Lecture 4: the cytoskeleton and cell movement (Actin microfilaments)

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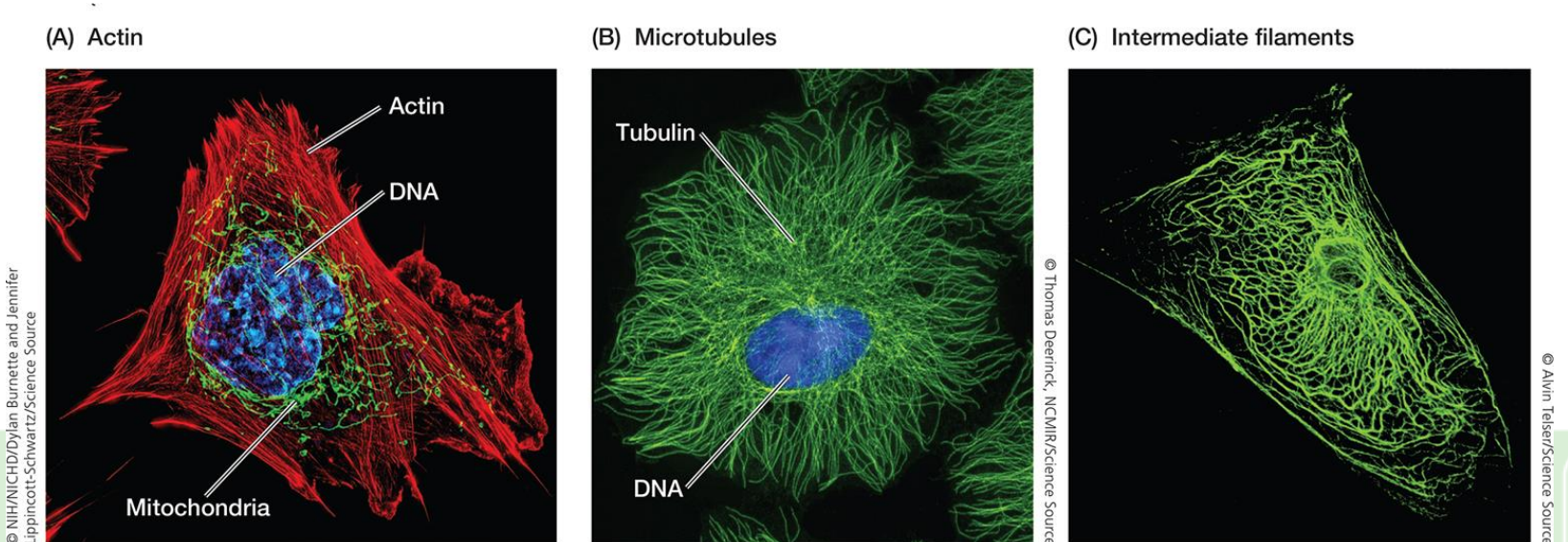
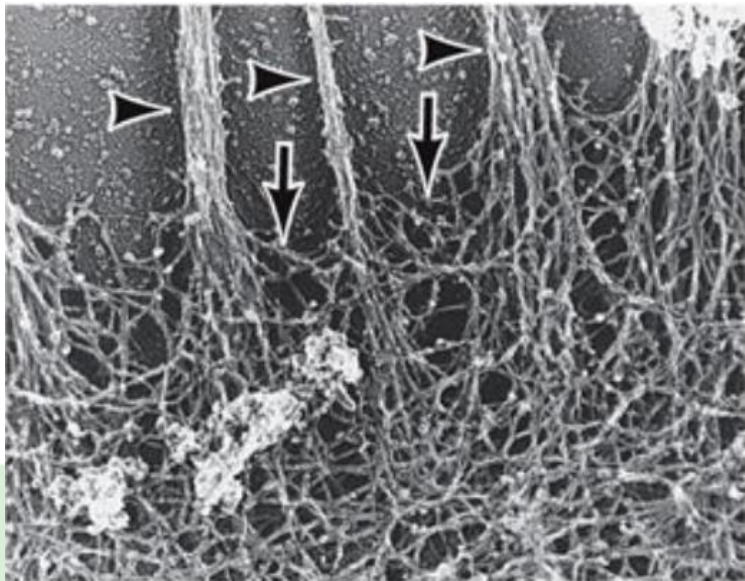
What is the cytoskeleton?

- What is it?
  - A dynamic network of protein filaments extending throughout the cytoplasm
  - Three types: actin microfilaments, microtubules, intermediate filaments
- Functions:
  - A structural framework of cells
  - A determinant of the overall organization of cytoplasm
  - A regulator of the internal movement of organelles
  - A determinant of cell shape and movement
  - A determinant of positions of organelles



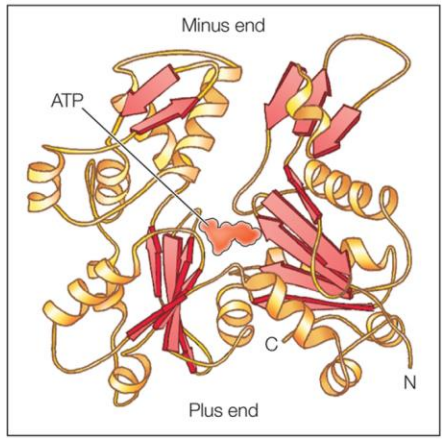
The actin (micro)filaments

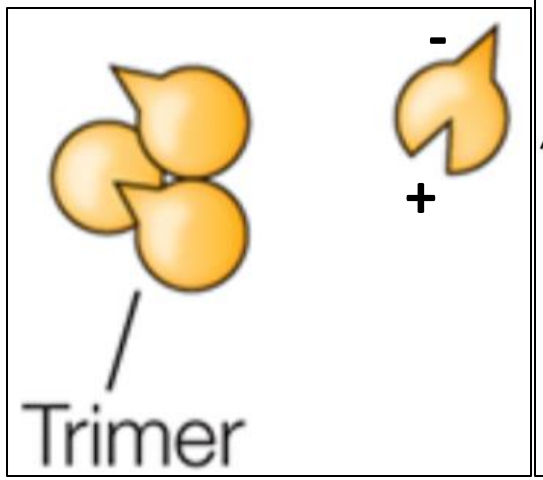
- Thin, flexible fibers that can be organized into bundles or networks.
- They form semisolid gels.
- They are bound to and regulated by various actin-binding proteins.
- They are abundant beneath the plasma membrane forming a network for cellular function.
- Mammalian cells have at least six distinct actin genes:
  - Four are expressed in different types of muscle.
  - Two are expressed in non-muscle cells.
- The actin proteins are conserved among species.
  - 90% similarity of amino acid sequence between yeast and human cells

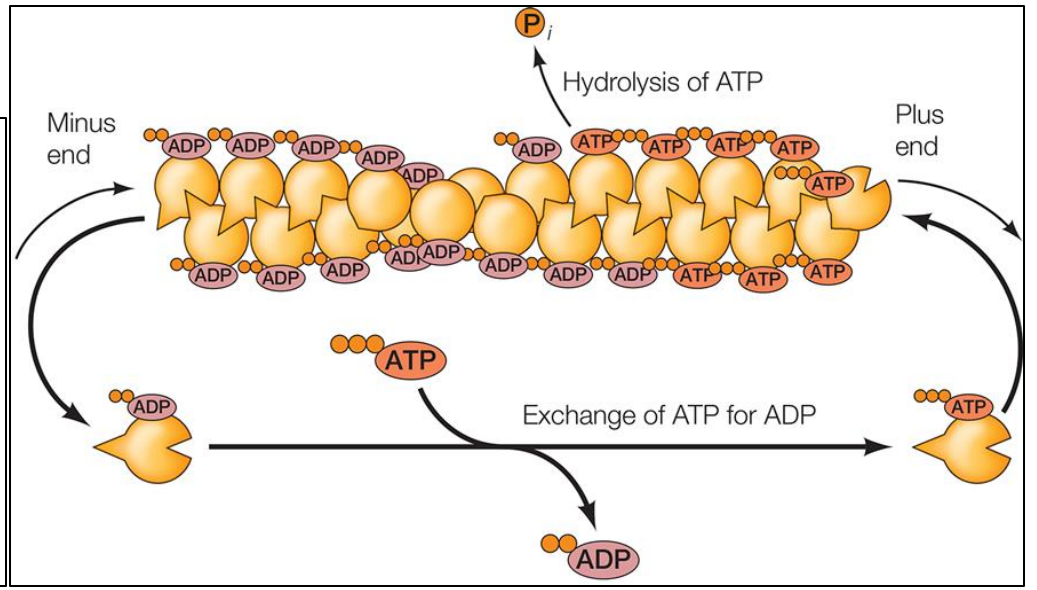


The actin polymerization

- **Nucleation** is the start of actin polymerization at both ends when a trimer of G-actin protein is formed.
- Actin filaments then grow by adding monomers to both ends.
- The ends are polar (the plus and minus ends).







