University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

● Slides ○ Sheet ○ Handout ○ other.....

Number # 6
Title: The cytoskeleton
Dr. Dr. Mamoun Ahram
Done By:
Date:
Price:

Designed by Nadeen Al-Freihat

Lecture 6: the cytoskeleton and cell movement

(Actin microfilaments)

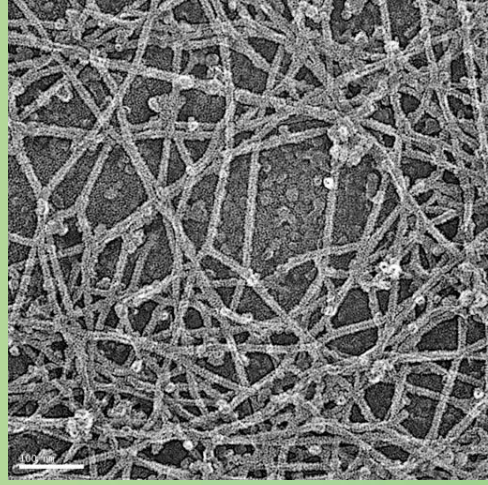
Dr. Mamoun Ahram
Faculty of Medicine
Second year, Second semester, 2014-2014

Principles of Genetics and Molecular Biology

What is the cytoskeleton?

- A dynamic network of protein filaments extending throughout the cytoplasm
- Three types: actin microfilaments, microtubules, intermediate filaments
- Function:
  - Structural framework of cells
  - Determines cell shape and movement
  - Determines positions of organelles
  - Determines overall organization of cytoplasm
  - Regulates internal movement of organelles

The actin filaments



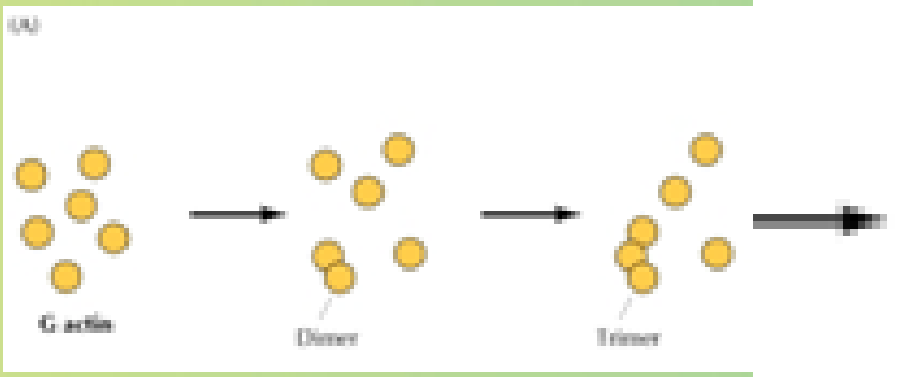
- They are also called microfilaments.
- They are thin, flexible fibers.
- They are organized into higher-order structures, forming bundles or three-dimensional networks.
- They form semisolid gels.
- They are regulated by a variety of actin-binding proteins.
- They are abundant beneath the plasma membrane, where they form a network for cellular function.

Actin genes

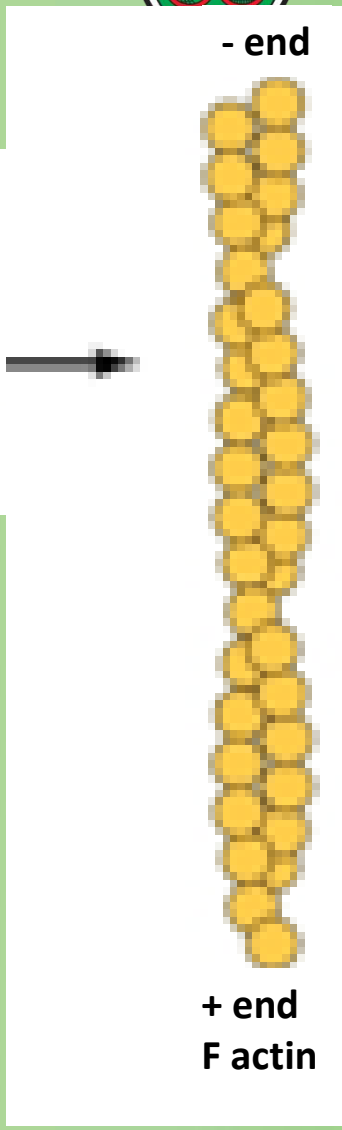
- Mammalian cells have at least six distinct actin genes:
  - Four are expressed in different types of muscle
  - Two are expressed in nonmuscle cells.
- Yeast actin is 90% identical in amino acid sequence to the actins of mammalian cells.

The actin protein

- An actin monomer (globular [G] actin) is tightly bound to two other actin monomer having a head-to-tail interactions

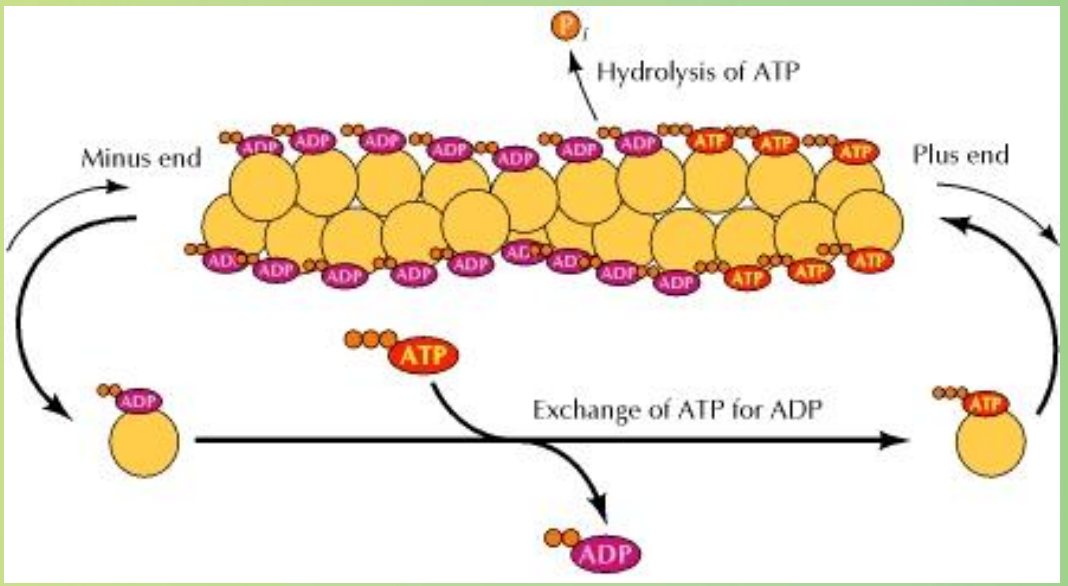


- Actin monomers polymerize to form filamentous [F] actin.
- Actin filaments have a distinct polarity and their ends (called the plus and minus ends).



