Lecture 7: The cytoskeleton and cell movement (Actin microfilaments)

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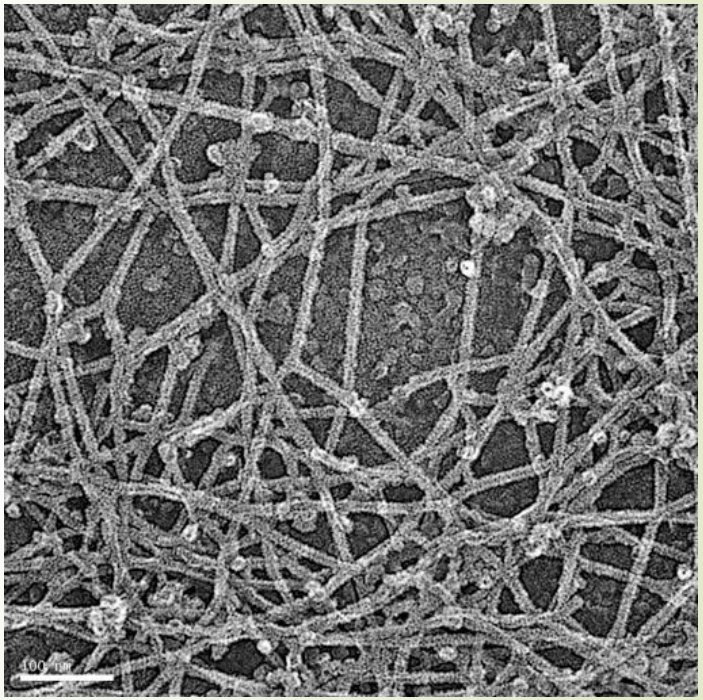
Principles of Genetics and Molecular Biology

What is the cytoskeleton?

- A dynamic network of protein filaments extending throughout the cytoplasm
- Three types of protein filaments: actin microfilaments, microtubules, intermediate filaments
- Functions:
  - Structural framework for cells
  - Determines cell shape and movement
  - Determines positions of organelles
  - Determines overall organization of cytoplasm
  - Regulates internal movement of organelles and other structures such as mitotic chromosomes

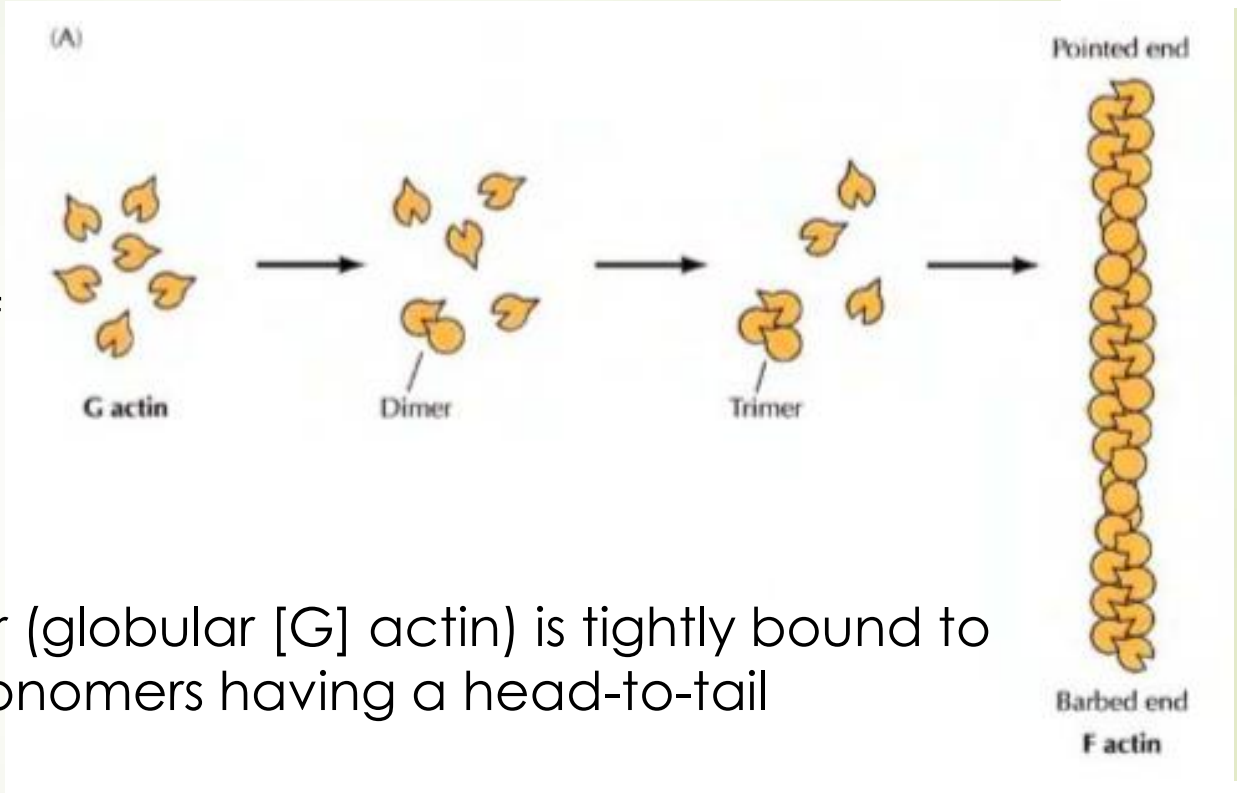
The actin filaments (microfilaments)

- Thin, flexible fibers (7nm diameter and several μm length).
- They are organized into higher-order structures, forming bundles or three-dimensional networks.
- They form **semisolid gels**.
- Their assembly, disassembly, cross-linking 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

- Mammalian cells have at least six distinct actin genes:
  - Four are expressed in different types of muscle
  - Two are expressed in nonmuscle cells.
- An actin monomer (globular [G] actin) is tightly bound to two other actin monomers having a 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 filament

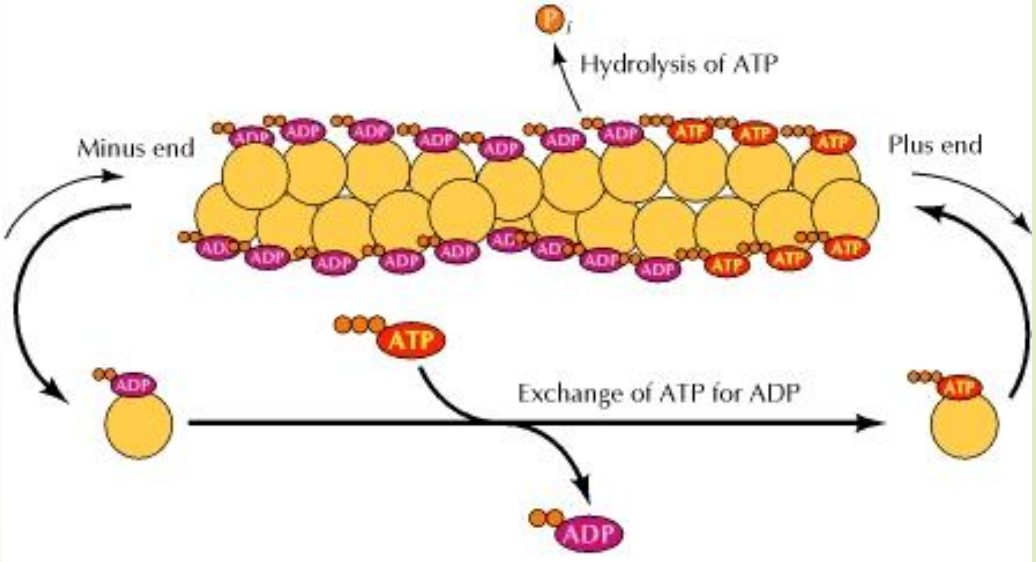
- Steps:

1. Nucleation: formation of an aggregate of 3 actin monomers.

2. Filament growth by adding additional monomers to both ends (barbed and pointed), but faster at barbed ends.

3. The monomers are bound to ATP, which is not required for nucleation, but

- **is hydrolyzed into ADP following assembly,**
- **Speeds polymerization**
- **Stabilizes binding.**



- **Treadmilling: ATP-actin is added to the barbed end while ADP-actin dissociates from the pointed end**
- **This illustrates dynamic behavior of actin filaments.**