https://www.youtube.com/watch?v=n-b7Zz-sfBk

The dynamic (de)polymerization of the actin filaments

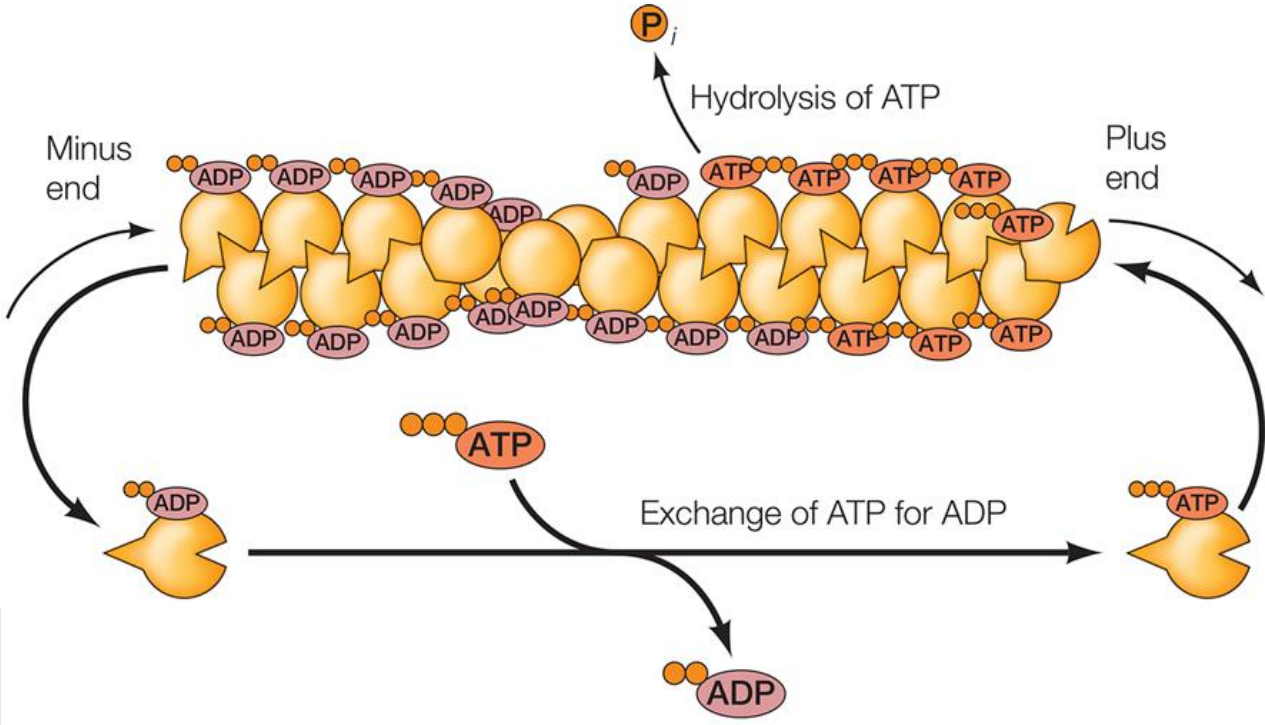
Polymerization

- The monomers are bound to ATP.
- ATP is not required for nucleation but:
  - It is hydrolyzed into ADP following assembly,
  - It speeds up polymerization,
  - It stabilizes binding.

Note that both ends can polymerize and depolymerize.

Treadmilling

- The filaments dissociate rapidly from
  - The ADP-actin
  - The minus end

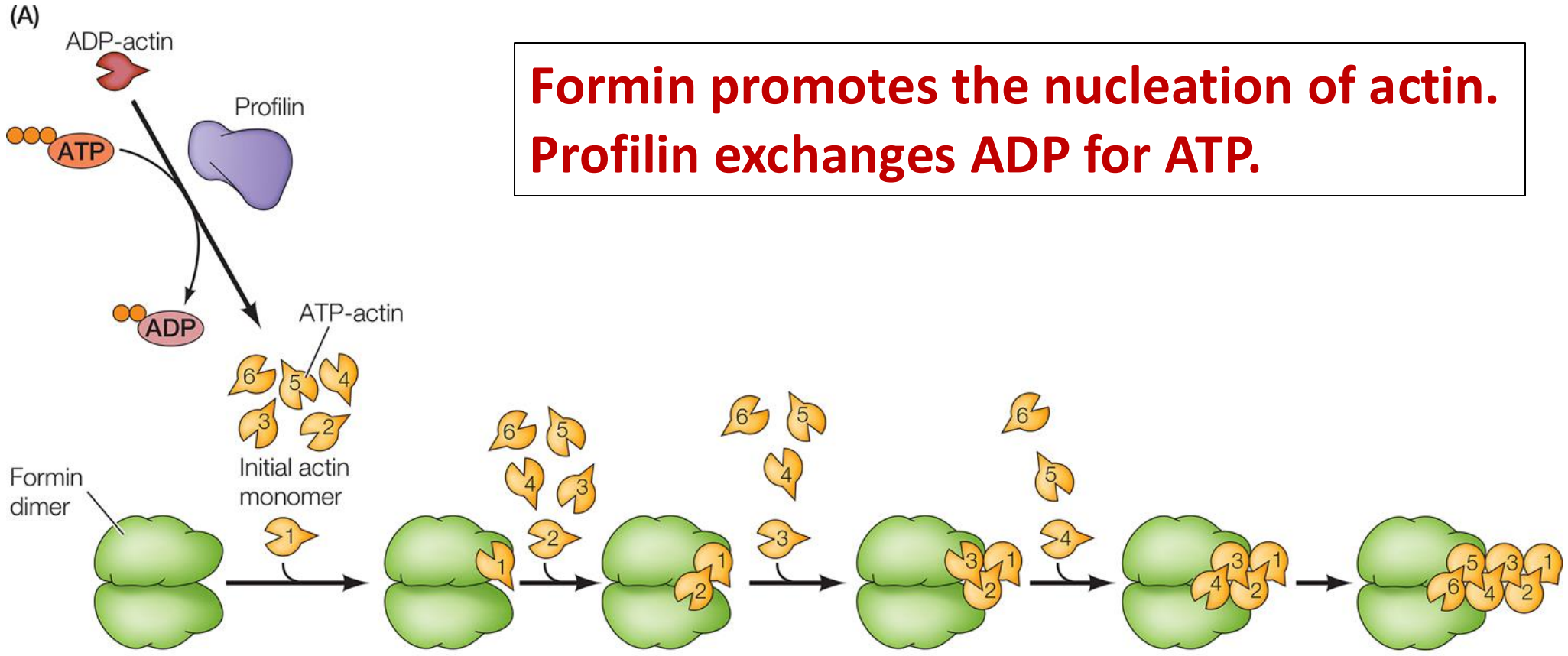


Actin-binding proteins

The (dis)assembly of actin filaments is regulated by actin-binding proteins.