Formation of filament

- Nucleation: formation of an aggregate of 3 actin monomers
- Additional monomers are added to both ends (barbed and pointed), but faster at barbed ends
- The monomers are bound to ATP, which is not required for nucleation, but
  - is hydrolyzed into ADP following assembly
  - Speeds polymerization
  - Stabilizes binding
- Treadmilling: dissociation of subunits mainly from
  - ADP-actin
  - from pointed end
- This illustrates dynamic behavior of actin filaments



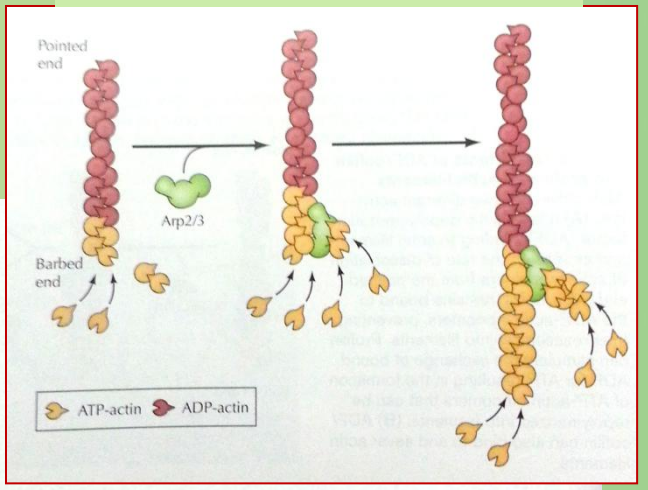
Actin-binding proteins

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

Examples

The rate-limiting step, nucleation, is facilitated by the actin-binding protein, formin.

