNA modification and the assembly of ribonucleoprotein particles.
4. Small RNA production such as tRNA, snRNA, RNase P RNA (the catalytic part of the tRNA processing enzyme), SRP (targets proteins to ER).
5. Cell division and responding to stress.

These functions are discussed in the next sections.

Most of our genome performs functions that are still not fully understood yet. And as some genes are transcribed to mRNA, which is in turn translated to amino acid sequence (polypeptide); other genes encode for functional RNA molecules; such as rRNA (ribosomal RNA).

Nucleoli are the cell factories to manufacture the rRNA and other RNA molecules, in addition to fulfilling the function of ribosomes subunits assembly.

The nucleolus is associated with chromosomal regions that contain about 200 copies of the genes for 5.8S, 18S and 28S rRNAs to synthesize large amounts of ribosomes. 5.8S, 18S and 28S rRNAs genes transcribed as a single unit by polymerase in the nucleolus. These genes are separated by spacers (non-coding sequences) which provide protection for the genes from deletions or damages on the sequence. 18S rRNA is a part of the small ribosomal subunit (40S); whereas the large ribosomal subunit (60S) has the 5.8S and 28S rRNAs, in addition to the 5S rRNA, which is produced outside the nucleolus (by RNA polymerase III).

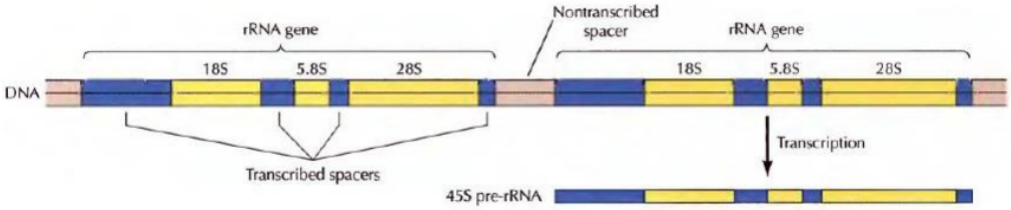


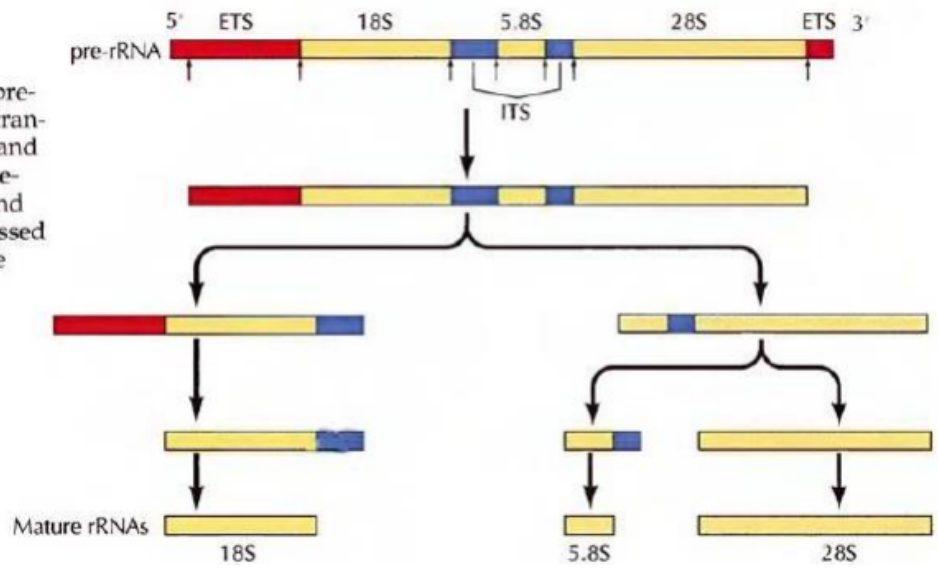
FIGURE 9.25 Ribosomal RNA genes Each rRNA gene is a single transcription unit containing the 18S, 5.8S, and 28S rRNAs as well as transcribed spacer sequences. The rRNA genes are organized in tandem arrays, separated by nontranscribed spacer DNA.

Ribosomal subunits preparation

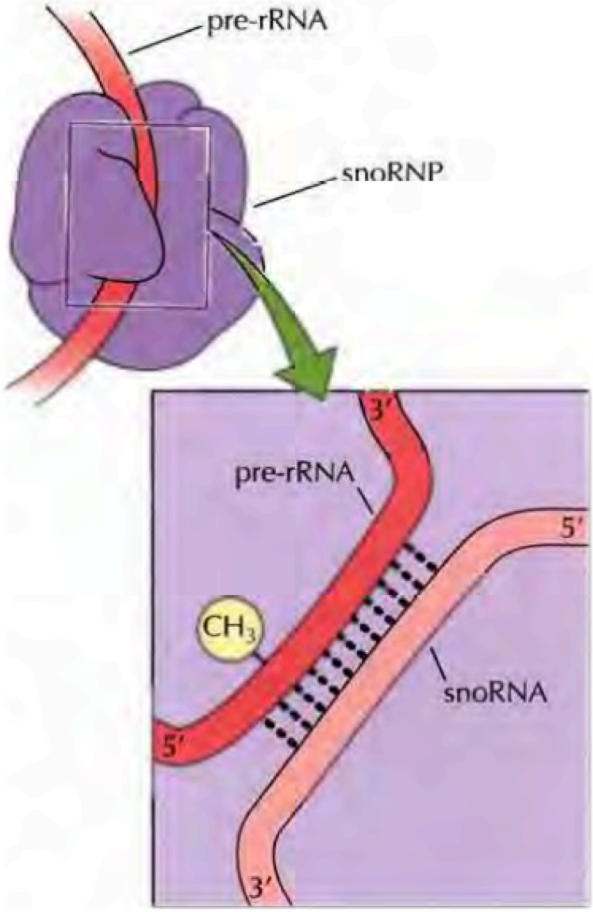
After the transcription of the genes mentioned with the separating spacers, a large molecule, which is the 45S pre-rRNA results. As we can see from the figure bellow, this molecule has 2 external spacers (at the 3' end and the 5' end), and 2 internal spacers.

This transcript will pass through cleavage steps (not for memorization) and base modification (methylation of ribose and some other bases). Such cleavage steps include cleavage within the external transcribed spacer (ETS) near the 5'end, and the removal of the ETS at the 3'end.

FIGURE 9.29 Processing of pre-rRNA The higher eukaryote 45S pre-rRNA transcript contains external transcribed spacers (ETS) at both ends and internal transcribed spacers (ITS) between the sequences of 18S, 5.8S, and 28S rRNAs. The pre-rRNA is processed via a series of cleavages to yield the mature rRNA species.