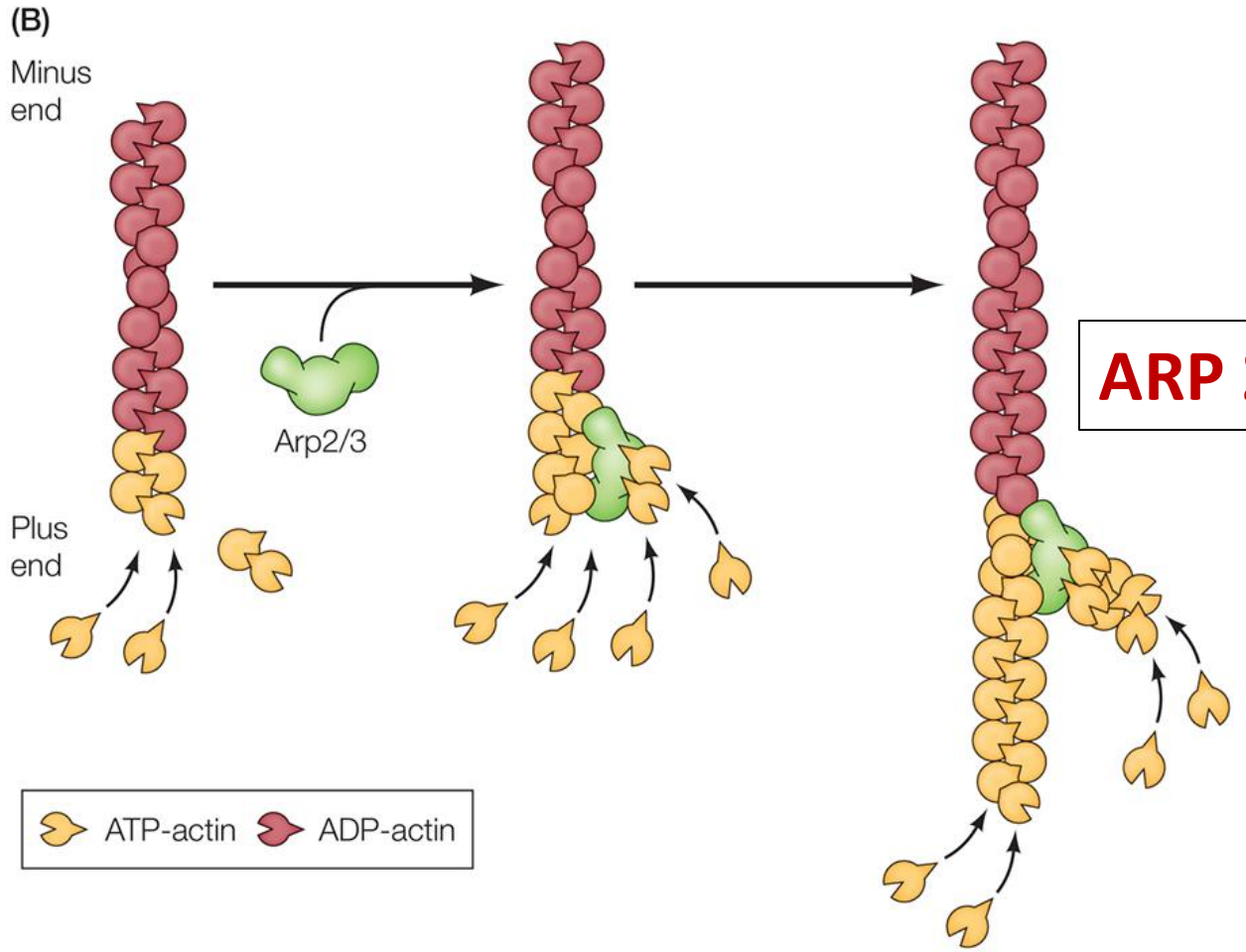
Cellular Role	Representative Proteins
Filament initiation and polymerization	Arp2/3, formin
Filament stabilization	Nebulin, tropomyosin
Filament cross-linking	α-actinin, filamin, fimbrin, villin
End-capping	CapZ, tropomodulin
Filament severing/depolymerization	ADF/cofilin, gelsolin, thymosin
Monomer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

Formin and profilin



Formin promotes the nucleation of actin.
Profilin exchanges ADP for ATP.

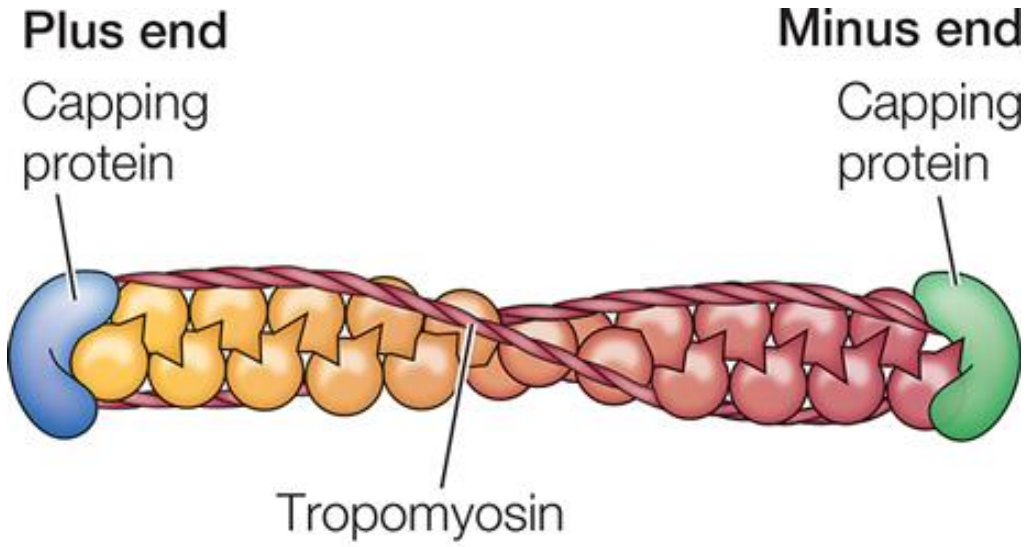
Branching by ARP 2/3



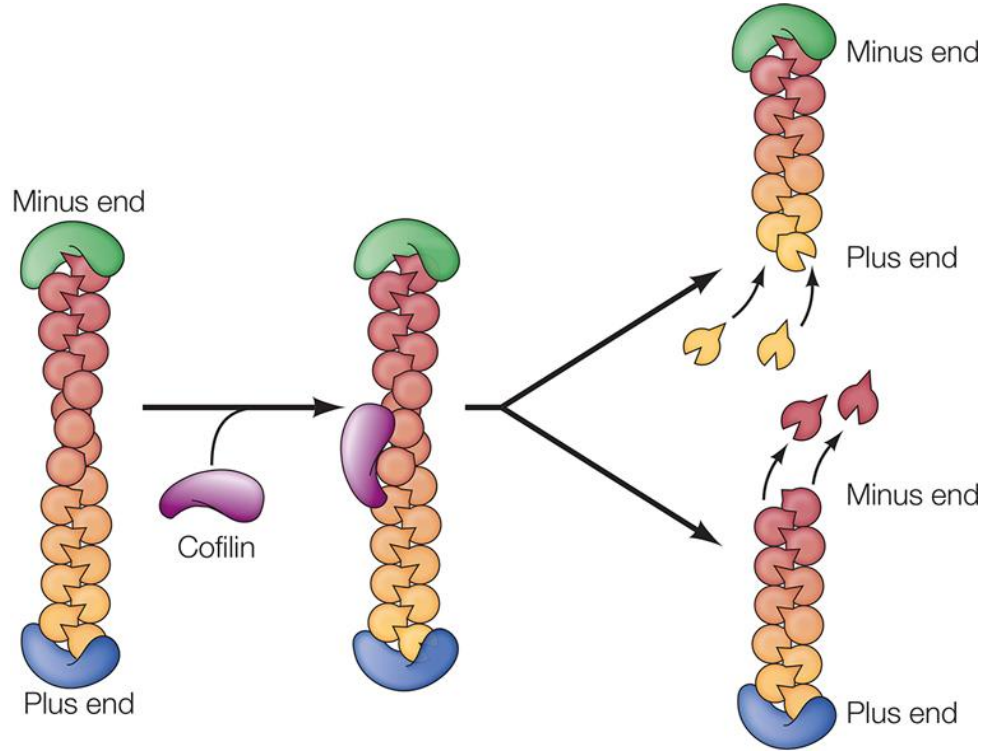
ARP 2/3 initiates branching.

Tropomyosin and cofilin

Tropomyosin stabilizes the ends by binding to capping proteins at both ends of the actin filament.