Branching is facilitated by the Arp 2/3 complex



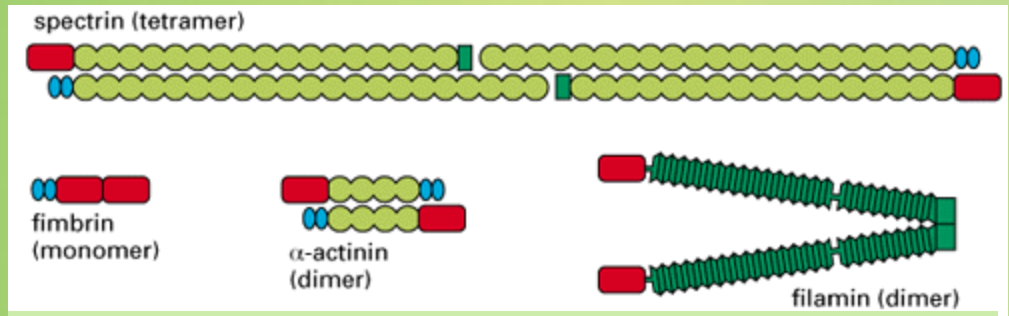
Tropomyosin

Cofilin

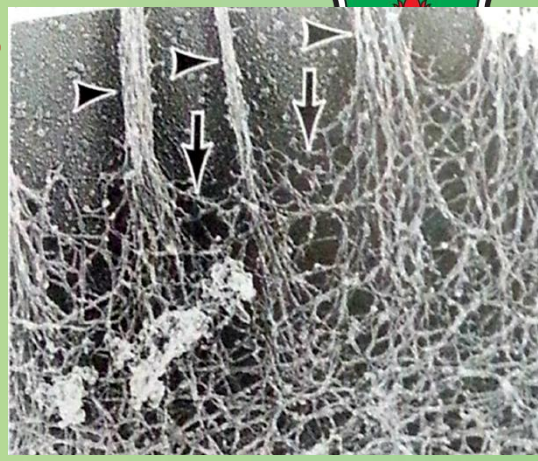
Proflin

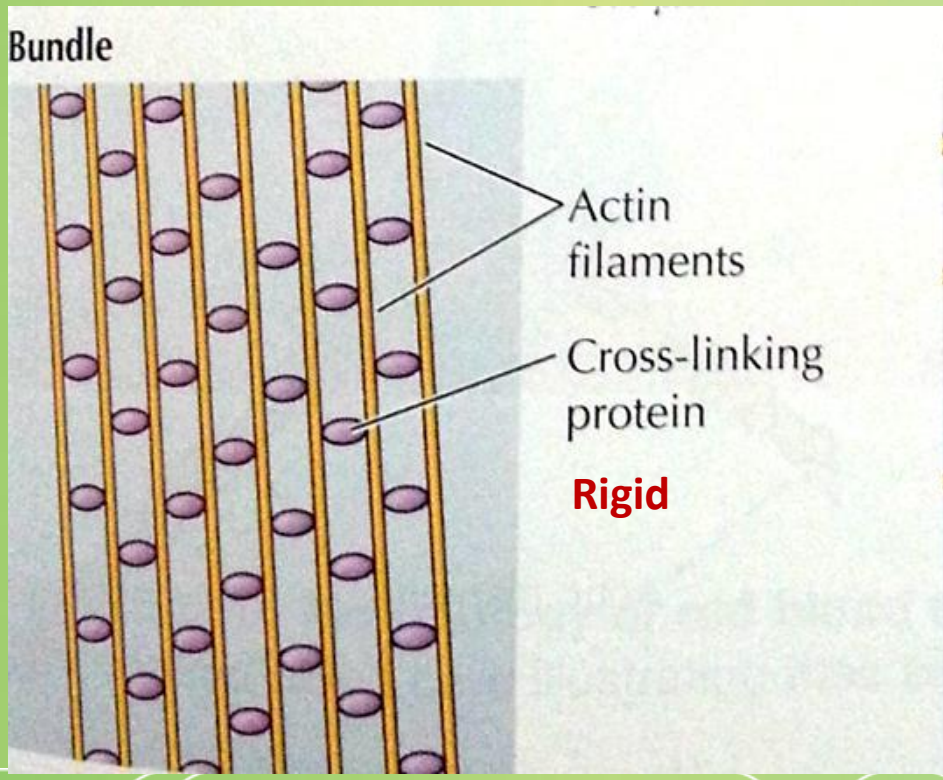
α-Actinin

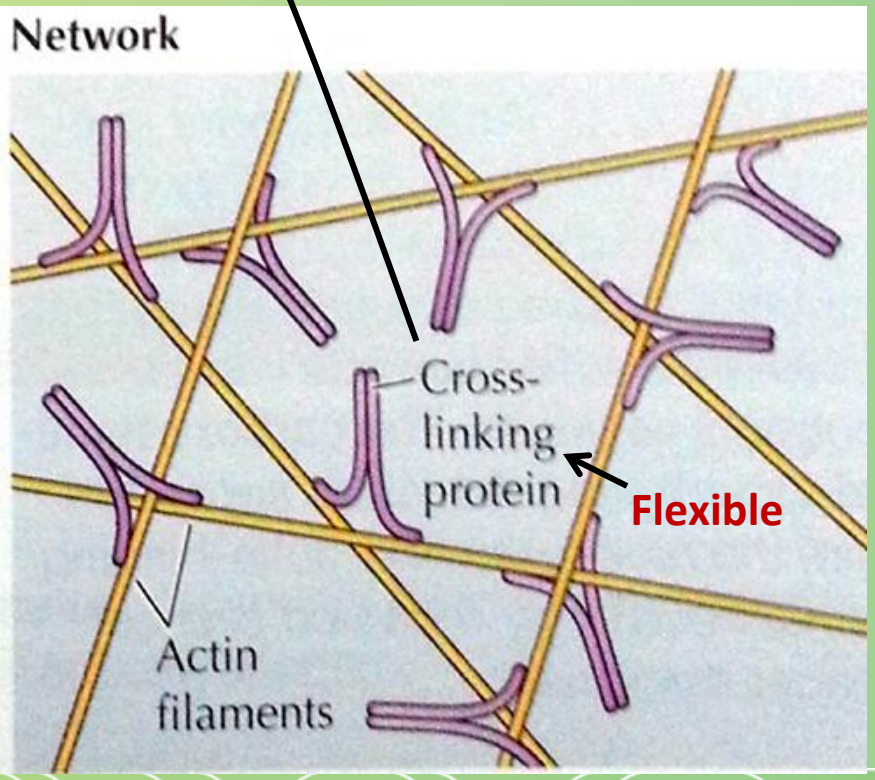
Organization of actin filaments



Differ in flexibiloty, spacer length and sequence

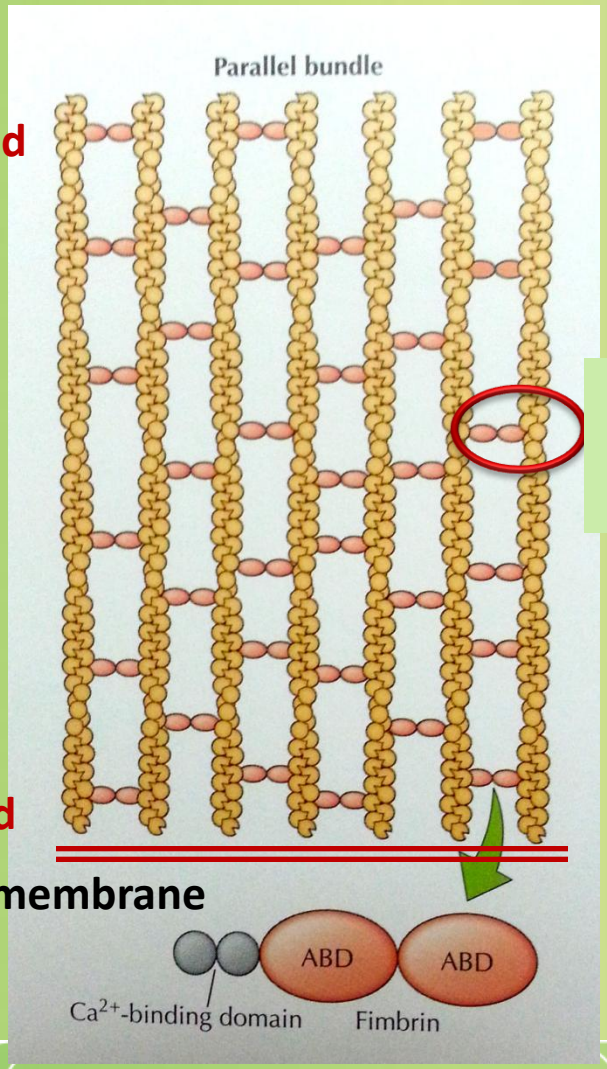






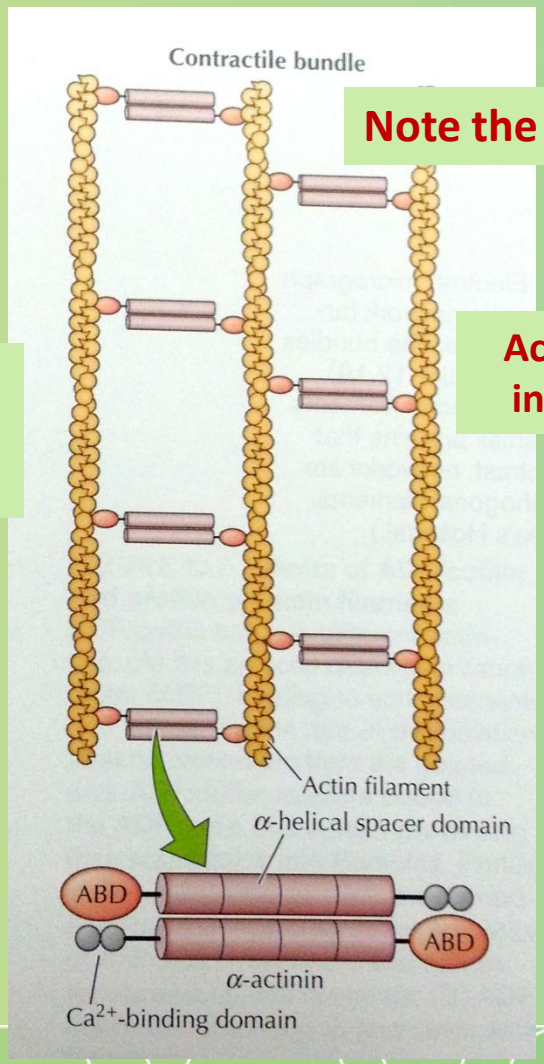
Types of actin bundles

Intestinal microvilli



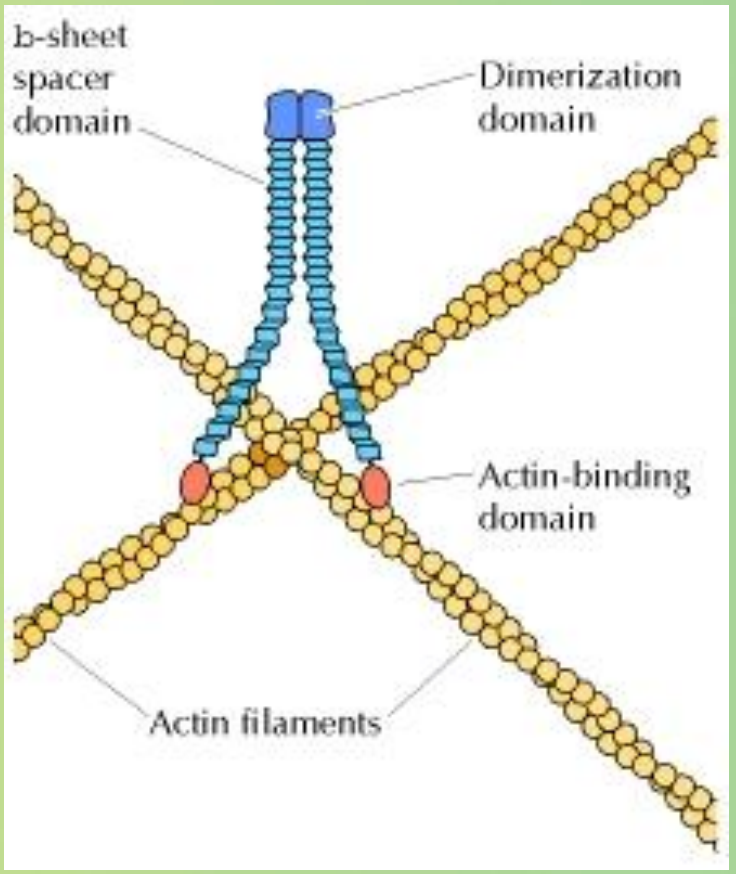
Actin-monomer interaction