Pre-rRNA Processing



Such functions need the action of both proteins and RNAs inside the nucleolus. One of such is snoRNP; which is a complex of several proteins and one molecule of small nucleolar RNAs snoRNA. snoRNP complexes with the pre-RNA to be processed. By base pairing with pre-rRNA (they contain ~15 nucleotides complementary to prerRNA), they target it by the enzymes that catalyze base modification, in addition to the cleavage of pre-rRNA into 5.8S, 18S and 28S products.

Ribosome assembly

Ribosomes formation involves the assembly of the ribosomal precursor RNA with the ribosomal proteins and the 5S rRNA. The ribosomal proteins are synthesized by the formation of the mRNAs inside the nucleus, which are trans-located to the cytoplasm, where they are translated to polypeptides. After that, such proteins enter the nucleus to go to the nucleolus for the assembly process. The assembly of the proteins with the rRNAs forms preribosomal particles. Although 5S rRNA is synthesized by RNA polymerase outside the nucleolus, it assembles similarly inside the nucleolus. Then, ribosomal subunits are processed. Small subunit processing is simpler than that of a large subunit, and is done by 4 endonuclease cleavages in the nucleus. Whereas, the large subunit (composed of 28S, 5.8S and 5 rRNAs) processing is more complex, and done with extensive nuclear cleavage in the nucleolus. Pre-ribosomal particles are exported to the cytoplasm to form the active 40S and 60S subunits of ribosomes, such process is called ***ribosomal subunit maturation*.**

Conclusion

By understanding such processes, we can conclude that the ribosomes subunits are ***conjugated proteins***; which means that they are composed of proteins with additional polymers; which are rRNAs.

0:00 – 10:00

The Cytoskeleton and Cell Movement

Actin Filaments

Introduction

The cell is made up of a watery cytoplasm, which is surrounded by a mosaic plasma membrane. In the cytoplasm dive many different organelles and compartments of the cell. And the cytoplasm is constructed by the diverse components of the cytoskeleton.

Cytoskeleton is a dynamic network of protein filaments extending throughout the cytoplasm. There are three types of protein filaments that make up the cytoskeleton; which are actin microfilaments, microtubules, and intermediate filaments.

The cytoskeleton performs essential functions. It is the structural framework for cells, and determines cell shape and movement, positions of organelles, and the overall organization of the cytoplasm. It also regulates the internal movement of organelles and other structures such as mitotic chromosomes. Furthermore, it gives the cell the needed structural stability and rigidity to resist trauma.

Actin filaments

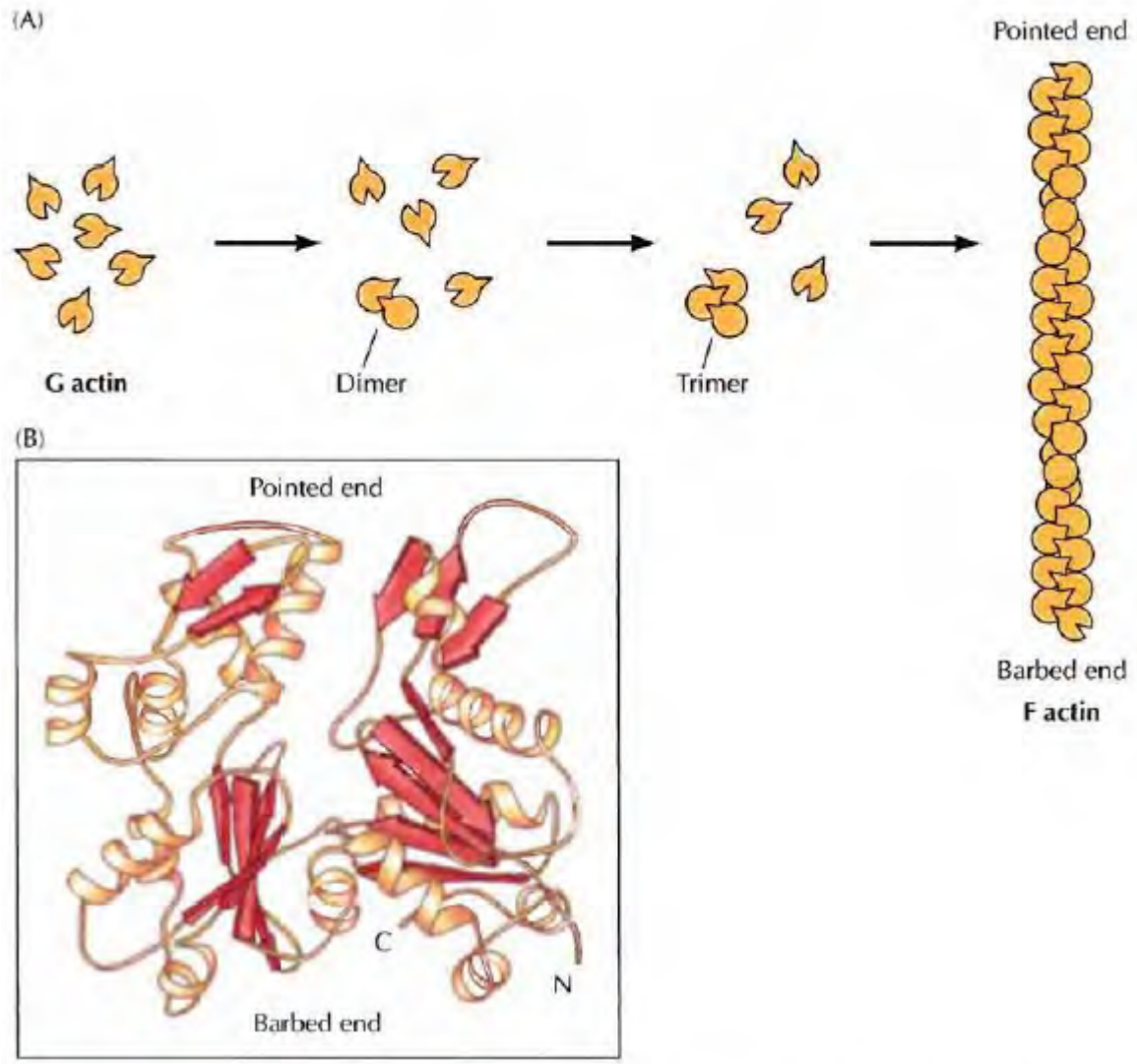
Definition

Actin filaments (also: *microfilaments*) are thin, flexible fibers (7nm in diameter and several µm long). They are organized into higher-order structures, forming bundles or three-dimensional networks, with the properties of semisolid gels. Their assembly, disassembly, crosslinking and association with cellular structures are regulated by a variety of actin-binding proteins. They are abundant beneath the plasma membrane to form a network that provides mechanical support, determines cell shape and allows cell movement.