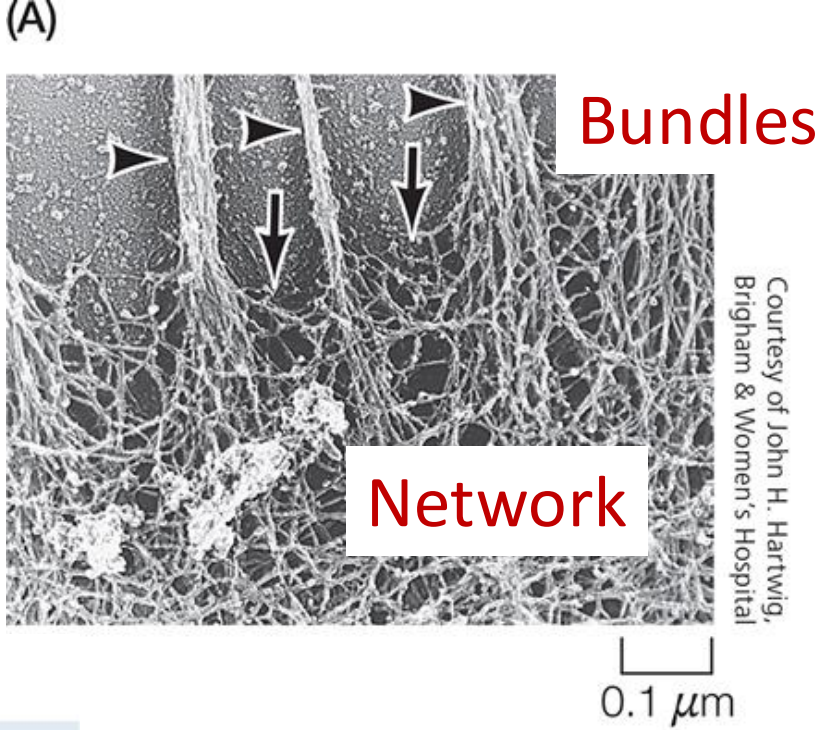
Cofilin breaks up the actin filament.

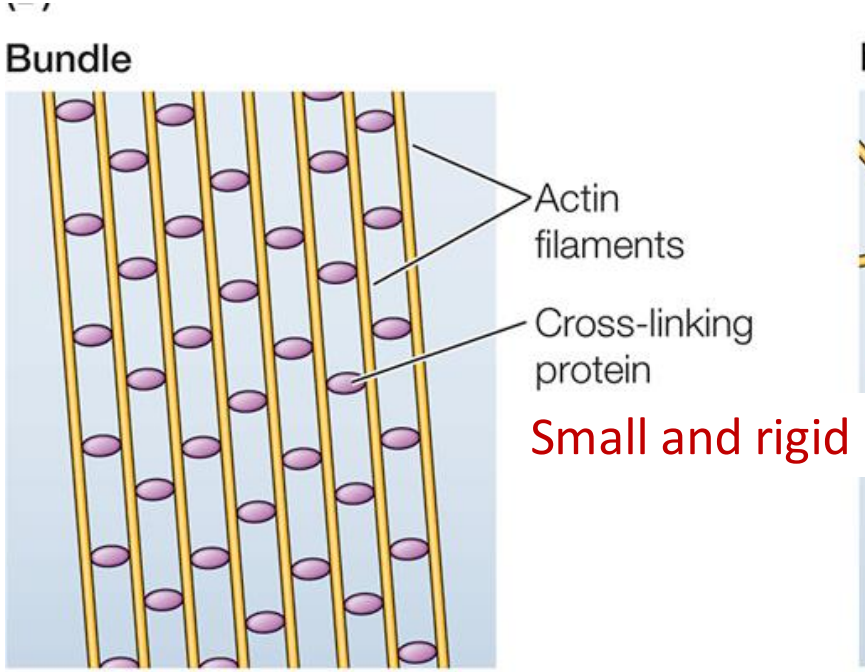


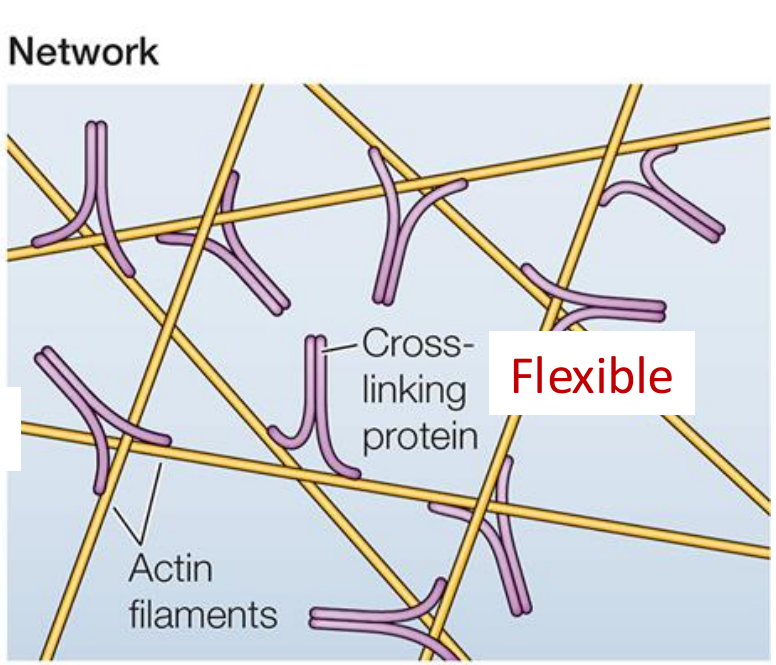
The cells cortex

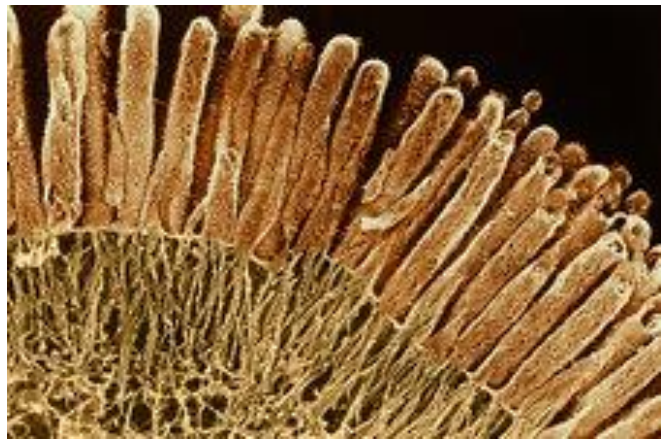
Bundles and networks

The cell cortex is made of an actin cytoskeleton that is organized as a three-dimensional network beneath the plasma membrane via actin-binding proteins determining cell shape and cellular activities.