Muscle

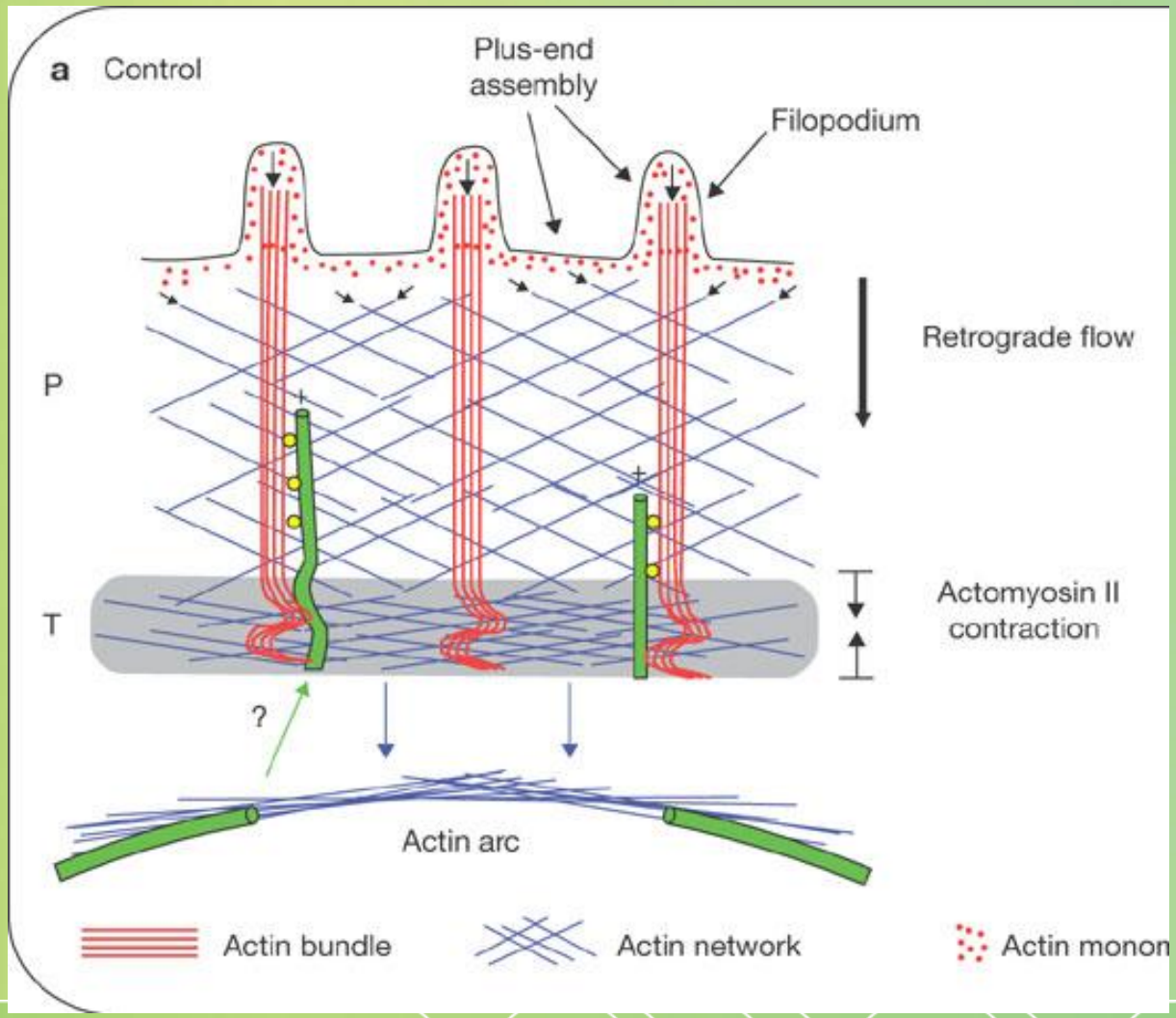


Actin networks

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



Again…where these structures are present intracellularly

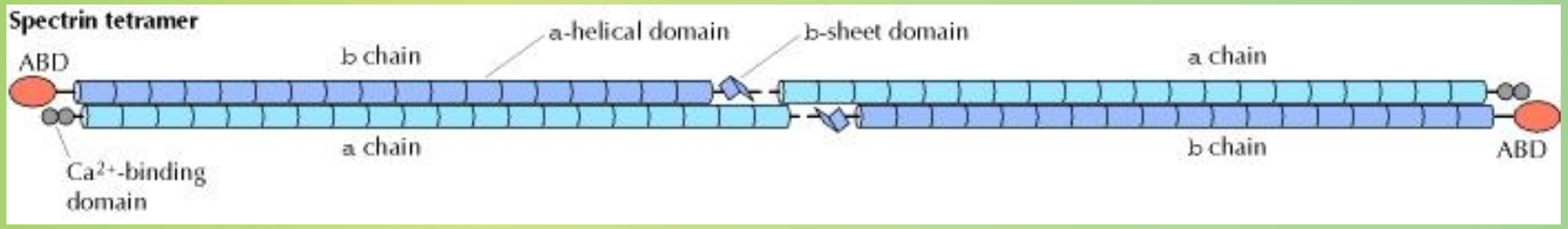


Actin filaments and plasma membrane

- Cell cortex: The network of actin filaments and associated actin-binding proteins.
- Studies in RBC because:
  - They do not have other cytoskeletal pstructures
  - They do not have organelles → no contamination
- The cytoskeleton is uniform with no specialized regions like in other cells