Actin-binding proteins

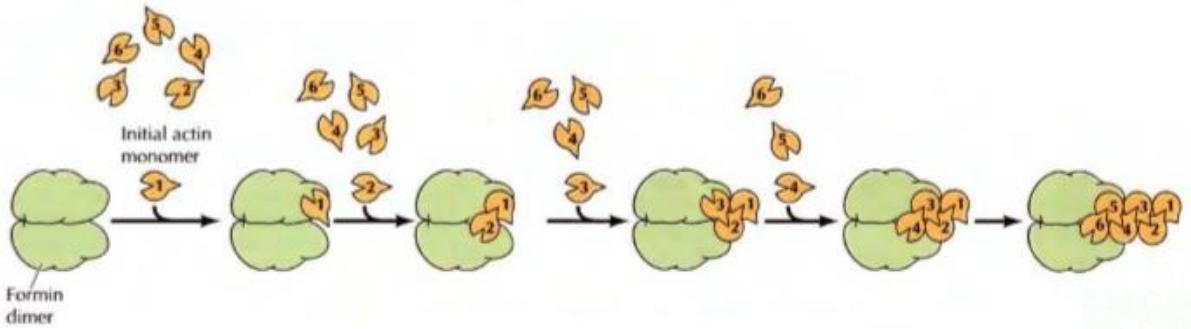
- **Regulate actin assembly and disassembly as well as stability of actin cytoskeleton.**

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

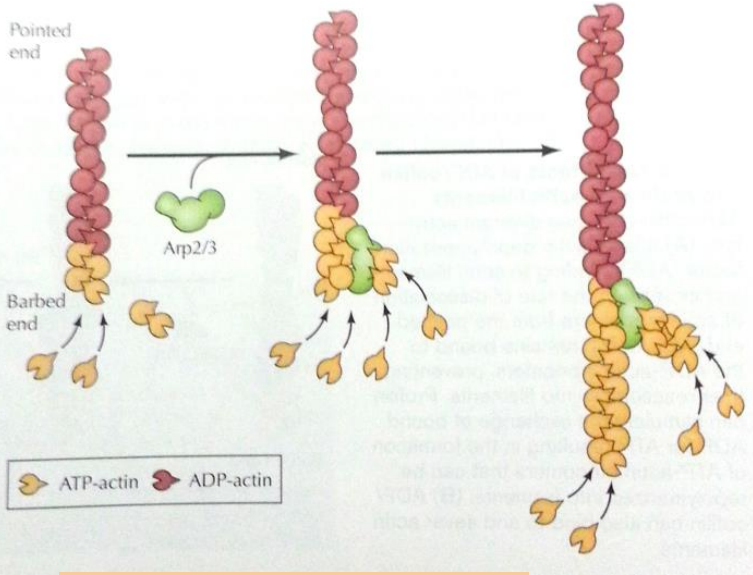
https://www.youtube.com/watch?v=VVgXDW_8O4U

https://www.youtube.com/watch?v=jonQiEtTHwY

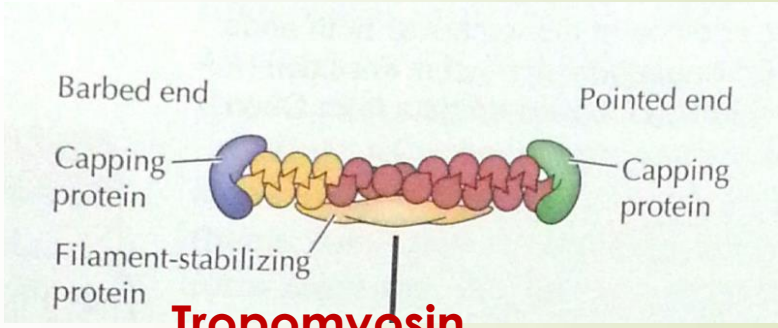
Actin binding proteins



The rate-limiting step of actin formation, nucleation, is facilitated by formin.



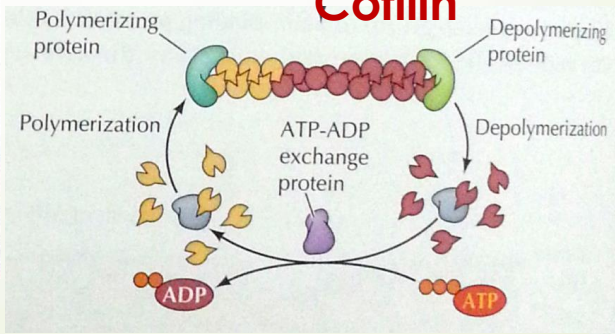
Branching is facilitated by the Arp 2/3 complex

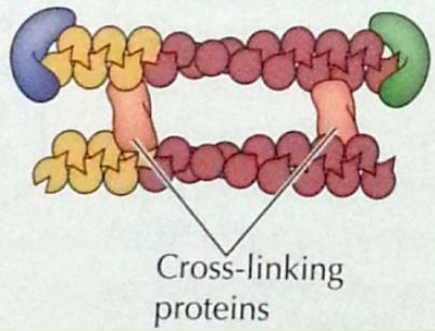


Tropomyosin

Proflin

Cofilin





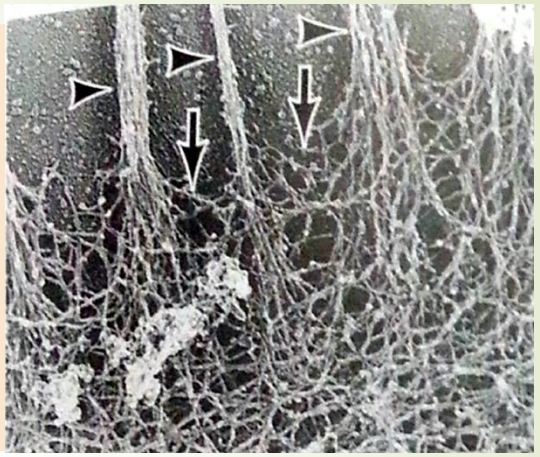
α-Actinin

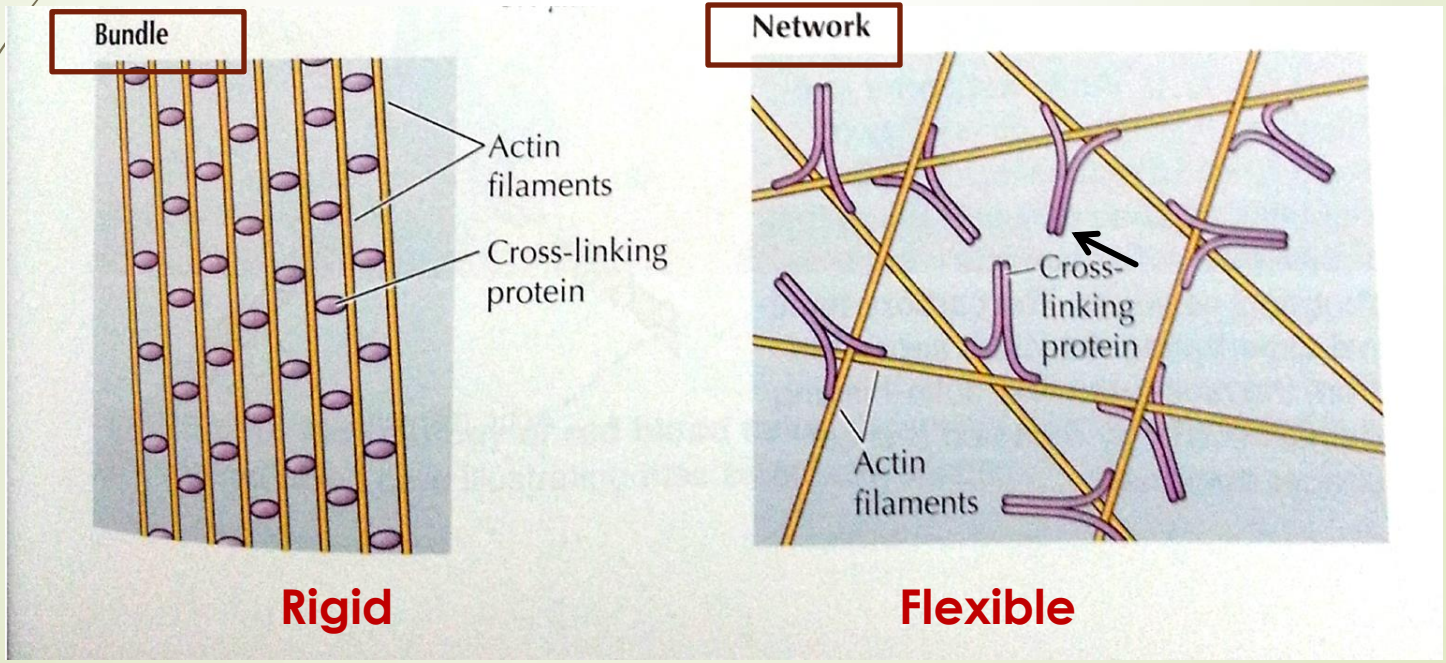
Organization of actin filaments

2 types of structures:

1. **Actin bundles**
2. **Actin networks**

Differ in flexibility and the size and shape of cross-linking proteins



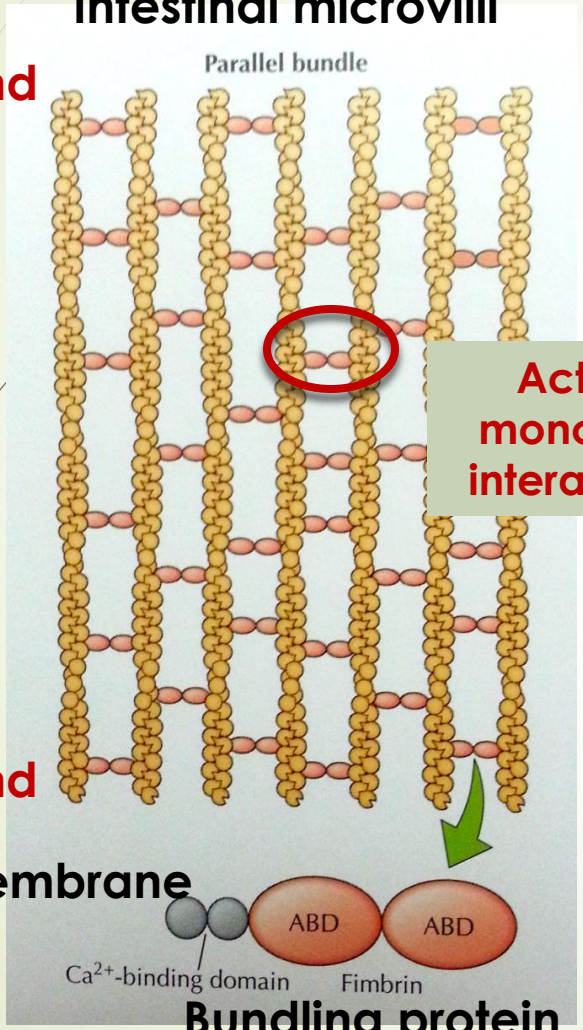


Rigid **Flexible**

Types of actin bundles

Intestinal microvilli

Pointed end



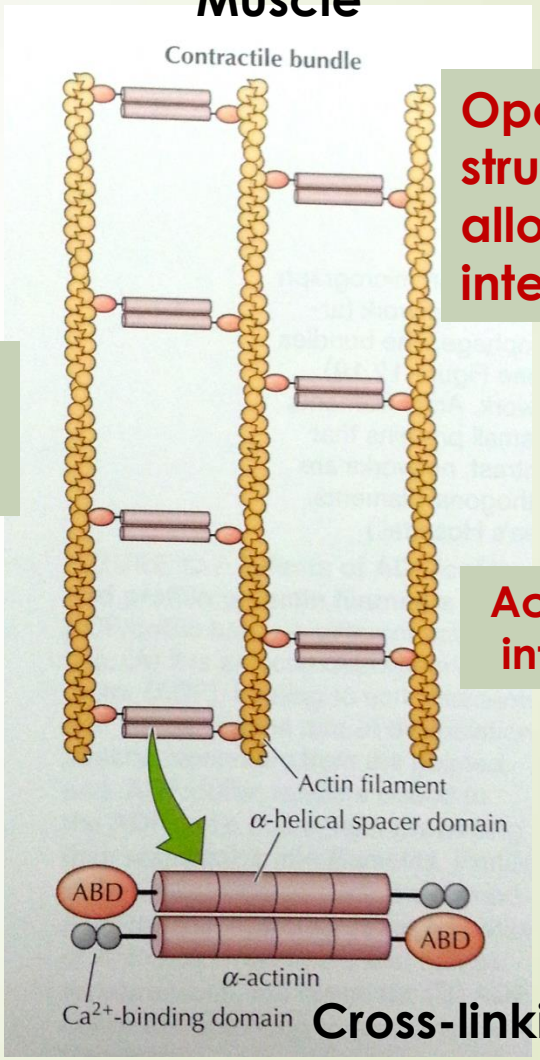
Actin-monomer interaction

Barbed end

Plasma membrane

Bundling protein

Muscle

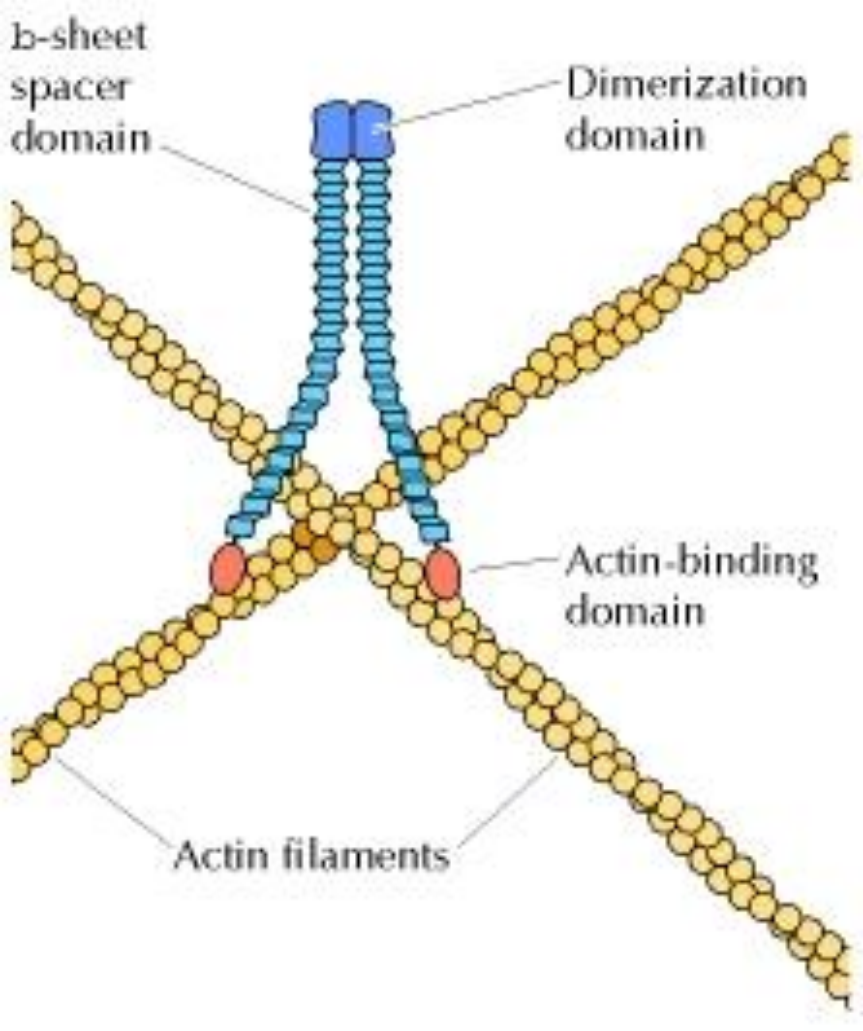


Open structure to allow myosin interaction

Actin-dimer interaction

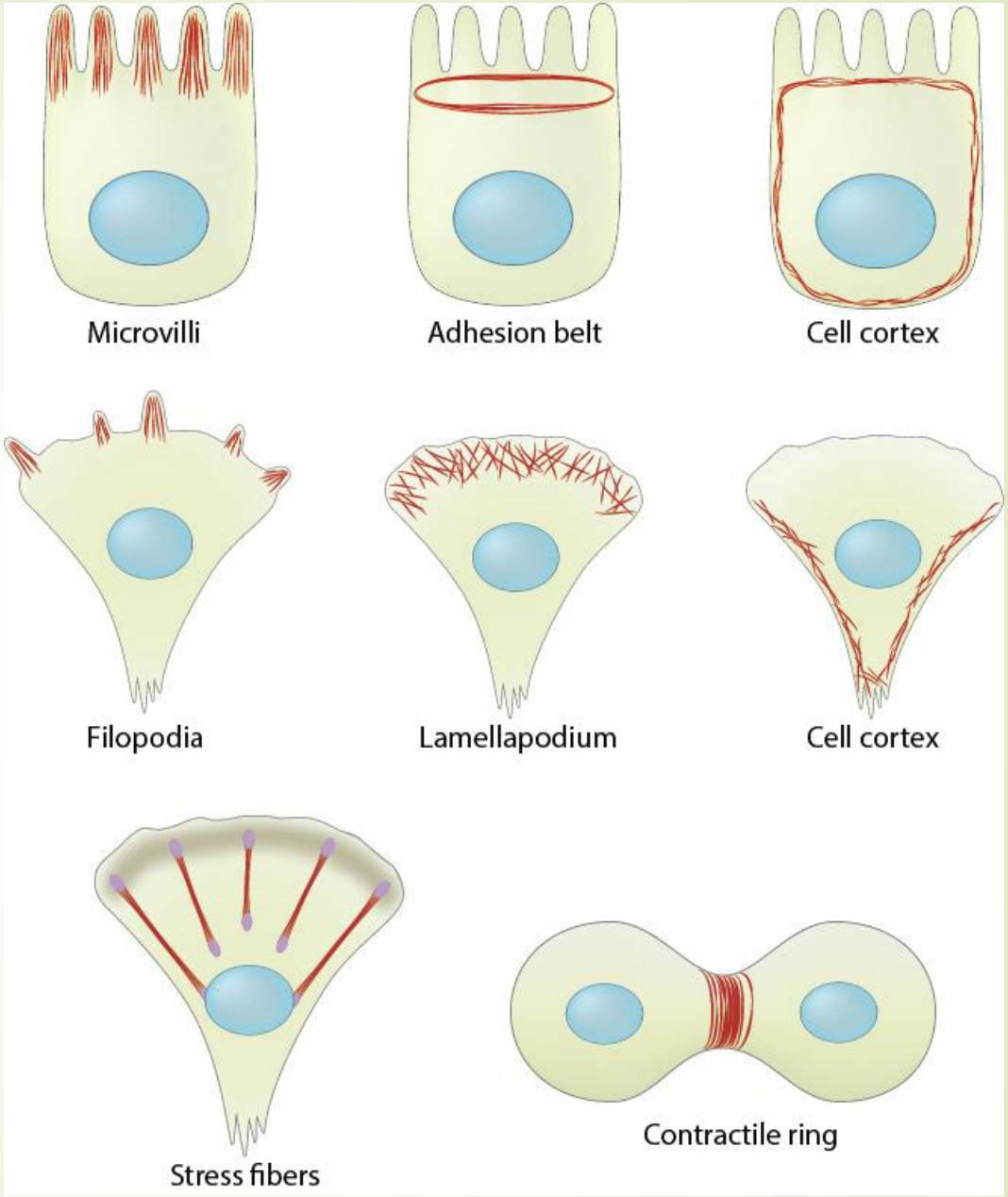
Cross-linking protein

Actin networks



- The actin filaments in networks are held together by large actin-binding proteins, such as filamin, which binds actin as a flexible dimer.
- Loose 3D meshwork
- Function: networks of actin filaments underlie the plasma membrane and support the surface of the cell.