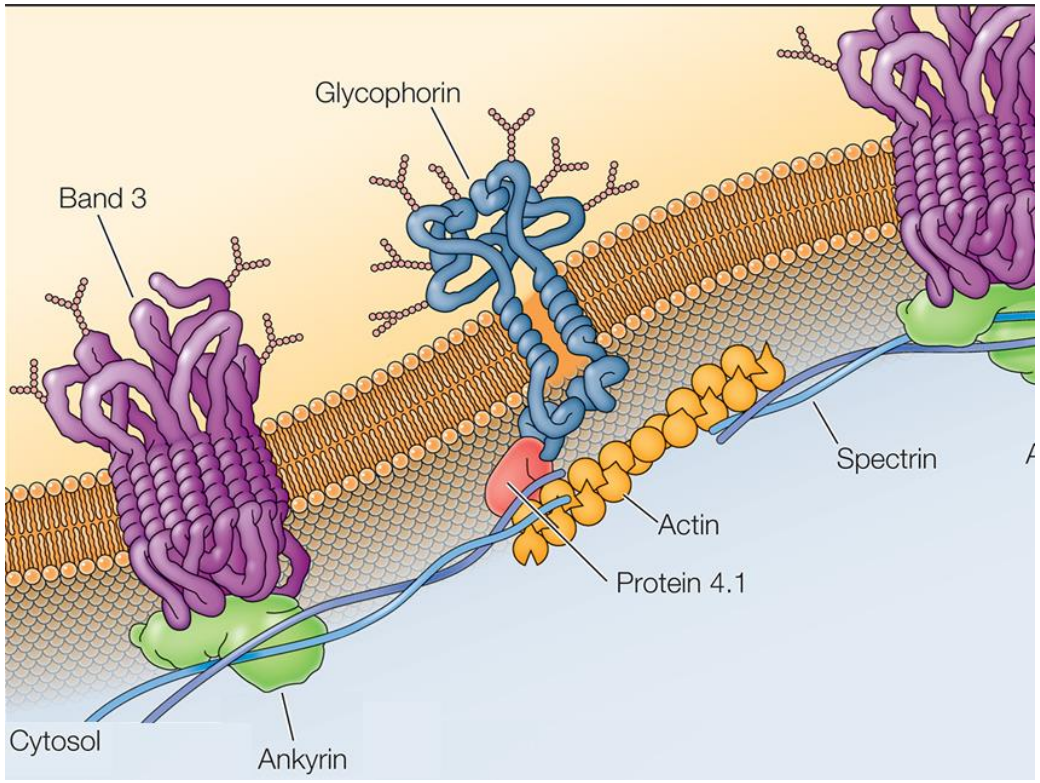




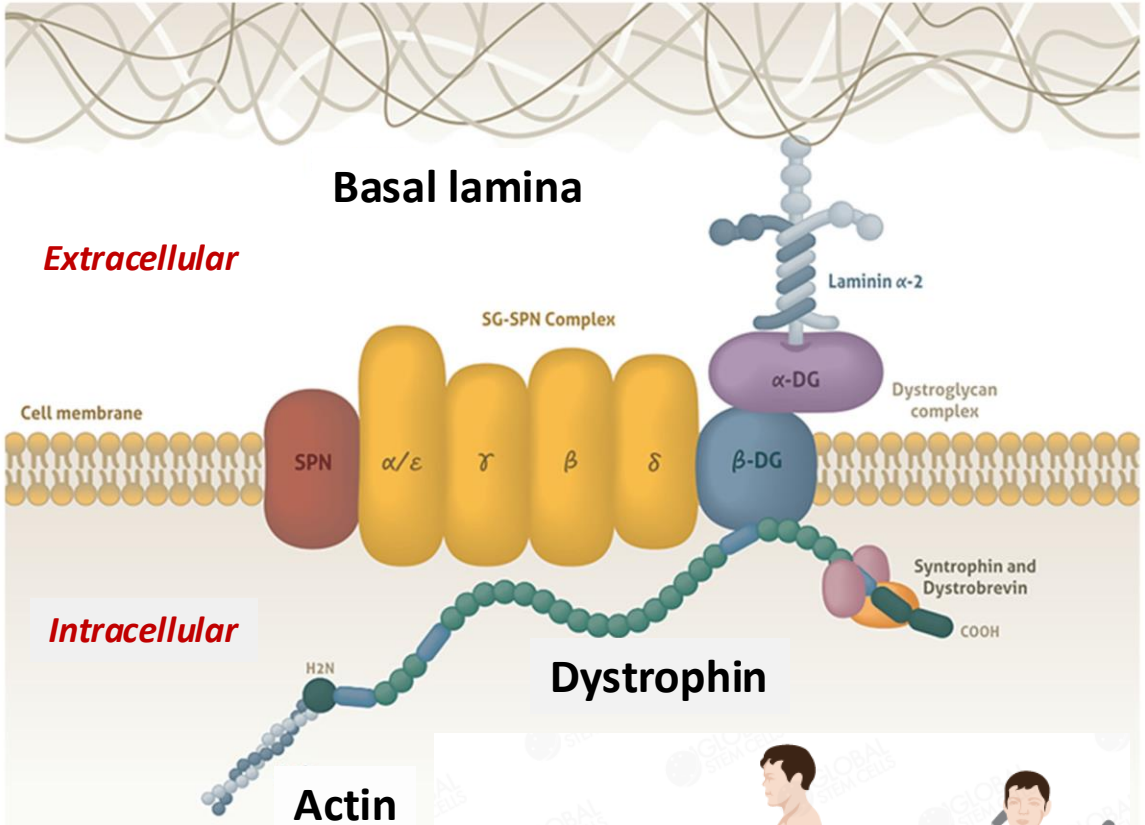
The red blood cells (*actin-membrane connection*)

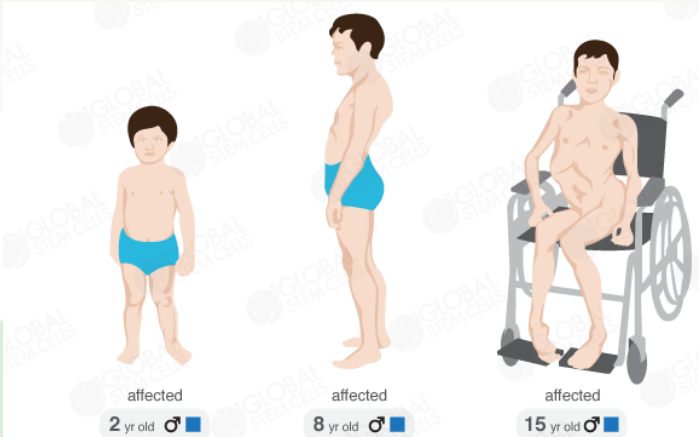
no nucleus, no organelles, no microtubules, no intermediate filaments

- The major actin-binding protein that provides the structural basis of RBCs is **spectrin**.
- Spectin links actin filaments to transmembrane proteins of RBC's plasma membrane via:
  - Ankyrin
  - Protein 4.1



Muscle cells and dystrophin

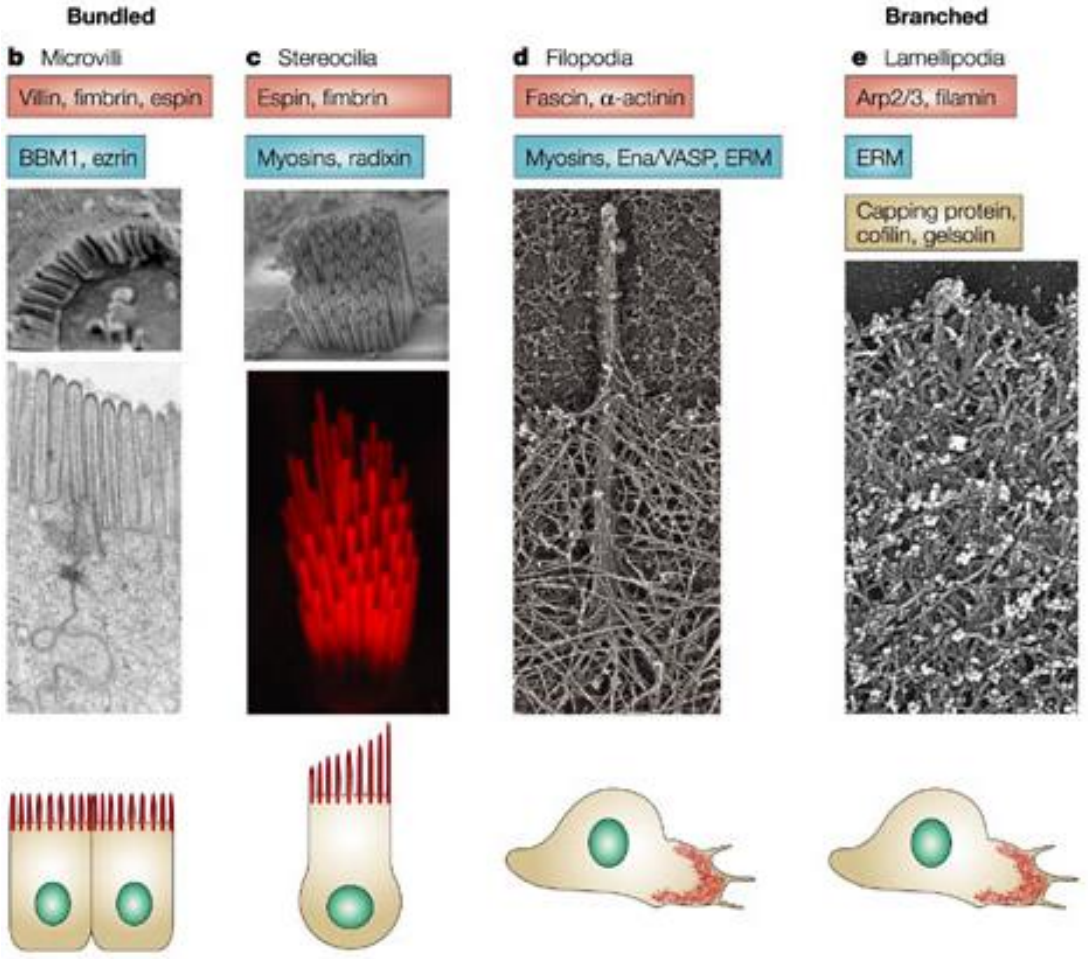




- Dystrophin is a large protein that links the actin filaments to transmembrane proteins of muscle cells.
- These linkages are important in maintaining cell stability during muscle contraction.
- Defective dystrophin is responsible for two X-linked, inherited, progressive, degenerative muscle diseases:
  - Duchenne muscular dystrophy
    - Absent protein; severe disease
  - Becker muscular dystrophy
    - Defective protein, less severe