Spectrin

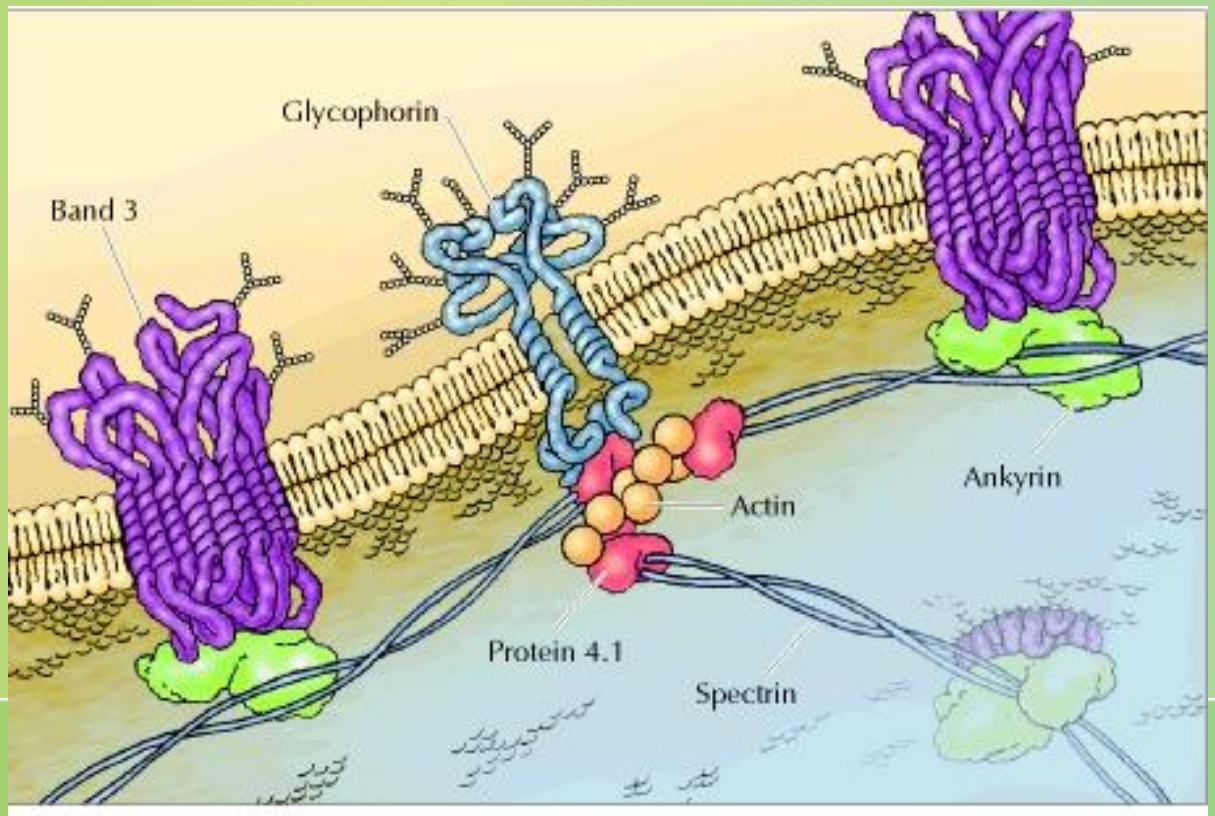
- The major protein that provides the structural basis for the cortical cytoskeleton in erythrocytes



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

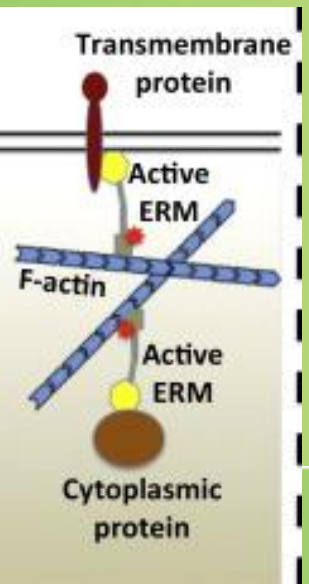
Actin filaments-plasma membrane interaction

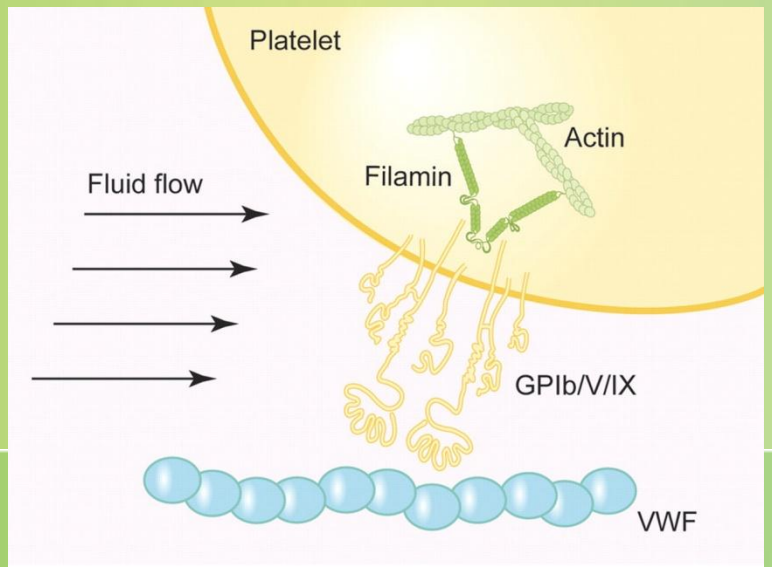
- **The spectrin-actin network is linked to the membrane by**
  - **ankyrin, which binds to both spectrin and the abundant transmembrane protein band 3.**
  - **protein 4.1, which binds to glycophorin**

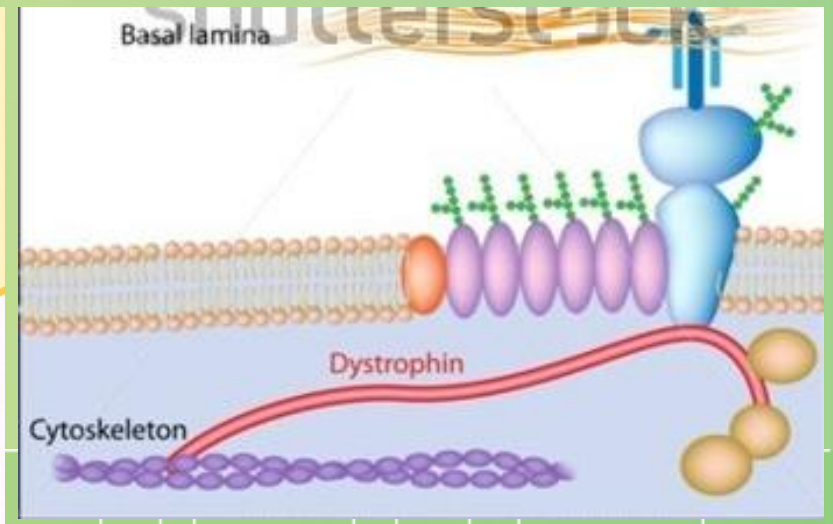


Other linkage in other cells

- 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 (a spectrin-related proteins) links actin filaments to transmembrane proteins of the muscle cell plasma membrane and the latter links the cytoskeleton to the extracellular matrix
  - This maintains cell stability during muscle contraction.