The Actin Protein

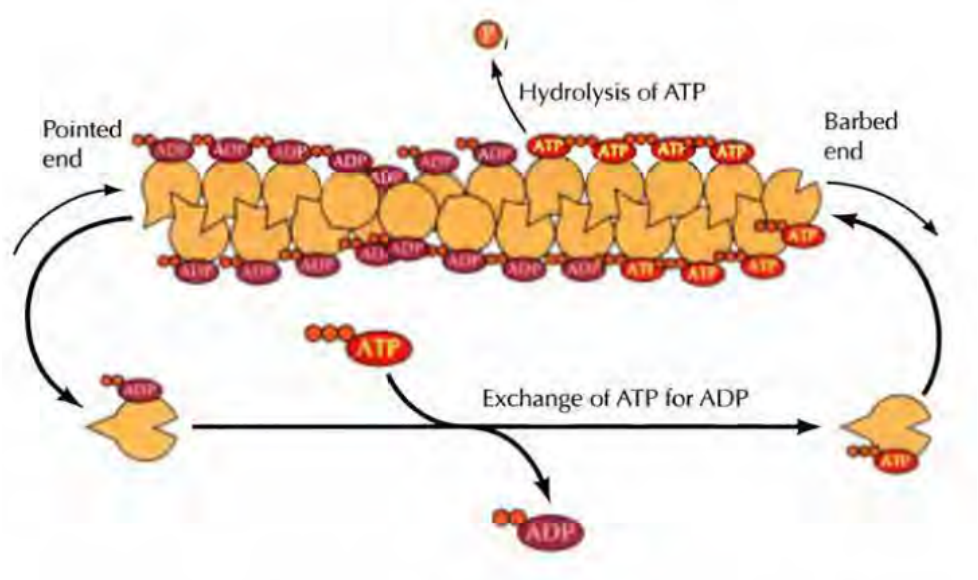
Actin protein is abundant in muscle cell; from which it was first isolated, but it is also present in all other cells. Mammalian cells have at least six distinct actin genes: four are expressed in different types of muscles, and two are expressed in non-muscle cells. All of the actins however, are similar in the amino acid sequence.

Actin filaments are polymers of actin monomers. An actin monomer (globular [G] actin) is tightly bound to two other actin monomers having head-to-tail interactions. Actin monomers polymerize to form filamentous [F] actin. Actin filaments have a distinct polarity and 2 different ends, the barbed (plus) and pointed (minus) ends. Polarity affects actin assembly and the direction of myosin movement relative to actin.



Formation of the filaments

Step 1. Nucleation: formation of an aggregate of 3 actin monomers. **Step 2**. Filament growth by adding additional monomers to both ends (barbed and pointed), but faster at barbed ends. **Step 3.** The monomers are bound to ATP, which is not required for nucleation, but is hydrolyzed into ADP following assembly. This speeds polymerization and stabilizes binding. **Tread-milling:** ATP-actin is added to the barbed end while ADP-actin dissociates from the pointed end; and that is due to conformational changes that occur to the actin after binding the ATP or ADP molecules. The length may stay the same if the addition rate equals the dissociation rate, and this explains the phrase "tread-milling". This illustrates dynamic behavior of actin filaments; shortening may increase in certain settings, while elongation may increase in other settings according to the needed function.



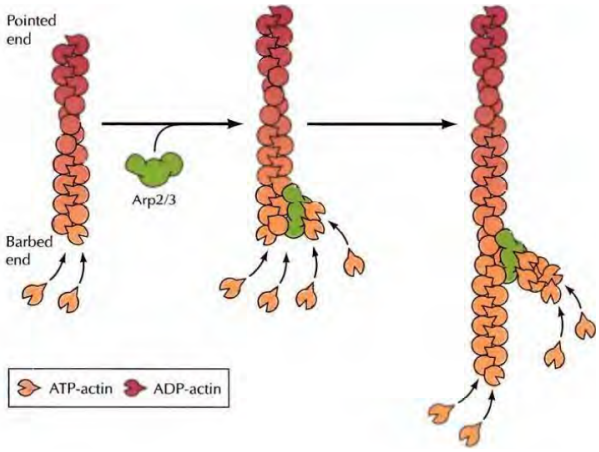
Actin-binding proteins

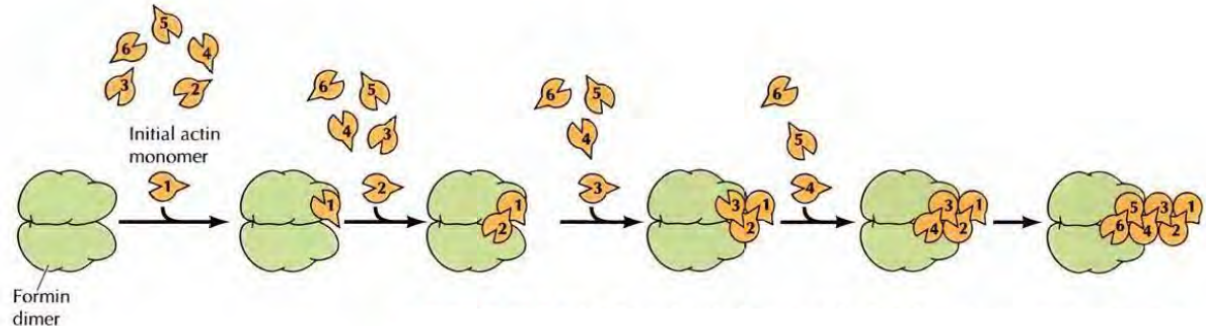
These accessory proteins regulate actin assembly and disassembly as well as stability of actin cytoskeleton. Such bindings increase the variety and the stability of actin filaments. Only mentioned examples are for memorization.

Cellular Role	Representative Proteins
Filament initiation and polymerization	Arp2/3, formin
Filament stabilization	Nebulin, tropomyosin
Filament cross-linking	α-actinin, filamin, fimbrin, villin
End-capping	CapZ, tropomodulin
Filament severing/depolymerization	ADF/cofilin, gelsolin, thymosin
Monomer binding	Profilin, twinfilin
Actin filament linkage to other proteins	α-catenin, dystrophin, spectrin, talin, vinculin

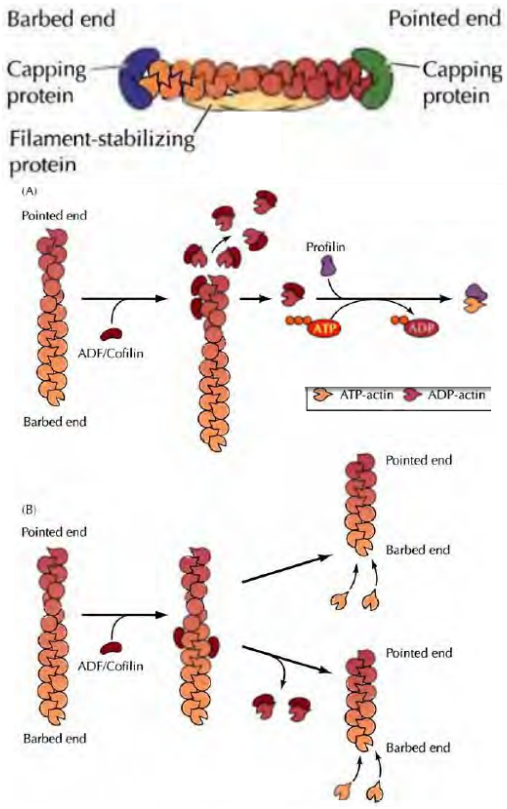
The following points discuss the previous table.