Actin fibers in the cell

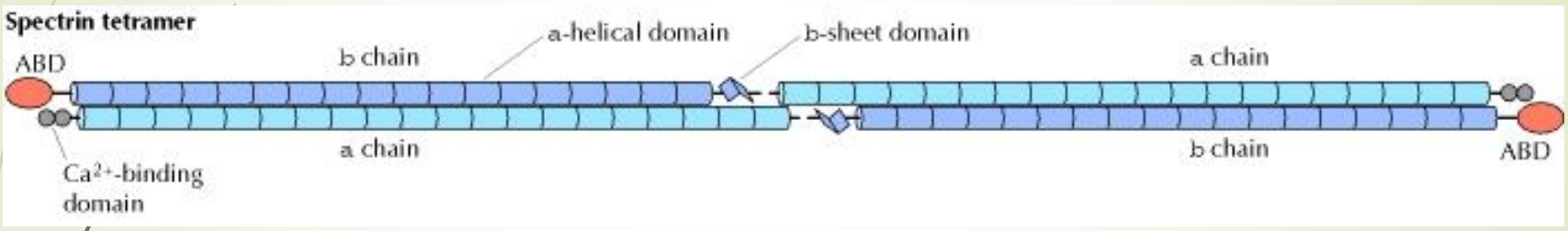


Actin filaments and plasma membrane

- **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.
- Studies in RBC because:
  - They do not have other cytoskeletal structures
  - They do not have nucleus or organelles → no contamination
- The cytoskeleton is uniform with no specialized regions like in other cells.

Spectrin as a structural component of cortical cytoskeleton

- The major protein that provides the structural basis for the cortical cytoskeleton in erythrocytes
- Is an actin binding protein.

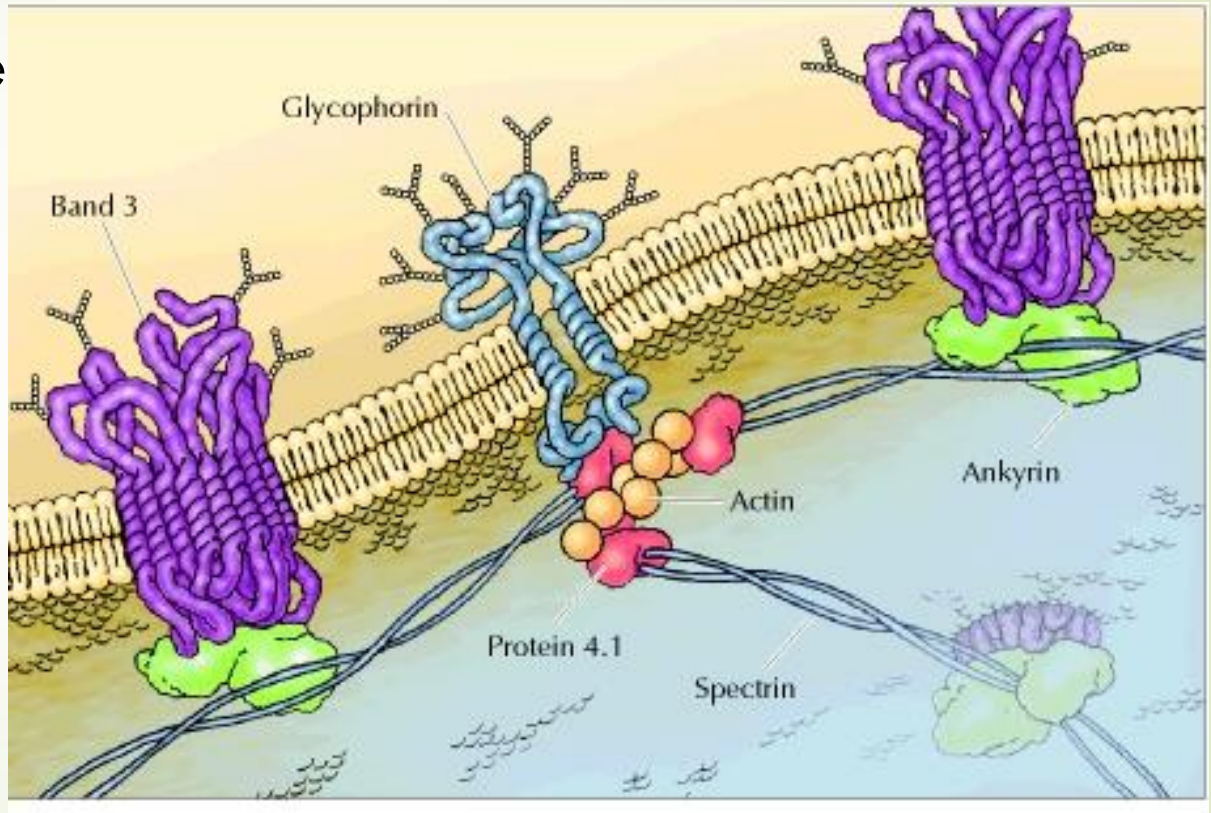


- A tetramer of two α and β polypeptides with the α chain having two Ca^{2+} binding domains at its C-terminus and the β chain having the actin-binding domain