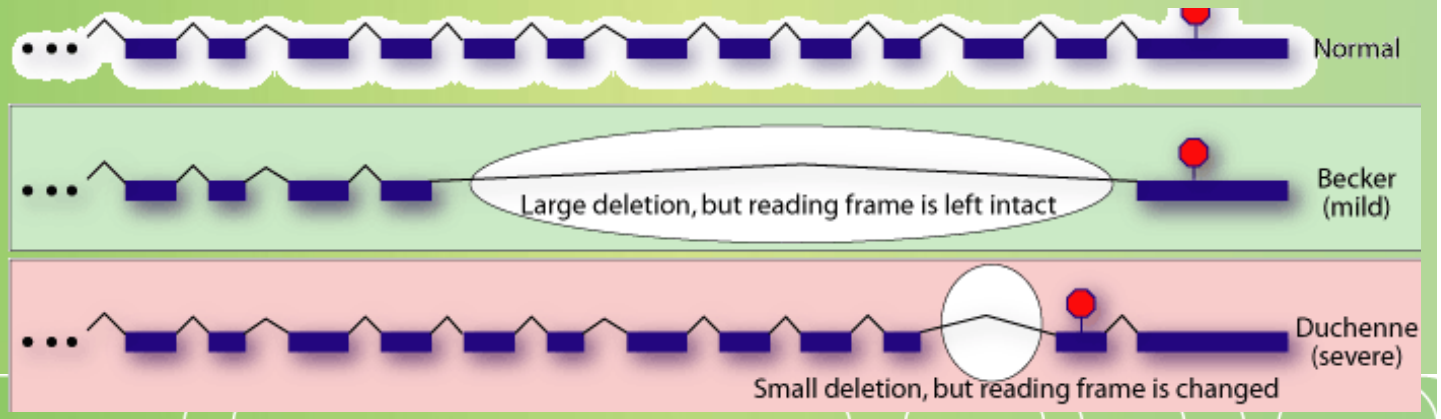


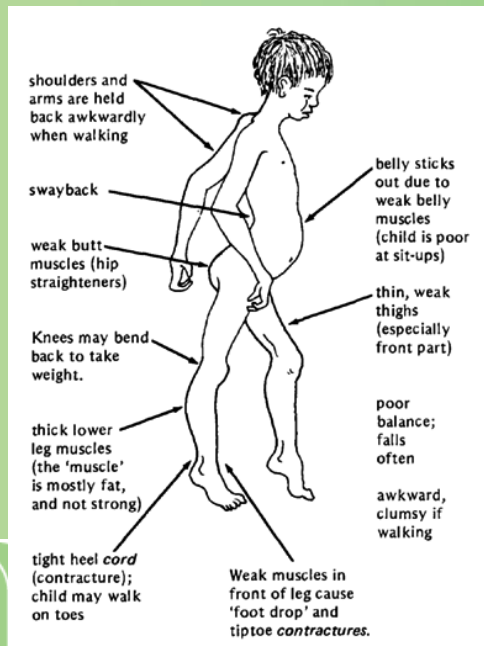




Dystrophin and disease

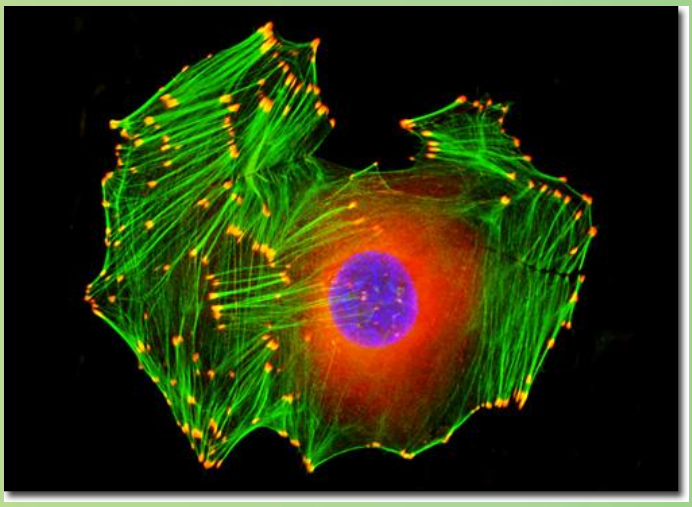
- The dystrophin gene encodes a large protein (427 kd).
- Mutations in the gene cause two types of muscular dystrophy, Duchenne's and Becker's, that either have severely of moderately dysfunctional protein in patients, respectively.
- These X-linked inherited diseases result in progressive degeneration of skeletal muscle, and patients with the more severe form of the disease (Duchenne's muscular dystrophy) usually die in their teens or early twenties.



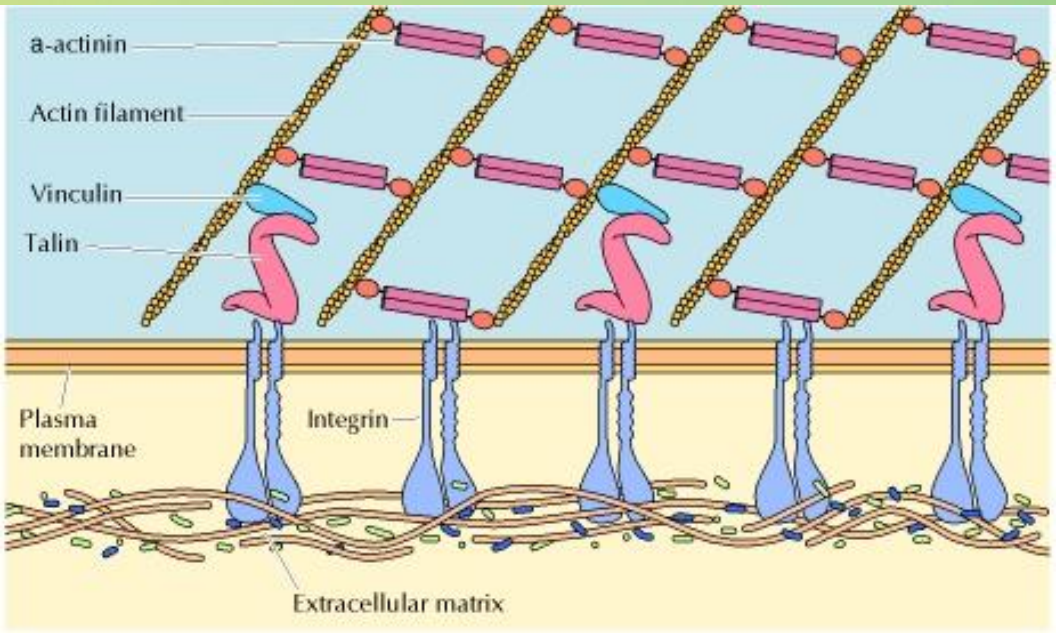


Focal adhesion

- These specialized regions that serve as attachment sites for bundles of actin filaments called stress fibers that anchor the cytoskeleton (and cells) to the extracellular matrix via the binding of transmembrane proteins (called integrins) to the extracellular matrix.