- *Branching is facilitated by the Arp 2/3 complex:* Arp2/3 has a structure that is complementary to actin monomers, but larger than them. When inserted, it allows the linear filament to extend a branch.
- *The rate-limiting step of actin formation, nucleation, is facilitated by formin:* Formin facilitates the formation of the linear filaments of actin; adding monomers to the barbed end.





- *Filament-stabilizing protein; tropomyosin:* Tropomyosin gives strength to the actin filaments, in addition to keeping them stable.
- *ADF/cofilin vs. profilin:* ADF/cofilin complex (ADF: actin depolarizing factor) enhances the rate of dissociation. It performs this function by binding to the pointed end of the actin filament. It also binds ADP-actin monomers to stabilize their binding to ADP instead of ATP. Moreover, it can sever actin filaments by binding the filament from an internal point; splitting the filament and creating new plus ends. However, profilin reverses these effects and enhances the elongation of the actin filaments. profilin binds to actin monomers dissociated under the effect of



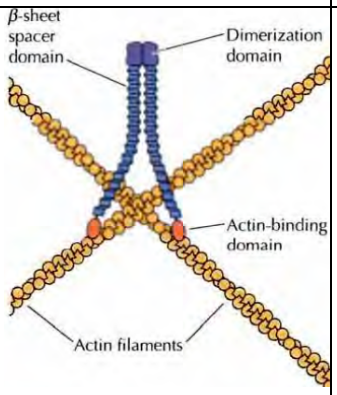
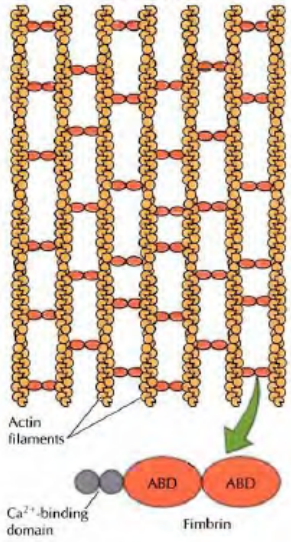
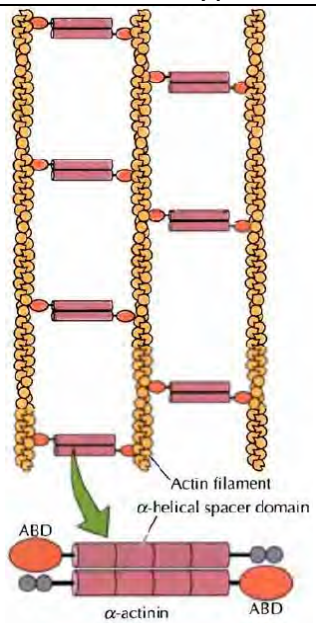
cofilin, stimulating the exchange of ADP for ATP. This provides active monomers for assembly into filaments.

- *α-actinin and filamin:* discussed in the next section.

For more information, review the links inserted in the slides.

Actin filaments arrangements

Different arrangements of actin filaments are required for actin to perform its various functions in the cell; including locomotion of the whole cell or parts of it. There are two main arrangements of actin filaments; **bundles** and **networks**. Each of these arrangements involves different actin-binding proteins to construct a cross-linkage. These arrangements differ in flexibility, size and shape of the cross-linking proteins.

Difference	networks	Bundles	
		First type	Second type
Illustration			
Cross-linking protein example	Large actin-binding proteins; *filamin*; binds actin as a flexible dimer	*Fimbrin*; Actin monomer interaction	*α-actinin*; Actin-dimer interaction
Features	Flexible; loose; three-dimensional meshwork; semisolid liquid properties; supports the cell membrane	Closely packed; rigid; supports membrane projections like microvilli	More widely spaced; allows contact and interaction (for example with myosin); **contractile bundles**
Example		Microvilli in intestinal epithelium	Muscle cells

20:00 – 27:00

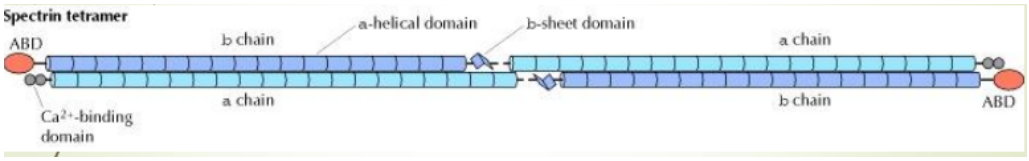
Actin fibers in the cell; Actin filaments association with the plasma membrane

Cell cortex or cortical cytoskeleton*:* The 3D network of actin filaments and associated actin-binding proteins at the cell periphery that determines cell shape and assist in cellular activities such as movement. This network supports the plasma membrane and exists in many cell types.

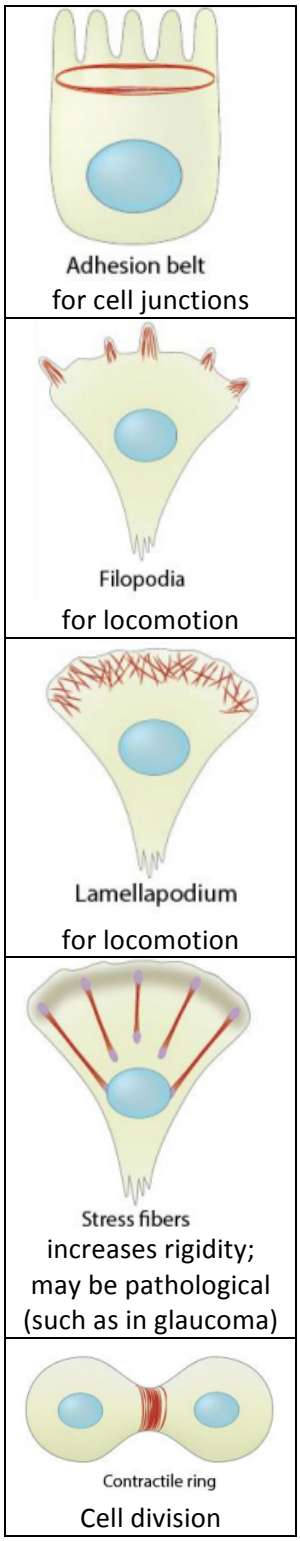
Studies of cortical cytoskeleton and plasma membrane were mainly conducted on the RBCs, where the cytoskeleton is uniform with no specialized regions like in other cells; they do not have other cytoskeletal structures and they do not have nucleus or other organelles, which prevents results contamination.