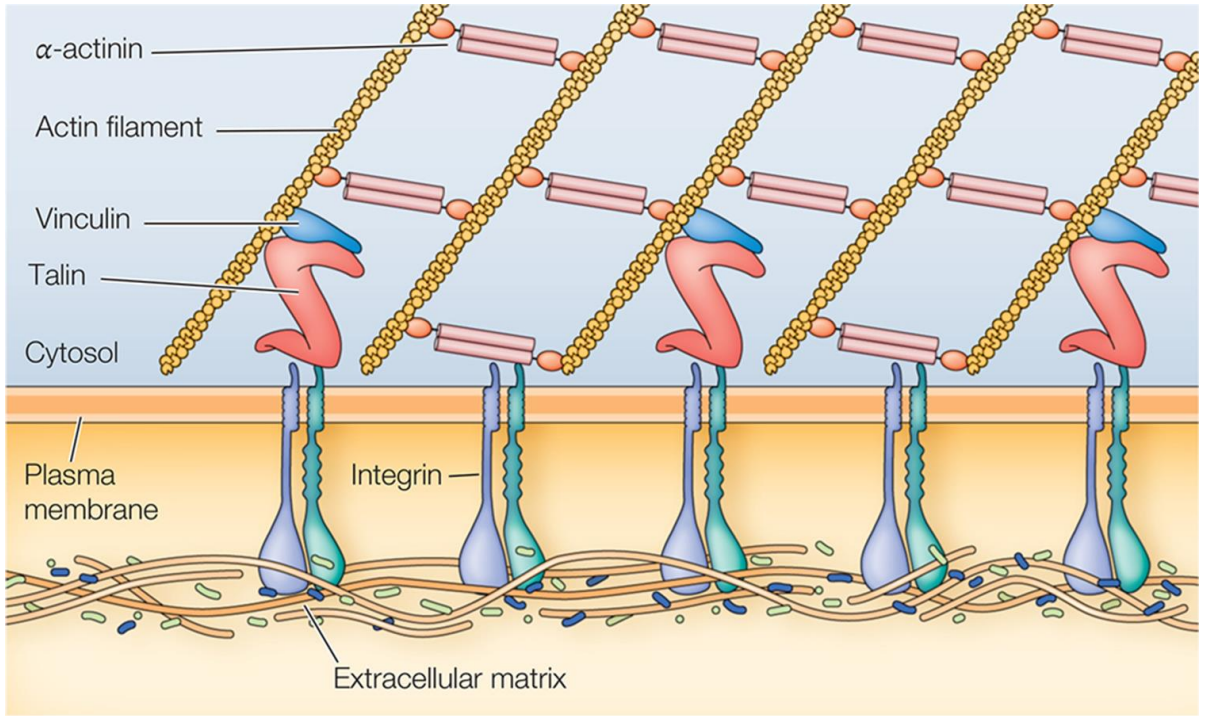
Specialized regions

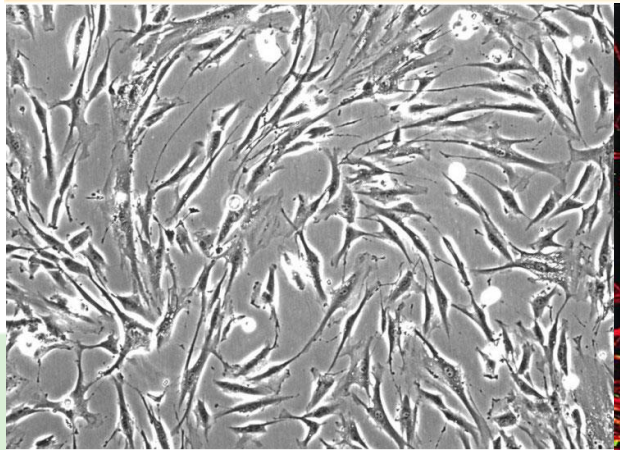
- The surfaces of most cells have a variety of protrusions or extensions that are involved in cell movement, phagocytosis, or specialized functions such as absorption of nutrients.
- Most of these cell surface extensions are based on actin filaments, which are organized into either relatively permanent or rapidly rearranging bundles or networks.

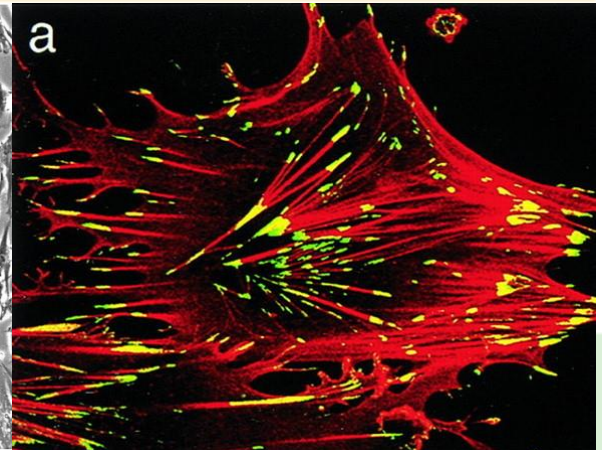


Specialized regions: focal adhesions

- Cultured fibroblasts secrete extracellular matrix proteins that cells bind to via **integrins**, and transmembrane proteins, at sites called **focal adhesions**, which themselves attach to bundles of actin filaments, called **stress fibers**.
- The actin bundles are stabilized by specialized actin-binding proteins.