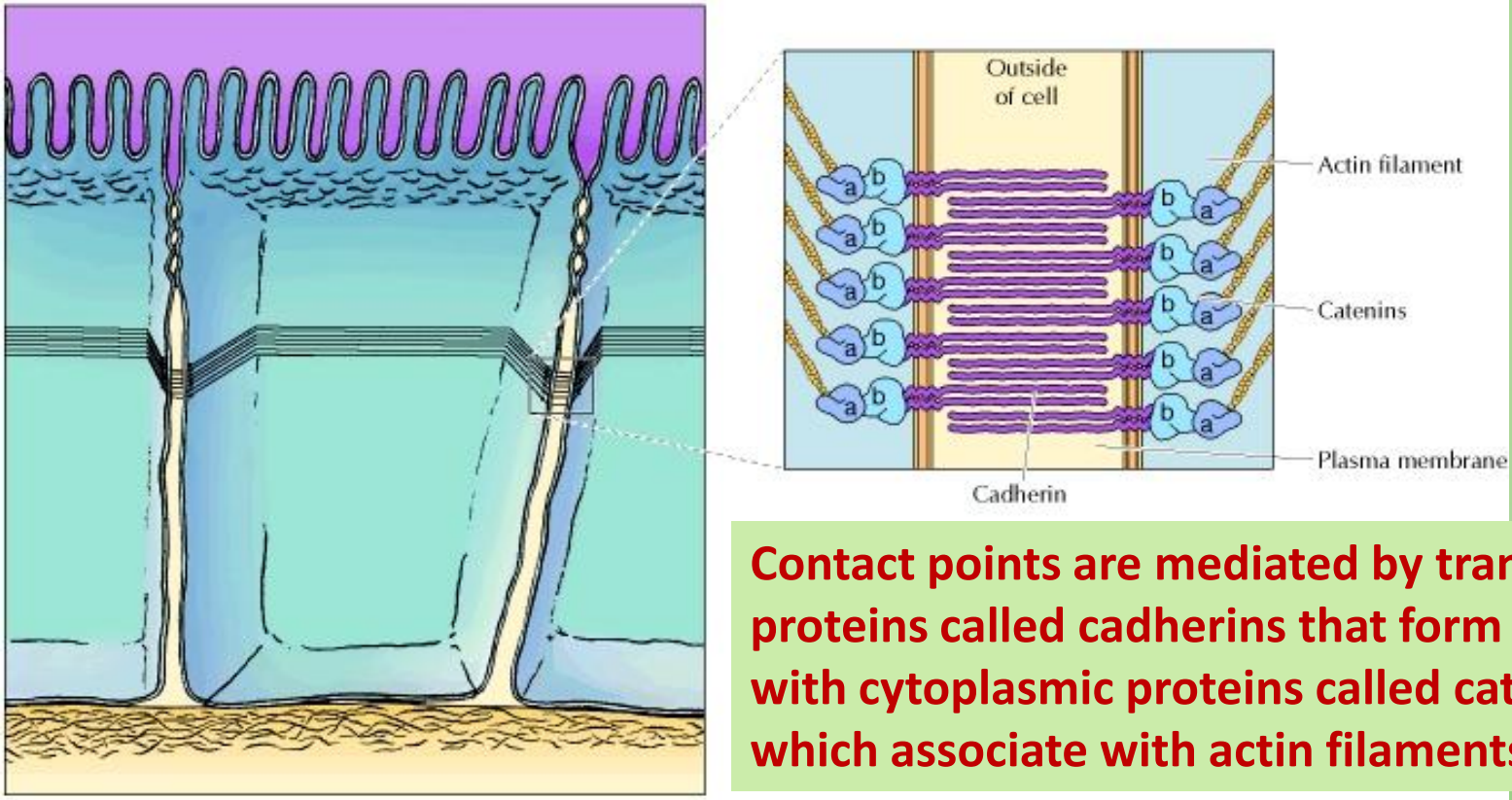


- They Stress fibers are crosslinked by α-actinin.
- These associations, are mediated by several other proteins, including talin and vinculin.



Adherens junctions

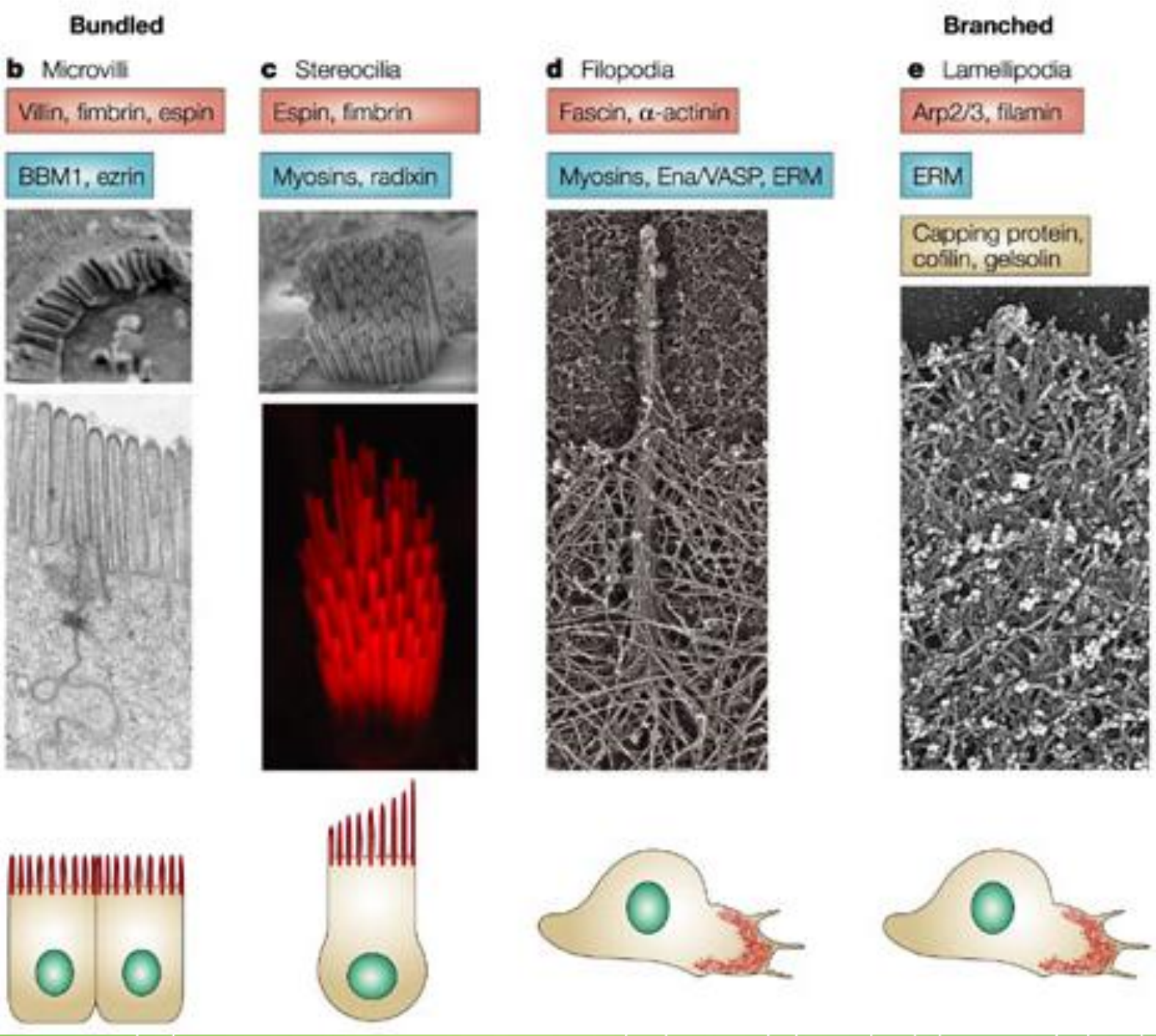
- Regions of cell-cell contact in sheets of epithelial cells with a continuous beltlike structure around each cell in which an underlying contractile bundle of actin filaments is linked to the plasma membrane.