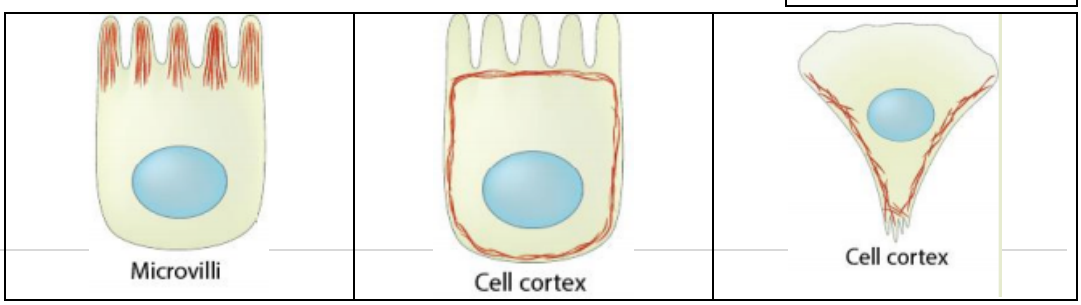
Cortex components

Actin filaments interact with many proteins depending on the cell type and the cell function, to form the cortex. ***Spectrin*** is an important example of such proteins. Spectrin is the major protein that provides the structural basis for the cortical cytoskeleton in erythrocytes. Structurally, it is a tetramer of two α and β polypeptides, with the α chain having two Ca2+ binding domains at it's C-terminus (for contractile functions), and the β chain having the actin-binding domain.

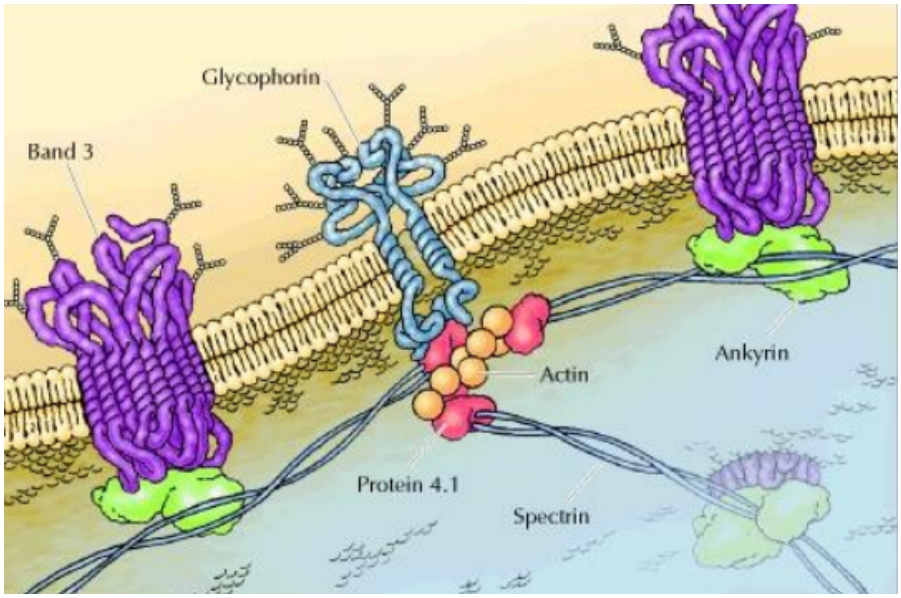


Spectrin interacts with actin filaments directly or with helping proteins; such as protein 4.1. Then, this helper protein interacts with membrane protein, like glycophorin, to increase the stability of the structure. The interaction with the membrane protein is preceded by an interaction with a peripheral protein; such as Ankyrin and 4.1, which, in turn, binds to the

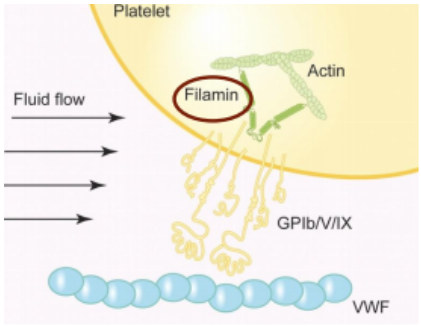


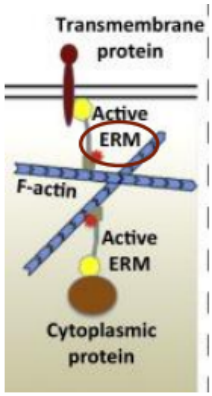


membrane protein.

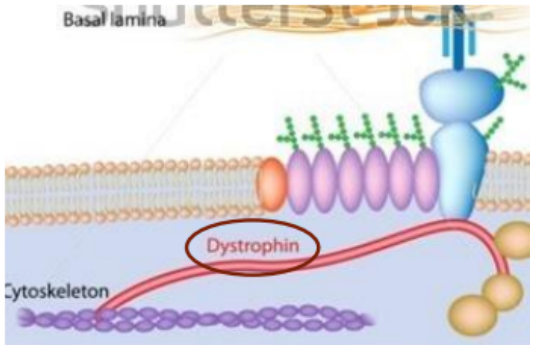


In muscle cells, instead of spectrin, ***Dystrophin*** (spectrin-related proteins) links actin filaments to transmembrane proteins of the muscle cell plasma membrane and the latter link the cytoskeleton to the extracellular matrix. ***Filamin*** (spectrin-related) links actin filaments with the plasma membrane of blood platelets. Furthermore, The *ERMproteins* (protein 4.1-related) link actin filaments to the plasma membranes of different kinds of cells.