Actin filaments-plasma membrane interaction

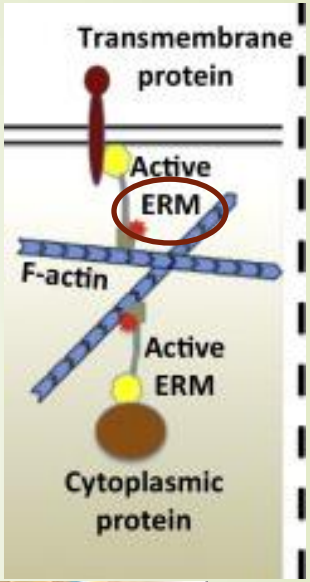
- The spectrin-actin network is linked to the membrane by

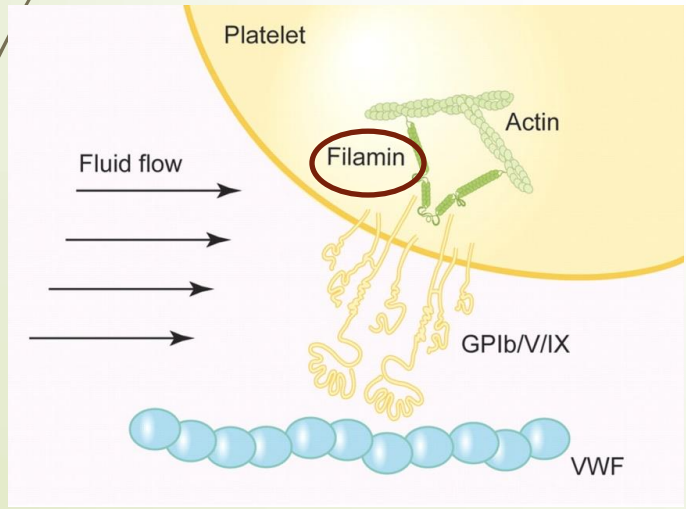
1. **Ankyrin**, which binds to both spectrin and the abundant transmembrane protein band 3.
2. **Protein 4.1** that binds to glycophorin
3. **Phospholipids**

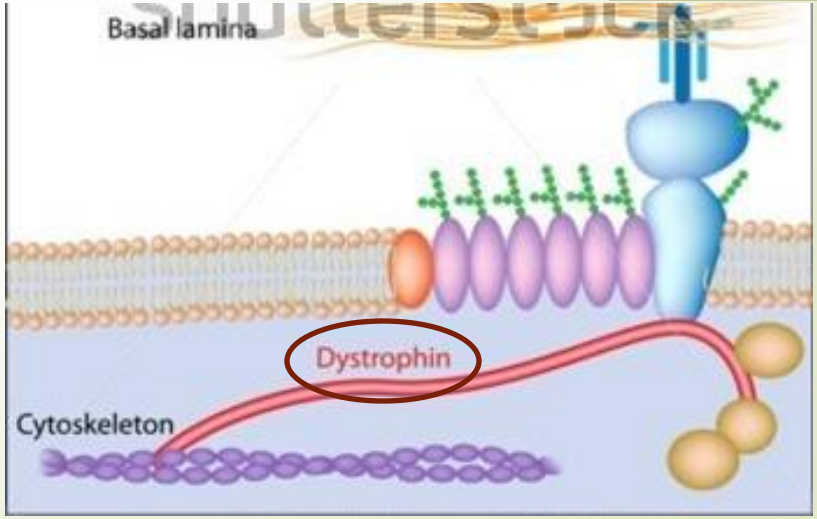


Other linkage to plasma membrane in other cells

- The ERM proteins (protein 4.1-related) link actin filaments to the plasma membranes of different kinds of cells.
- Filamin (spectrin-related) links actin filaments with the plasma membrane of blood platelets.
- 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.
  - This maintains cell stability during muscle contraction.

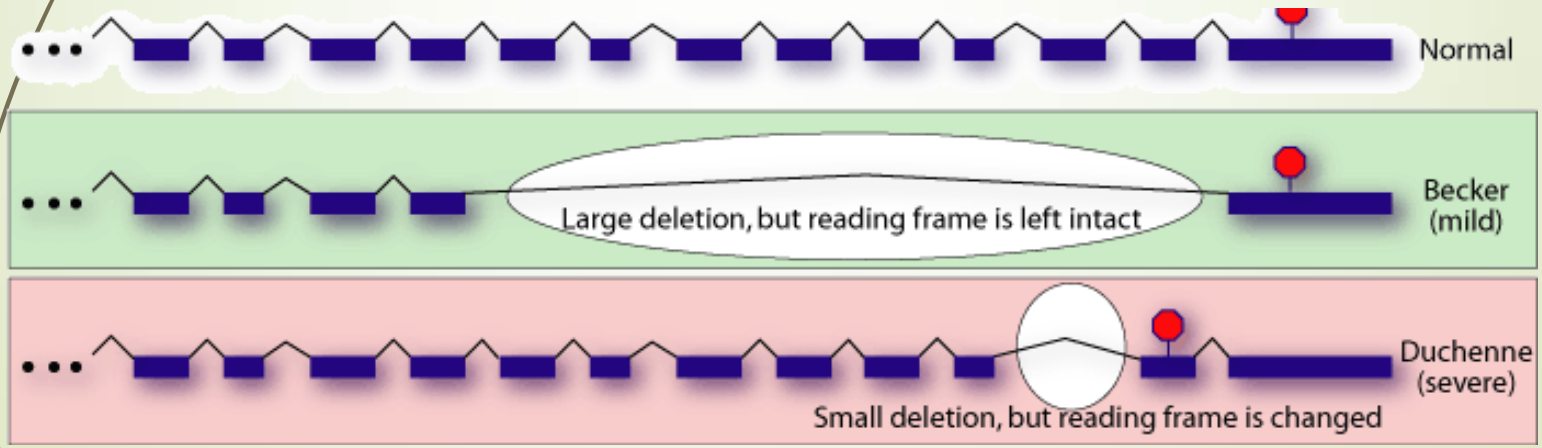




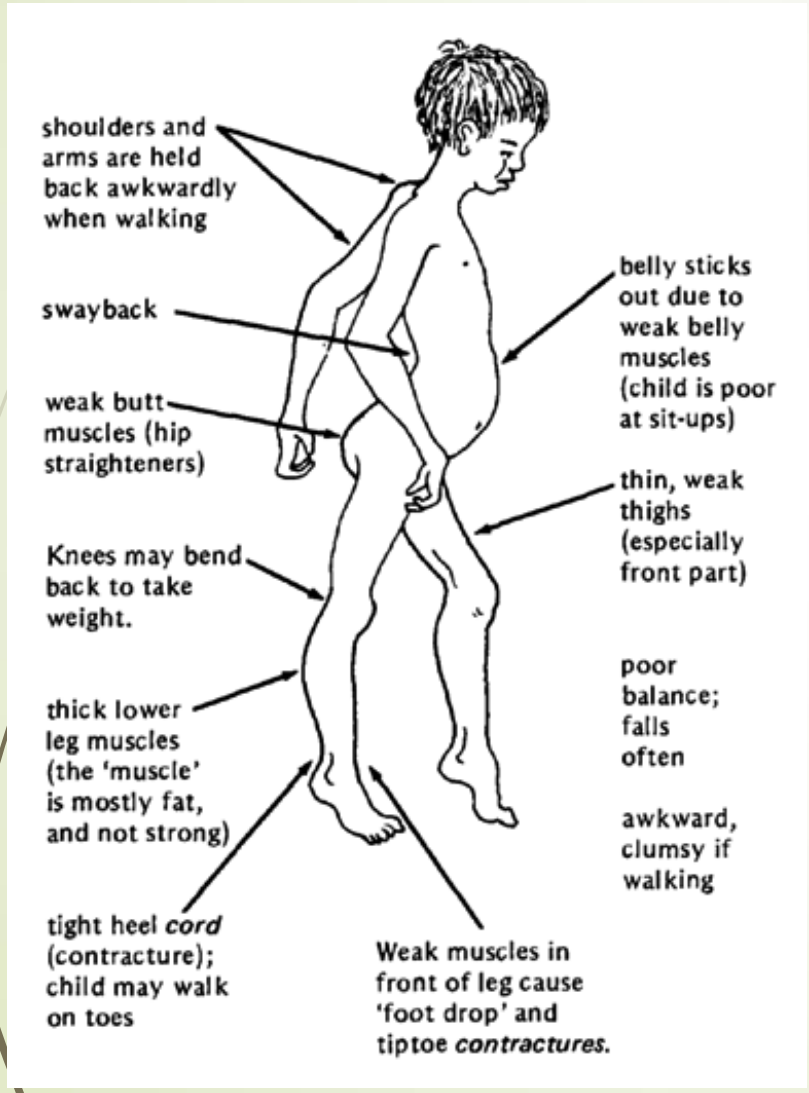


Dystrophin and muscular dystrophies

- The dystrophin gene encodes a large protein (427 kd).
- Mutations in the gene cause two types of muscular dystrophy, Duchenne's (severe) and Becker's (moderate).
- X-linked inherited diseases
- Progressive degeneration of skeletal muscle
- Patients with Duchenne's muscular dystrophy usually die in their teens or early twenties.