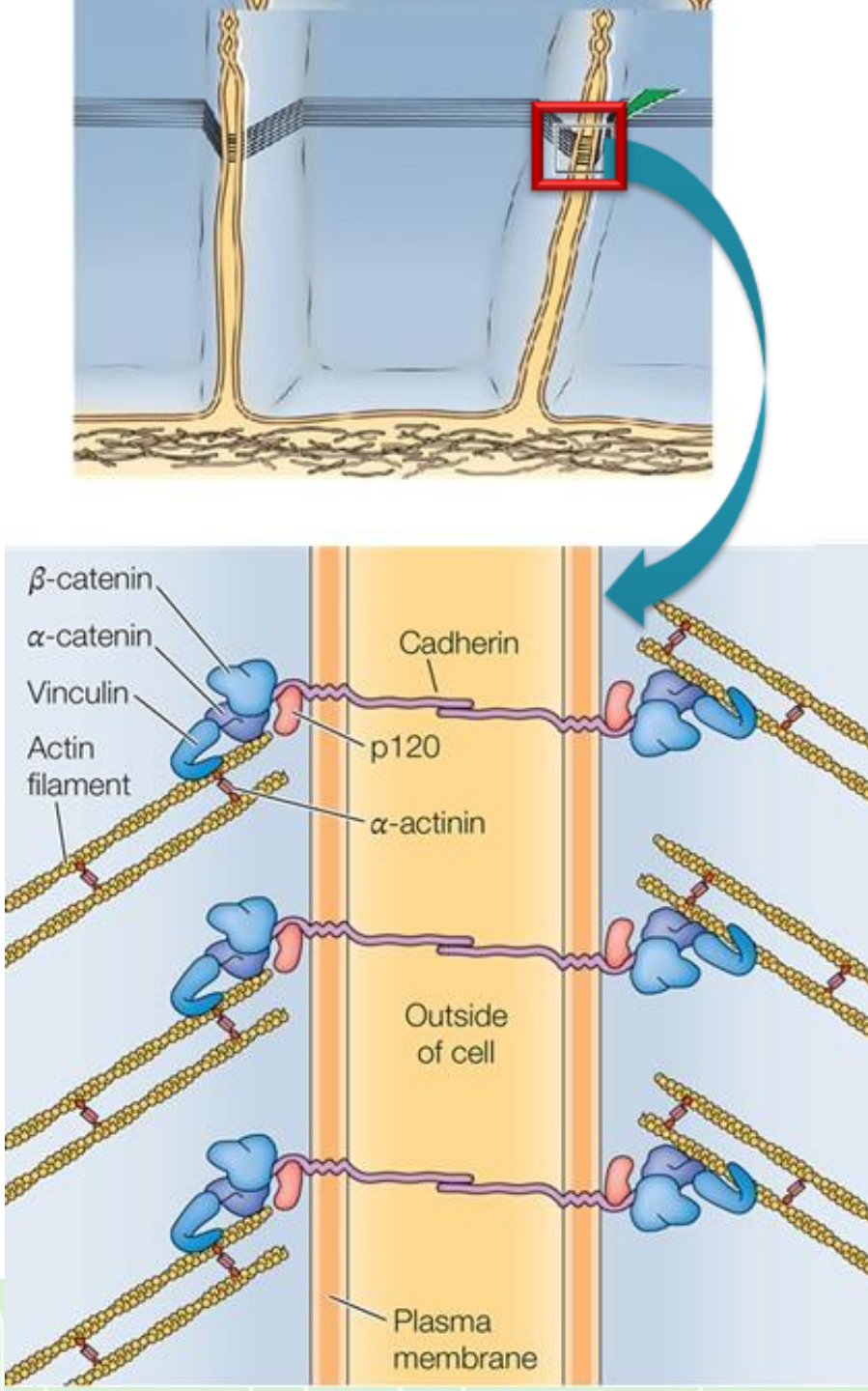




Specialized structure:
Adherins junctions

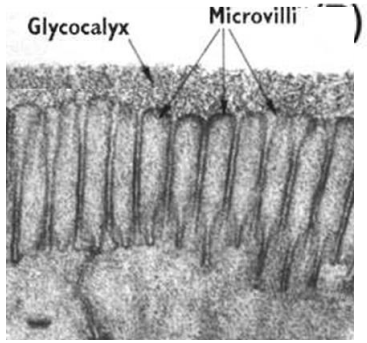
- Cells interact with each other at **adherens junctions**, which are mediated by the transmembrane **cadherins**, which interact indirectly with actin filaments.
- The junctions form a continuous belt of actin filaments around each cell.
- Cadherins attach to actin filaments via **catenins** stabilizing the junctions.
- **Epithelial cells lose cadherins when they become cancerous becoming fibroblast-like.**

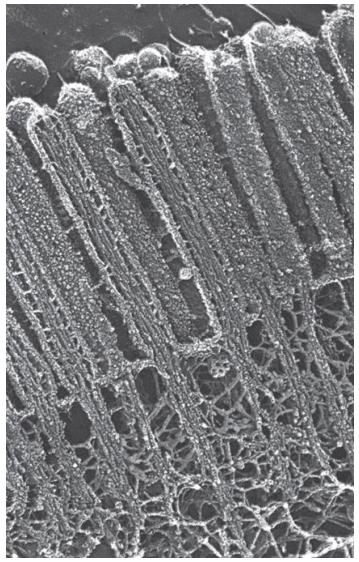
https://sketchfab.com/3d-models/intercellular-junctions-c49bf33b4efe4d32ae0bdfab5d344b4d

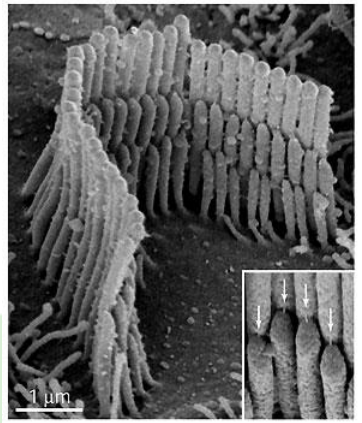


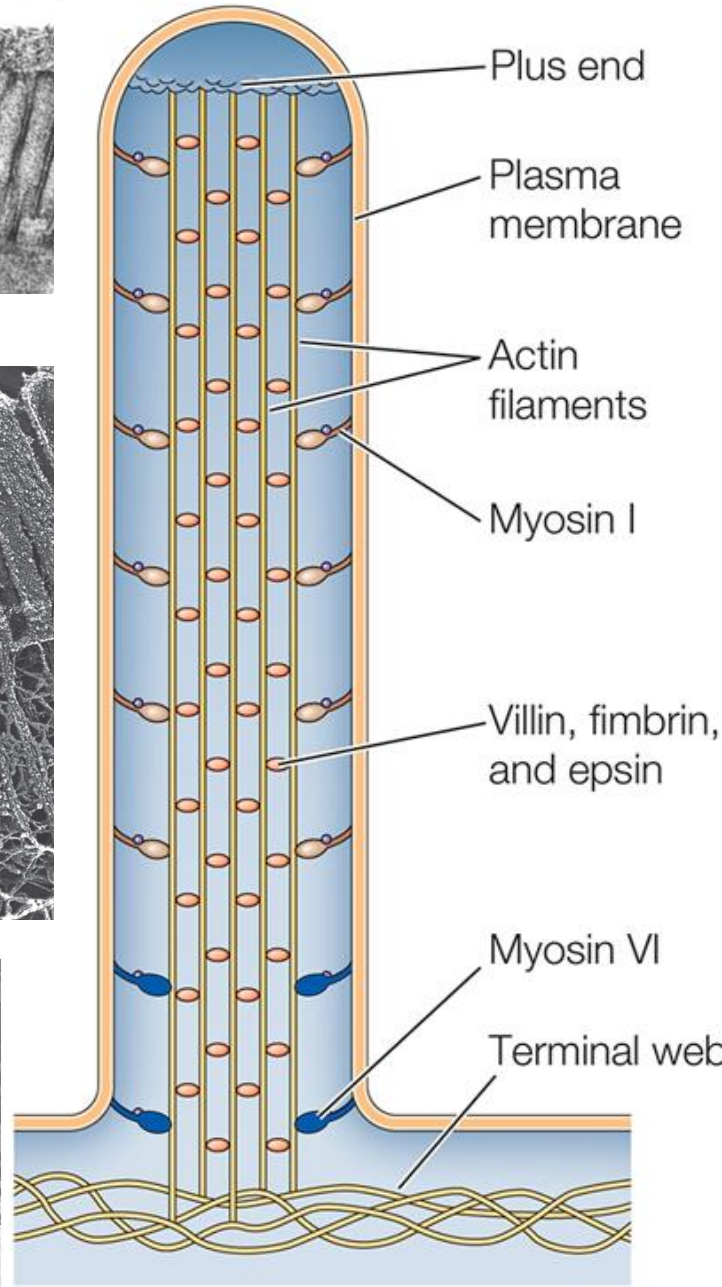
Specialized structures:
Microvilli

- Finger-like extensions of the plasma membrane that are found on the surfaces of cells involved in absorption such as:
  - The apical surface (brush border) of intestinal epithelial cells.
  - Stereocilia: a specialized form of microvilli on the surface of auditory hair cells.
- The microvilli are stabilized by the anchorage of the actin bundles to the spectrin-rich actin cortex.