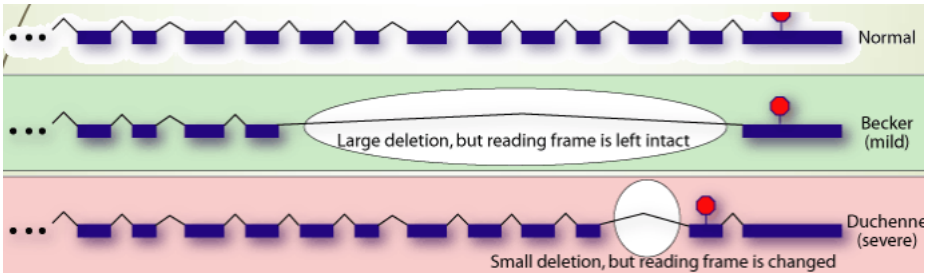


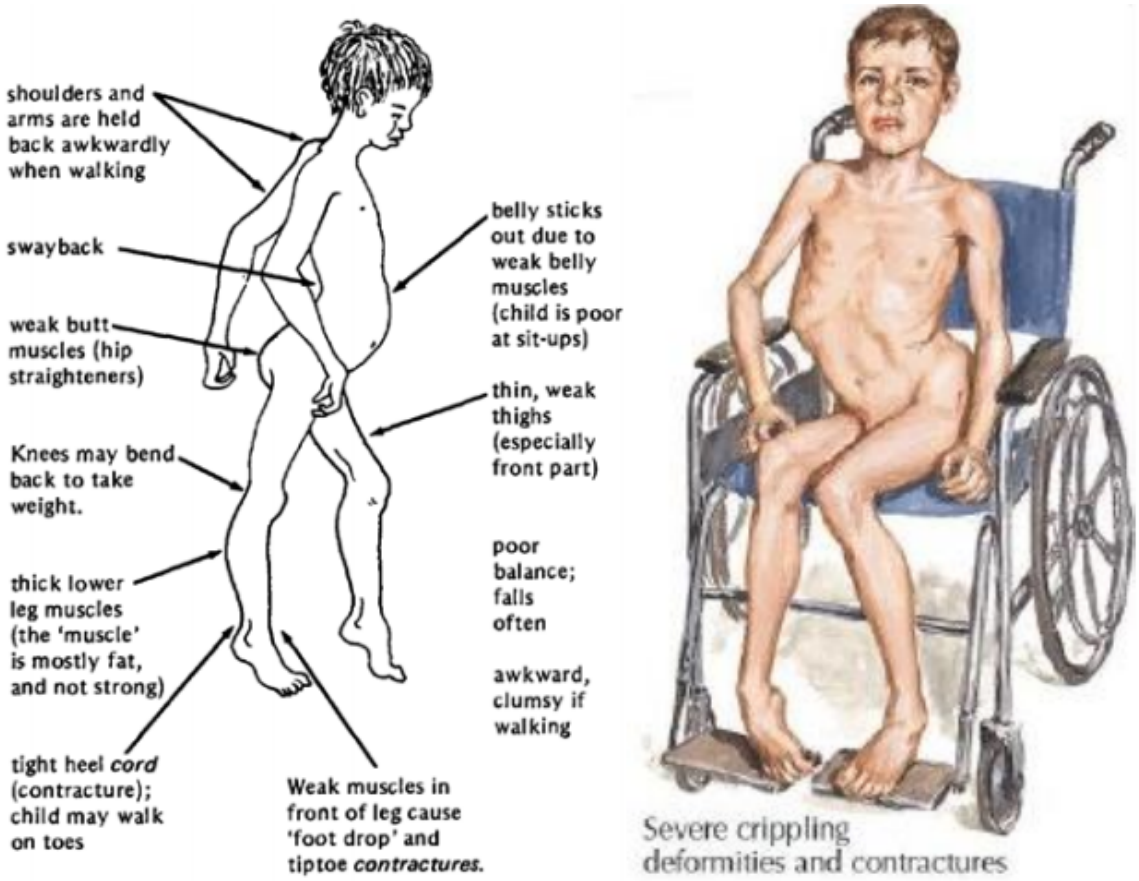
<u>Clinical application; Dystrophin and muscular dystrophies</u>



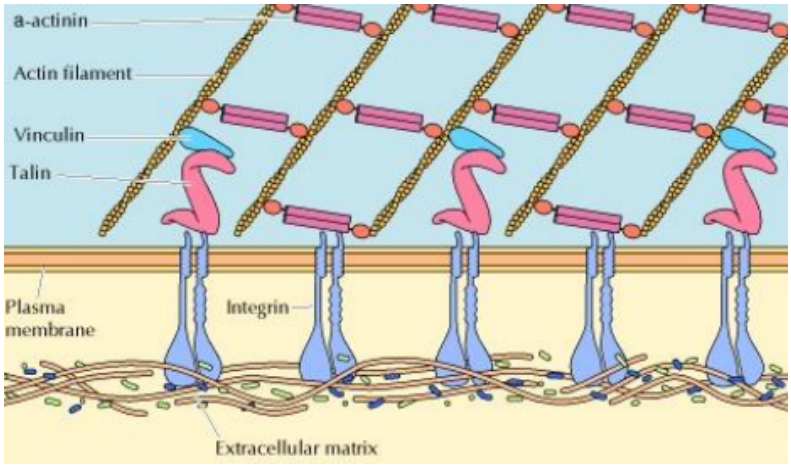
The dystrophin gene encodes a large protein (427 kd). Mutations in the gene causes two types of muscular dystrophy, **Duchenne's** (severe; frameshift mutation; more common) and **Becker's** (moderate; deletion mutation). These diseases are X-linked inherited diseases.

<u>Manifestations</u>: include progressive degeneration of skeletal muscle, with small-sized bodies and curved musculoskeletal system. Patients with Duchenne's muscular dystrophy usually die in their teens or early twenties.



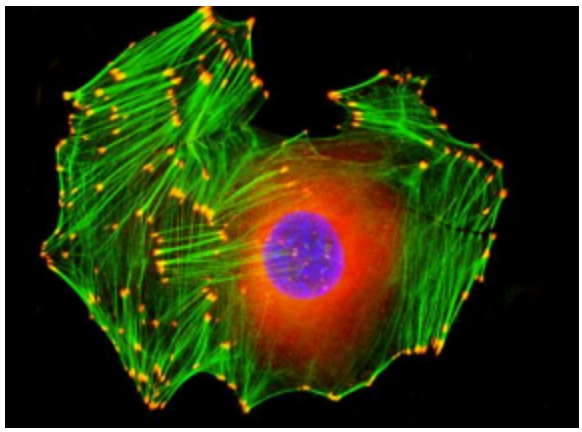


Focal adhesion



Focal adhesion is a specialized local region that serves as attachment sites for bundles of actin filaments (stress fibers) that anchor the cytoskeleton (and cells) to areas of cell contact or to extracellular matrix via the binding of transmembrane proteins (called integrins) to the extracellular matrix. Focal adhesion, like other cell-ECM connections, has a great role in the stabilization of cells and providing them with the needed mechanical strength, especially for epithelial cells.