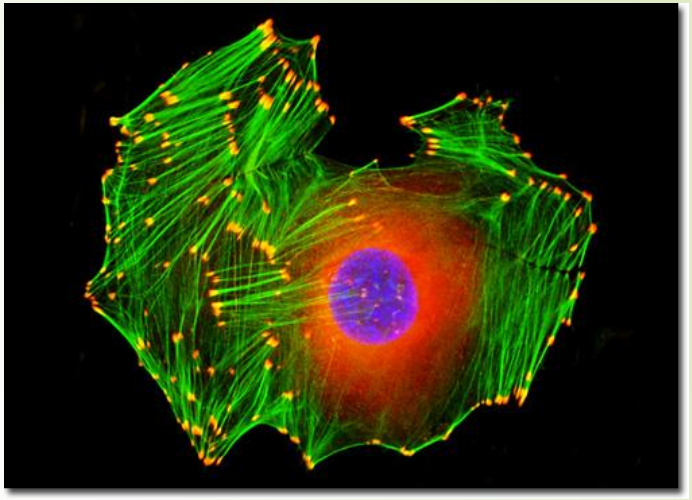


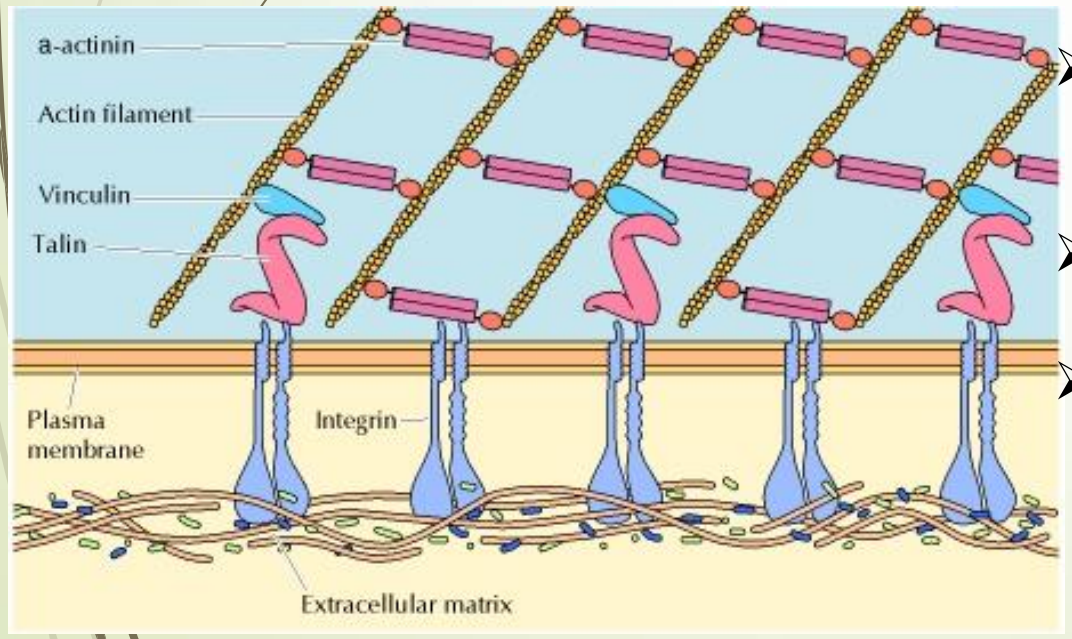
Dystrophin and muscular dystrophies



Focal adhesion

- Specialized regions that serve 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.

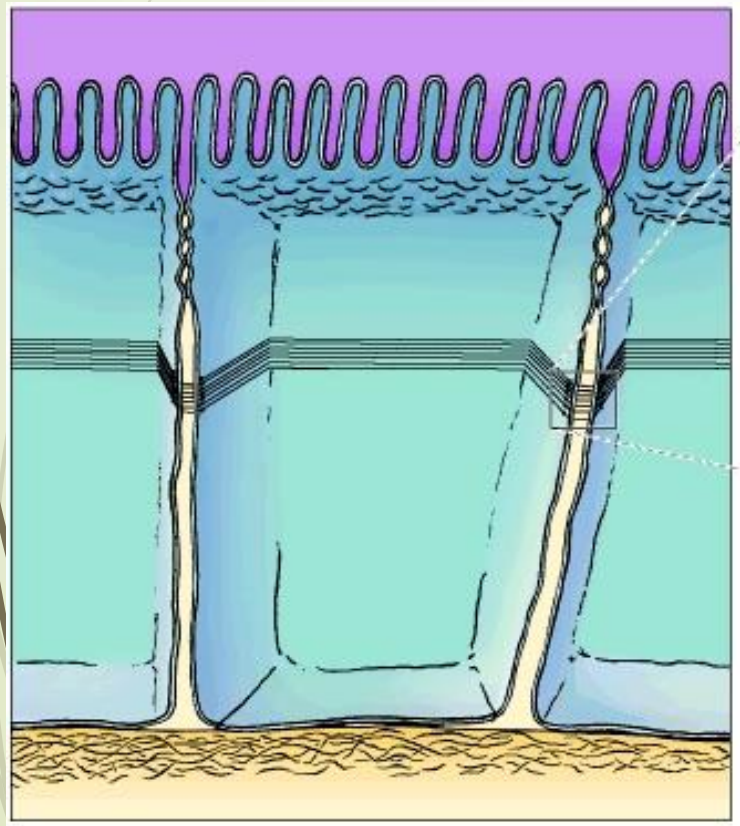


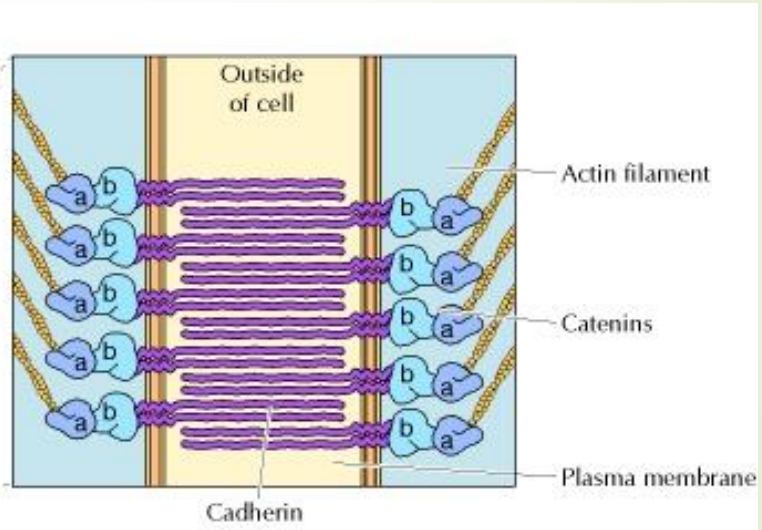


- **Stress fibers are contractile bundles of actin filament**
- **The stress fibers are crosslinked by a-actinin.**
- **These associations, are mediated by several proteins, including talin and vinculin.**

Adherens junctions

- Regions of **cell-cell contact** to which actin cytoskeleton is anchored.
- They form a continuous beltlike structure **(adhesion belt)** around each cell in which an underlying contractile bundle of actin filaments is linked to the plasma membrane.