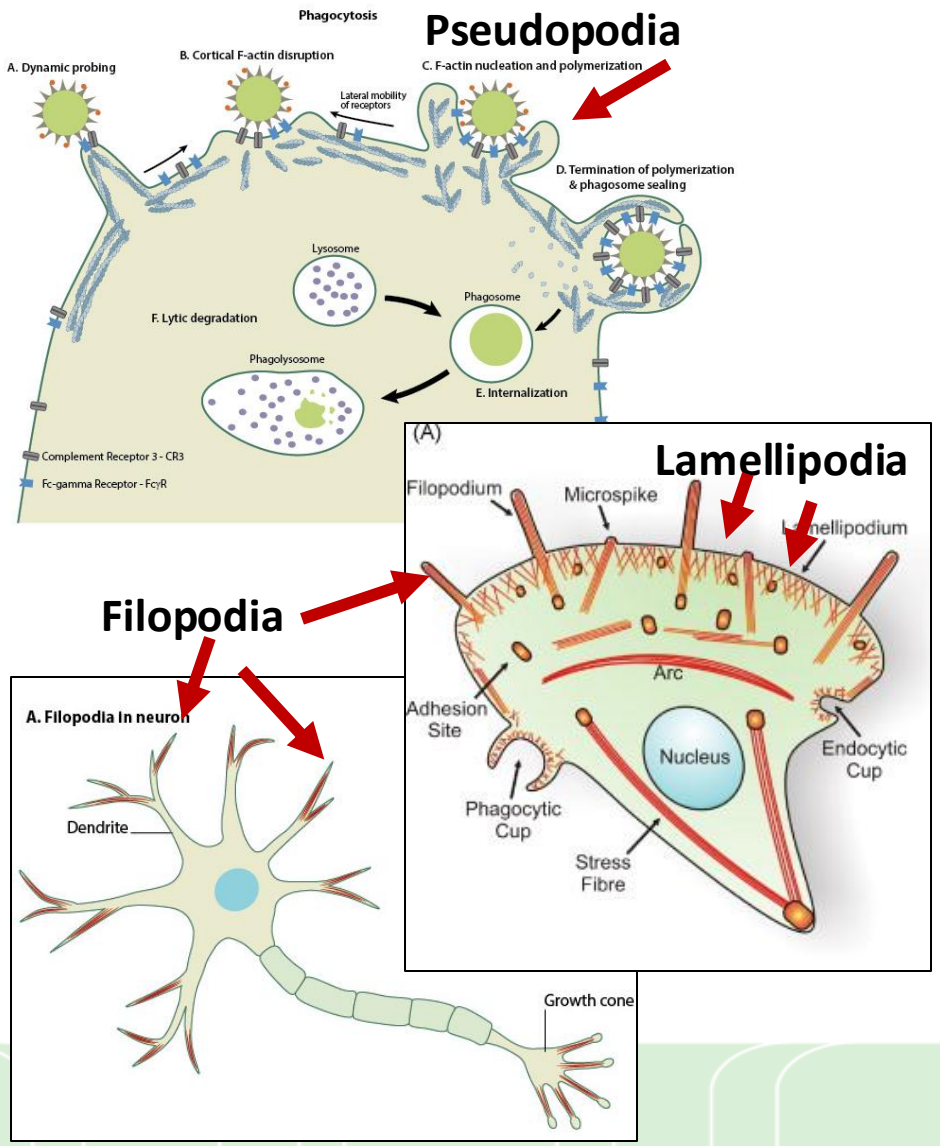




Specialized regions:

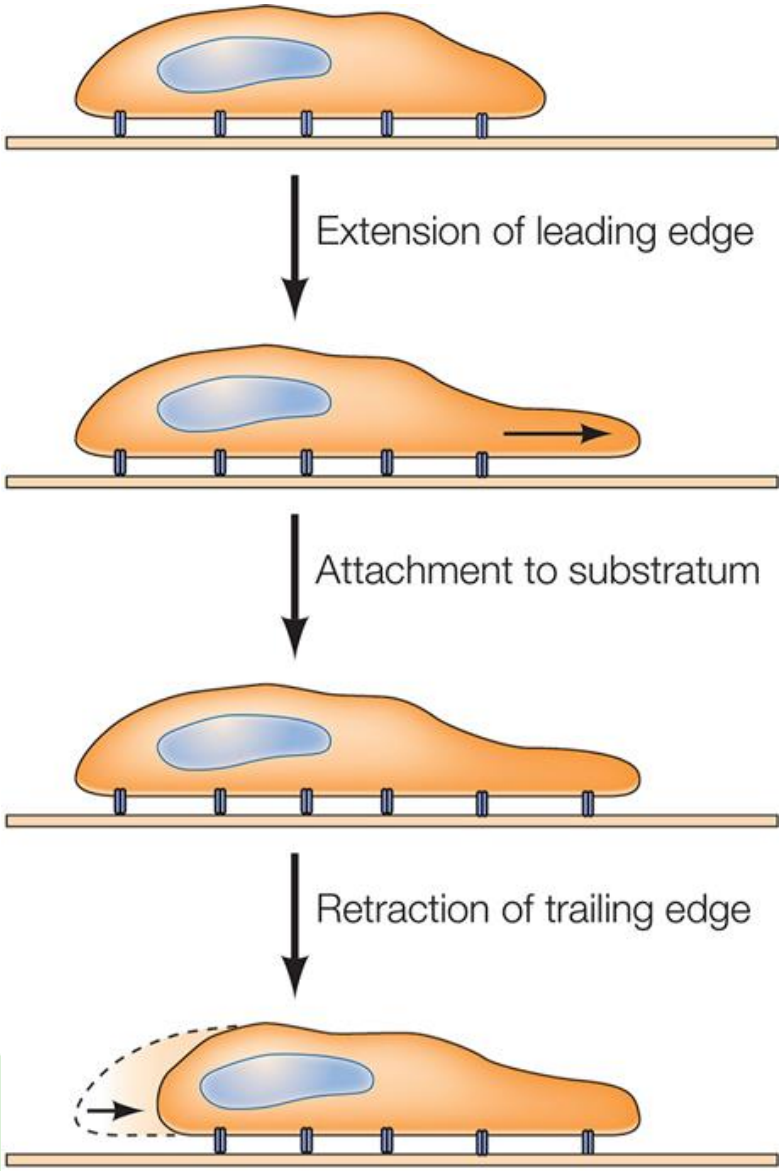
Transient surface protrusions for cell movement, phagocytosis, nerve cell extensions

- **Pseudopodia**: Extensions of actin filaments cross-linked into a network for **phagocytosis**.
- **Lamellipodia**: Broad, sheetlike extensions made of a network of actin filaments at the leading edge of **moving** fibroblasts.
- **Filopodia**: Very thin projections of the plasma membrane, supported by actin bundles, that extend from lamellipodia for **sensory purposes**.
- The formation and retraction of these structures during cell movement is based on the regulated assembly and disassembly of actin filaments.



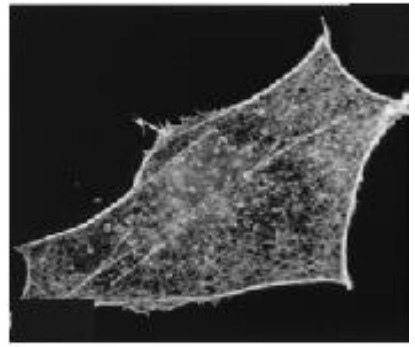
Cell migration

- Cell-substratum attachment is normally maintained via stress fibers and focal adhesions.
- Movement is initiated via sensing an attractant (wounds, chemokines, etc.) and polarity is established.
- Actin-bundling proteins and focal adhesion proteins are transported to the leading edge in connection with integrins forming protrusions (lamellipodia, filopodia, psuedopodia).
- Actin filaments are extended via polymerization and branching.
- At trailing end, focal adhesions are broken down and cells dissociate.

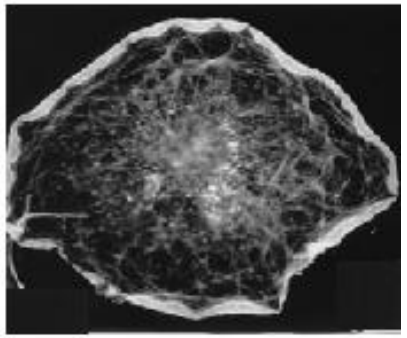


Rho family proteins

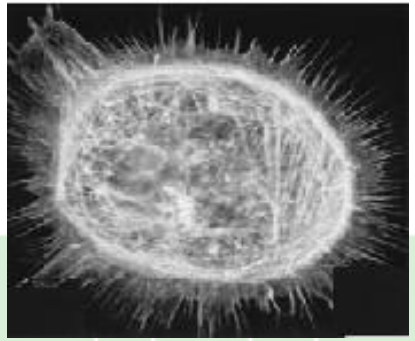
- The formation of cell surface protrusions in response to extracellular stimuli is regulated by small GTP-binding proteins of the Rho family.
  - Rho: formation of stress fibers and contraction
  - Rac: formation of lamellipodia
  - Cdc42: formation of filopodia (cell direction)



Rho



Rac



Cdc42

Actions of the Rho family

- Profilin
- Formin
- Arp2/3
- Cofilin