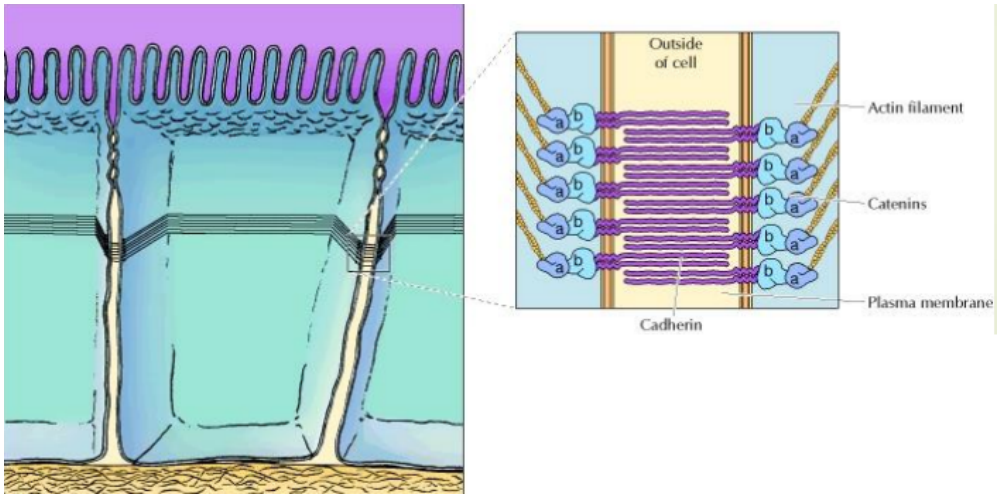
Integrins are transmembrane proteins that have two subunits (α; β). Such subunits are diverse, and are usually denoted by numbers. Integrins are very important for cellular interactions.

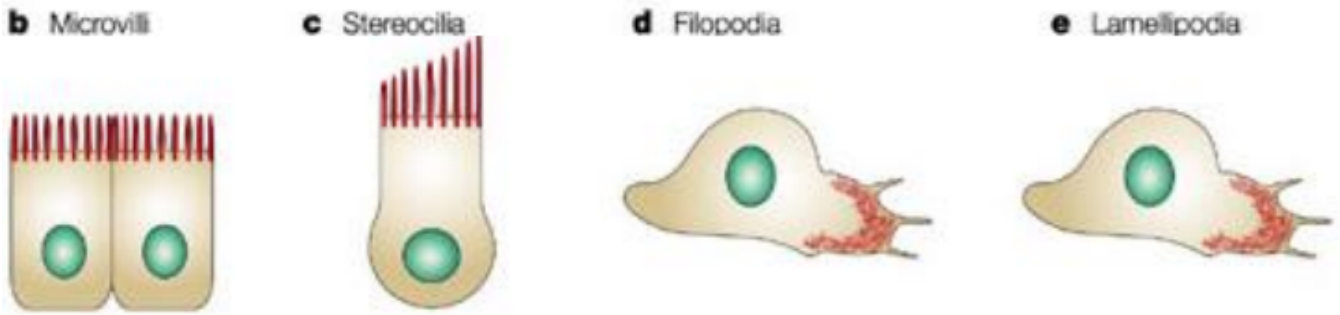


Stress fibers are contractile bundles of actin filament. The stress fibers are arranged in packed bundles cross-linked by α-actinin(increasing the cell rigidity ,to support its function such as regulating blood pressure). These associations are mediated by several proteins, including talin, which binds integrins, and vinculin, which binds actin bundles. Then, these two linker proteins interact with each other. Lastly, integrins stick the cells with the ECM, by the interaction with its components, or with other cells, by interacting with their integrins. They may increase in many diseases affecting the rigidity of the cells.

Adherens junctions

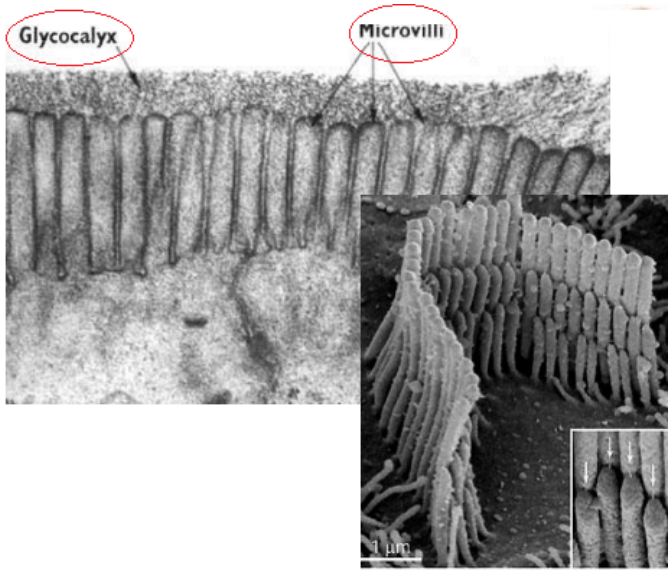
These junctions are regions of **cell-cell contact** to which actin cytoskeleton is anchored. They form a continuous belt-like structure (adhesion belt) around each cell in which an underlying contractile bundle of actin filaments is linked to the plasma membrane. Such junctions help holding cells together. Contact between cells is mediated by cadherins transmembrane proteins that forms a complex with cytoplasmic proteins called catenins. Catenins have two subunits; α which binds to actin bundles, and β which binds to cadherins.





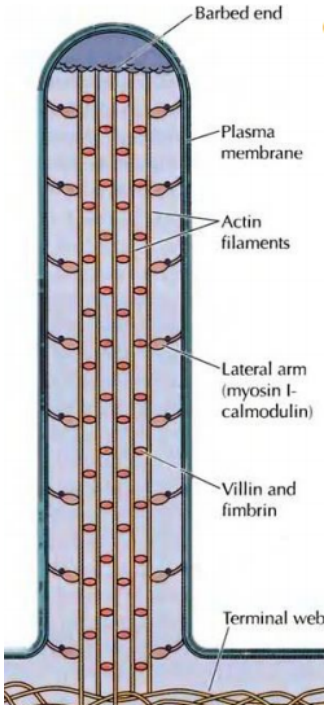
Protrusions of the cell surface

A variety of protrusions or extensions are present on cell surfaces. Cell surface protrusions are involved in cell movement, phagocytosis, or specialized functions such as absorption of nutrients. Most of these cell surface extensions are based on actin filaments organized into either relatively permanent or rapidly rearranging bundles or networks.



Microvilli

Microvilli are fingerlike extensions of the plasma membrane that are particularly abundant on the surfaces of cells involved in absorption, such as the epithelial cells lining the intestine. They form a layer on the apical surface (called a **brush border**) to increase the exposed surface area available for absorption.



Organization of microvilli:

- 20-30 closely packed parallel bundles
- Filament bundles are linked by villin (major) and fimbrin.
- Attachment to plasma membrane is mediated by calmodulin and myosin I to assist in movement.
- Filaments are linked to the cortex at the base via a spectrin-rich region called the terminal web.

  Notice that the structure of microvilli is stabilized by many mechanisms; which include the closely-packed bundle of actin filaments; the interaction with the cortex of the membrane; and having myosin interactions.