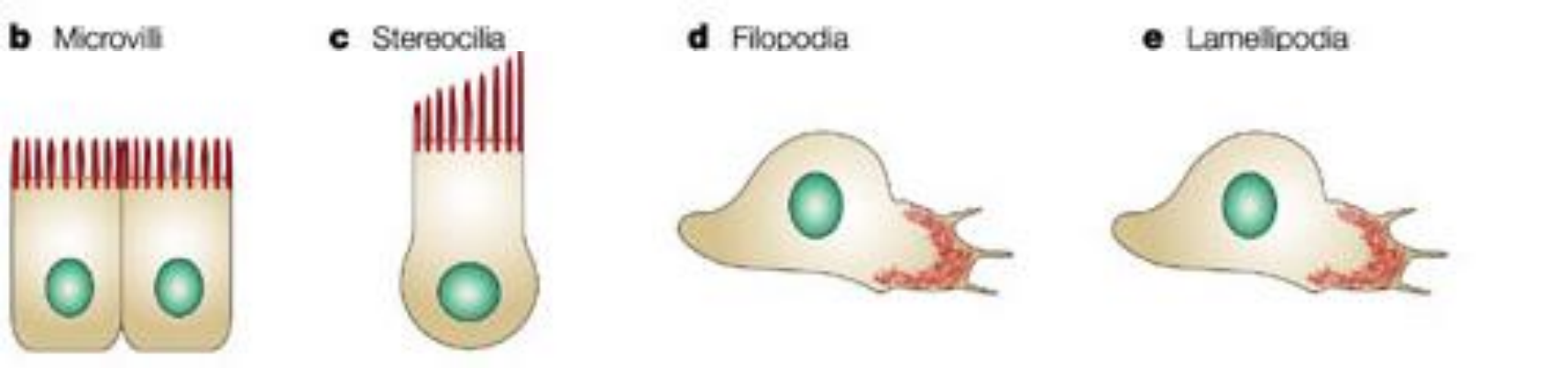




Contact between cells is mediated by cadherins transmembrane proteins that form a complex with cytoplasmic proteins called catenins, which associate with actin filaments.

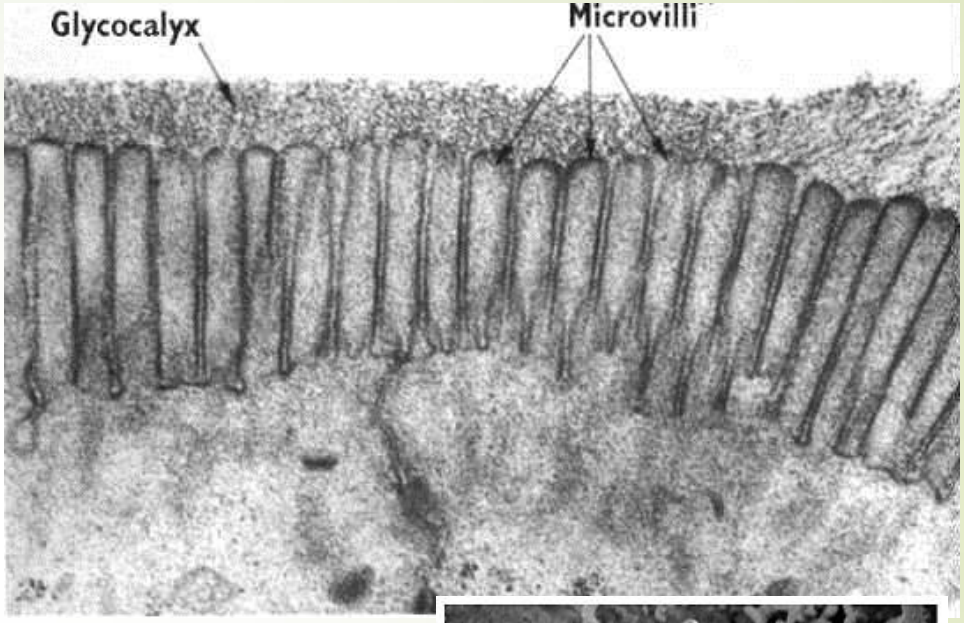
Protrusions of the cell surface

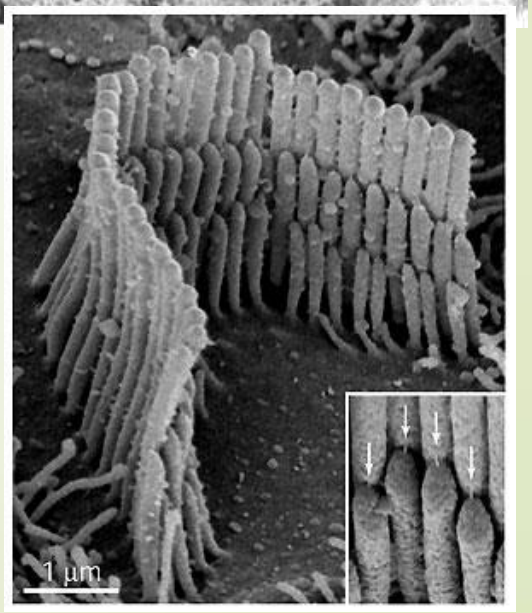
- A variety of protrusions or extensions are present on cell surfaces
- Cell surface protrusions are involved in cell movement, phagocytosis, or specialized functions such as absorption of nutrients.
- Most of these cell surface extensions are based on actin filaments organized into either relatively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



Microvilli

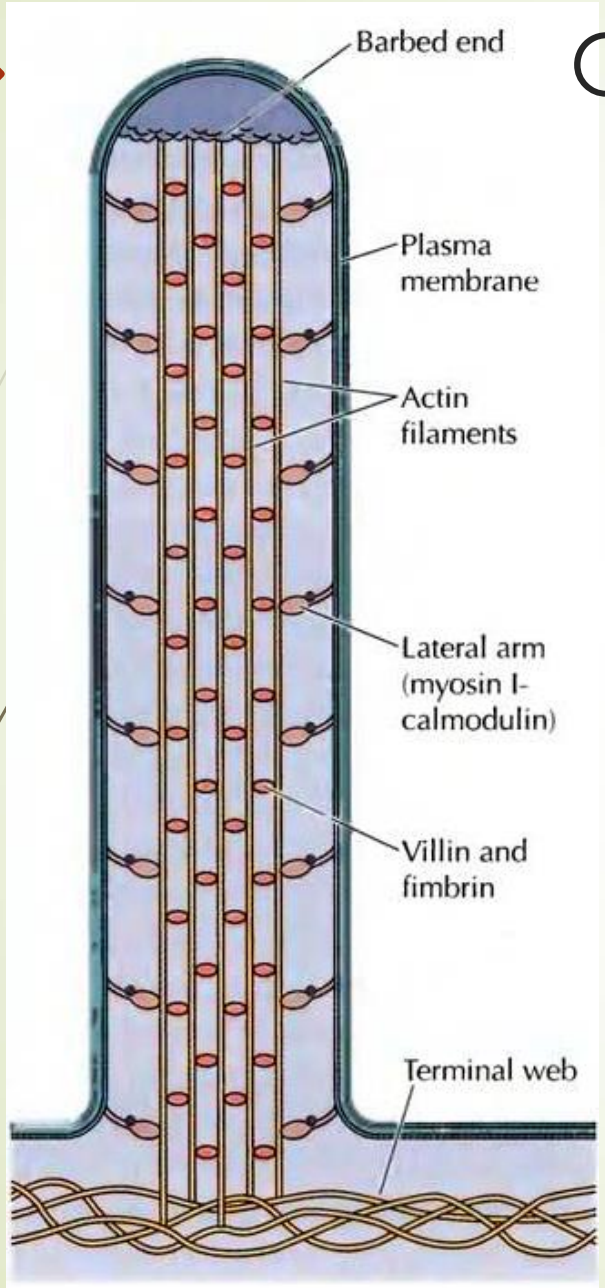
- Fingerlike **extensions of the plasma membrane** that are particularly abundant on the surfaces of cells involved in absorption, such as the epithelial cells lining the intestine.
- They form a layer on the apical surface (called **a brush border**) to increase the exposed surface area available for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





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

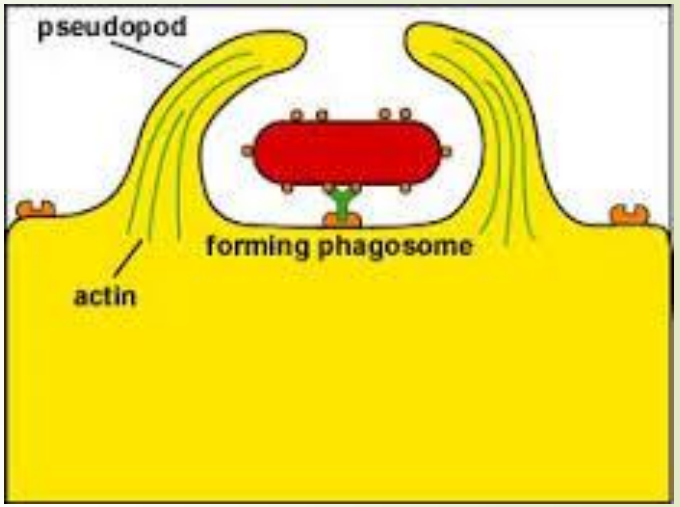
Organization of microvilli

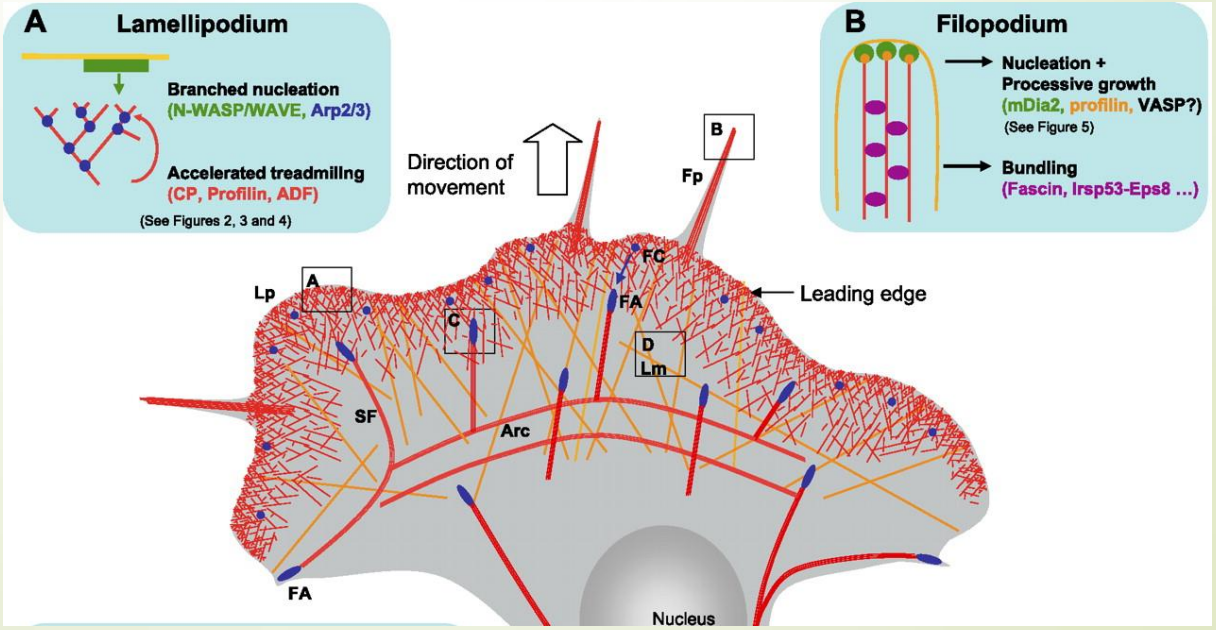


- 20-30 closely packed parallel bundles
- Filament bundles are linked by villin (major) and fimbrin.
- Attachment to plasma membrane is mediated by calmodulin and myosin I to assist in movement.
- Filaments are linked to the cortex at the base via a spectrin-rich region called the terminal web.

Other protrusions

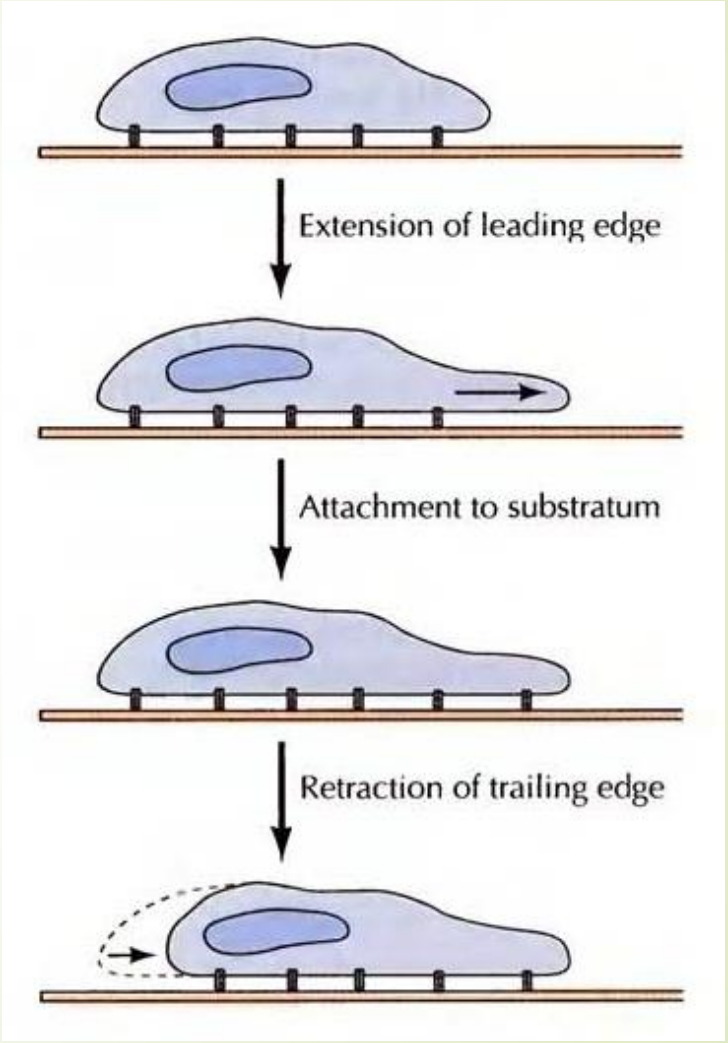
- Psuedopodia: extensions of moderate width responsible for phagocytosis
- Lamellipodia: broad, sheet-like networks of actin leading edge of moving fibroblasts
- Filopodia: thin projections extending from lamellipodia





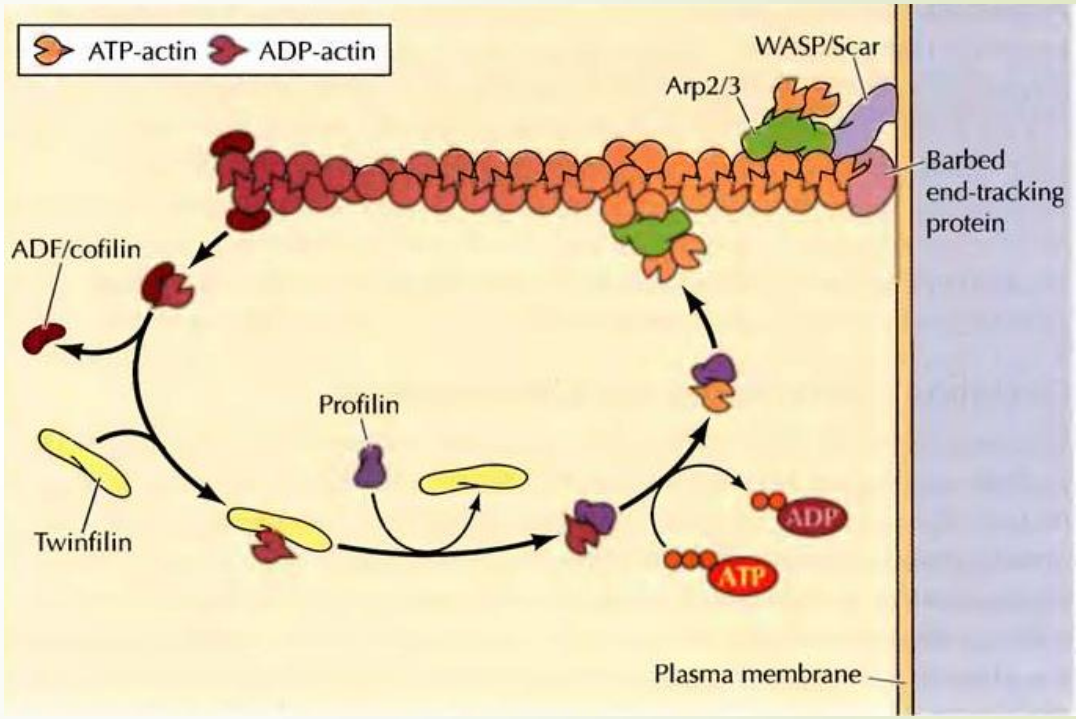
Cell migration

1. Develop polarity via specialization of the plasma membrane or the cell cortex.
2. Extend protrusions (lamellipodia, filopodia, psuedopodia) at the leading edge via the force of branching and polymerization of actin filaments.
3. Attach to substratum (e.g. focal adhesions)
4. The trailing edge dissociates from substratum and retract into the cell body



Dynamics of actin filament at the leading edge

- 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 leading edge by twinfillin and reactivated by profilin.

Modification of focal adhesions

- 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 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.