Stereocilia

These are specialized forms of microvilli located on the surface of auditory hair cells, and are responsible for hearing by detecting sound vibrations.

Psuedopodia

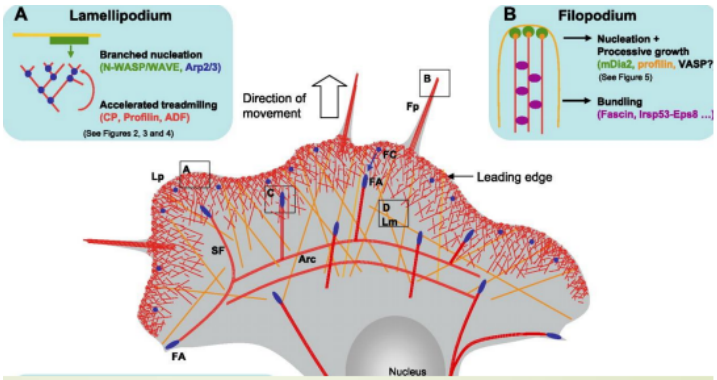
These are extensions of moderate width responsible for phagocytosis.

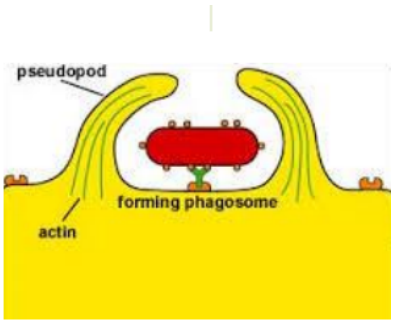
Lamellipodia

Lamellipodia are broad, sheet-like networks of actin leading edge of moving fibroblasts.

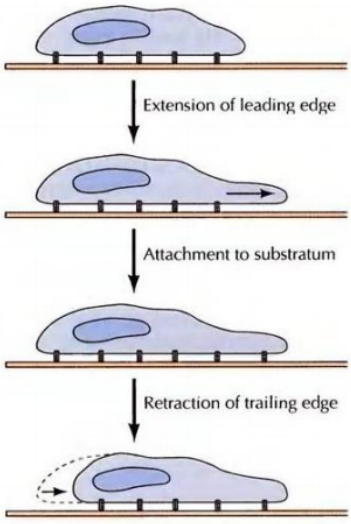
Filopodia

Filopodia are thin projections extending from lamellipodia. They form a local compacted area of condensed actin filaments. It precedes the lamillopodia in the cell movement.





Cell migration

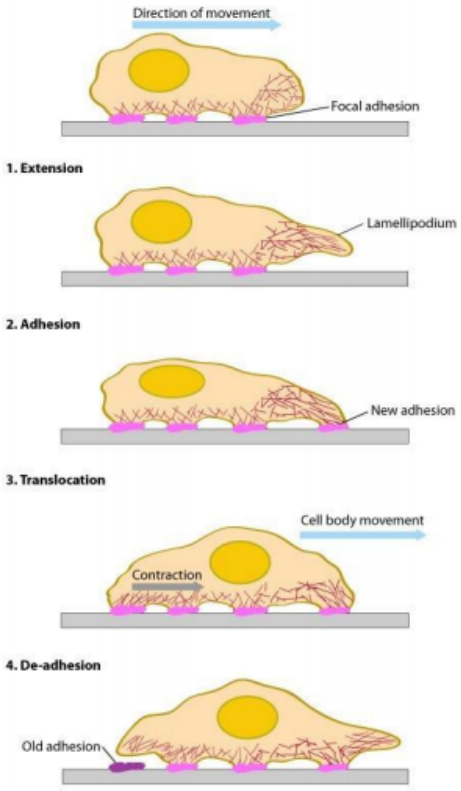


Cells tend to move in many circumstances; such as the attraction of inflammatory cells; such as the leukocytes and the macrophages, under the effect of chemotactic agents. The steps of cell locomotion are:

1. Developing polarity via the specialization of the plasma membrane or the cell cortex.
2. Extending protrusions (lamellipodia, filopodia, psuedopodia) at the leading edge (specific area) via the

force of the branching and polymerization of actin filaments. Branching the filaments increase the efficacy of this step.

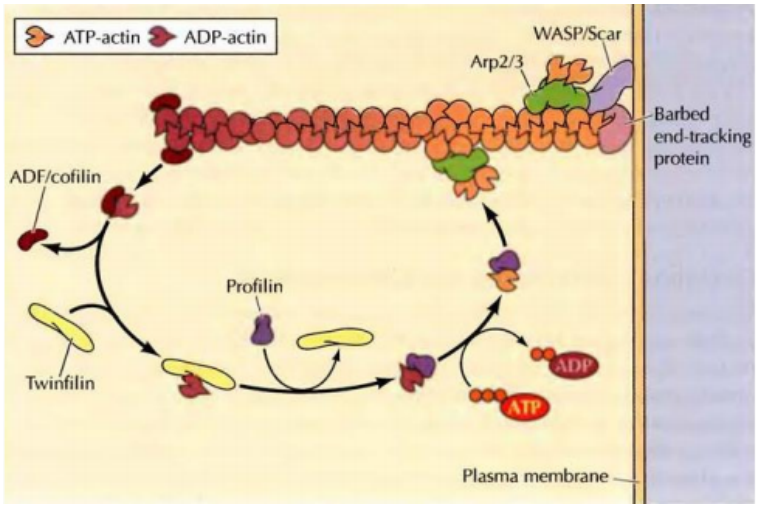
3. Attaching to substratum (e.g. focal adhesions) from the extended region.
4. The trailing edge (lagging edge) dissociates from substratum and retracts into the cell body.



Certain signals lead to the recruitment of Arp2/3, WASP/Scar, and barbed-end tracking proteins to the leading edge. WASP/Scar activates Arp2/3; initiating filament branching to provide more force to push against the membrane. At the pointed end, ADP-actin is disassembled by ADF-cofilin. ADP-actin monomers are carried to the leading edge by twinfillin and reactivated by profilin.

Cell-substratum attachment is initiated via transporting actin-bundling proteins and focal adhesion proteins (e.g., vinculin and talin) to the leading edge in connection with integrins. At the trailing end, focal adhesions are broken down.

This is true for slow moving cells like fibroblasts and epithelial cells, but rapidly moving cells like macrophages form diffuse contacts with the substratum whose composition is unknown.



40:00 – 50:00

FINALLY we finished this sheet!! I am quite amazed that all this has been discussed in one lecture… whatever; let us end with the usual ending ☺ : This sheet is done by your colleagues; so if you notice any editing or mistakes, do not hesitate to contact the writer and the corrector of the sheet. Thank you and good luck.

"Let food be thy medicine and medicine be thy food."