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

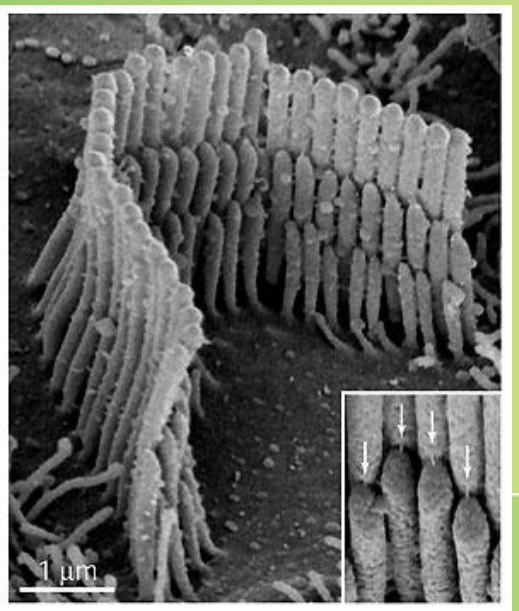
Protrusions of the cell surface

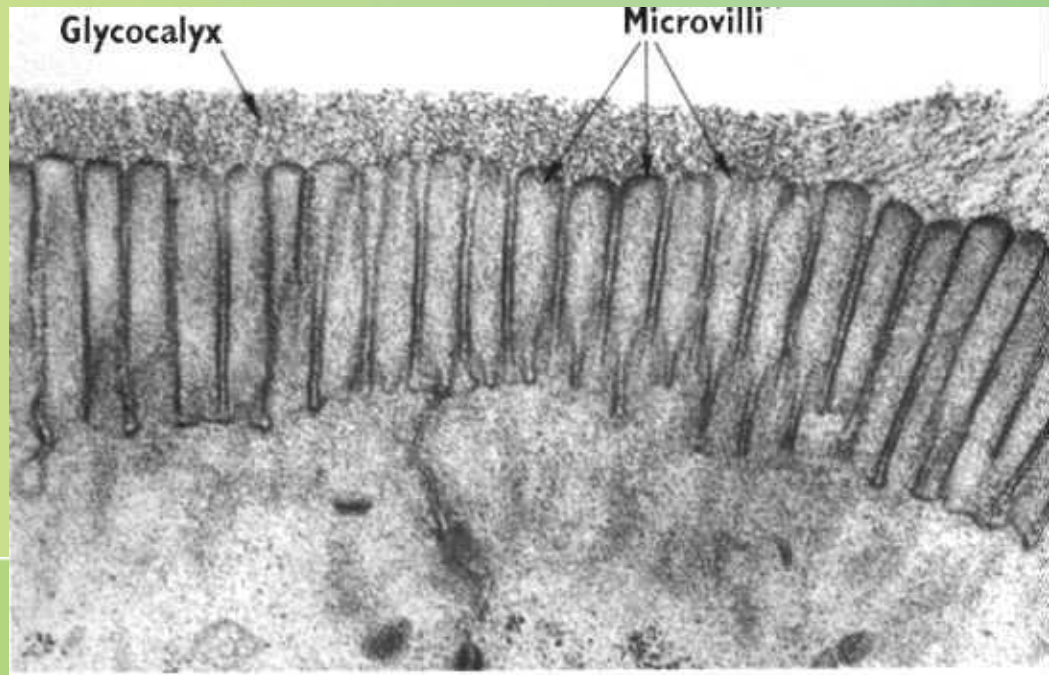
- The surfaces of most cells have a variety of protrusions or extensions that are involved in cell movement, phagocytosis, or specialized functions such as absorption of nutrients.
- Most of these cell surface extensions are based on actin filaments, which are organized into either relatively permanent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


Bundled
Branched
b Microvilli
c Stereocilia
d Filopodia
e Lamellipodia
Villin, fimbrin, espin
Espin, fimbrin
Fascin, α-actinin
Arp2/3, filamin
BBM1, ezrin
Myosins, radixin
Myosins, Ena/VASP, ERM
ERM
Capping protein, cofilin, gelsolin

Microvilli

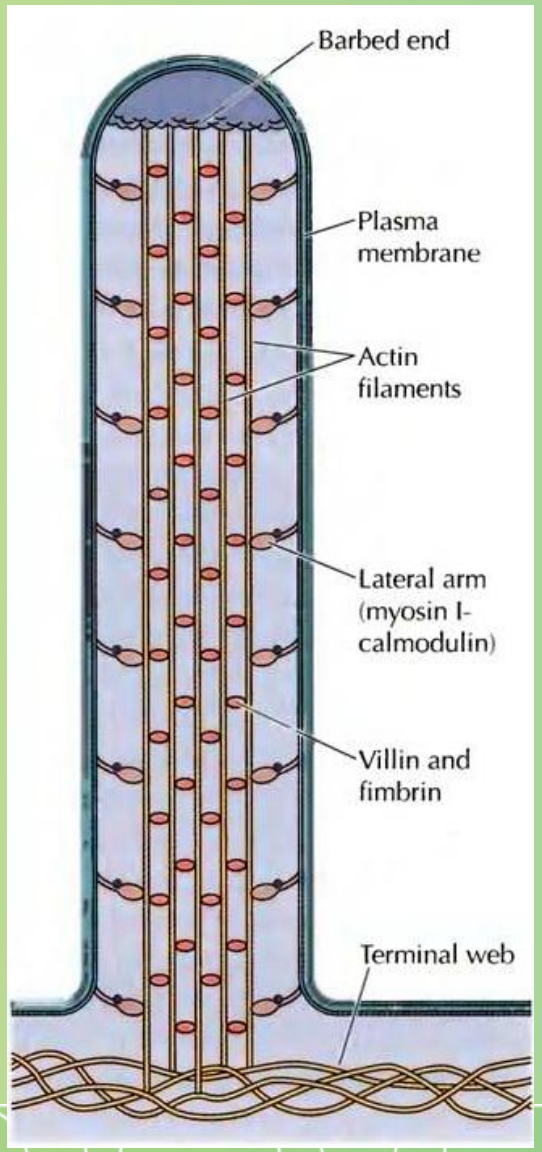
- Fingerlike extensions of the plasma membrane that are particularly abundant on the surfaces of cells involved in absorption, such as the epithelial cells lining the intestine.
- They form a layer on the apical surface (called a brush border) to increase the exposed surface area available for absorption.
  - Stereocilia : specialized forms of microvilli on the surface of auditory hair cells, are responsible for hearing by detecting sound vibrations.





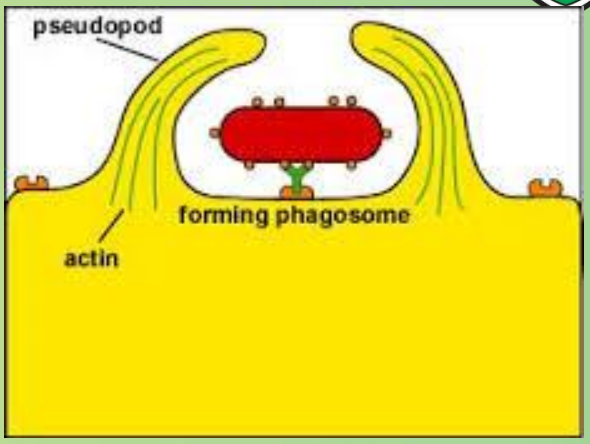
Organization of microvilli

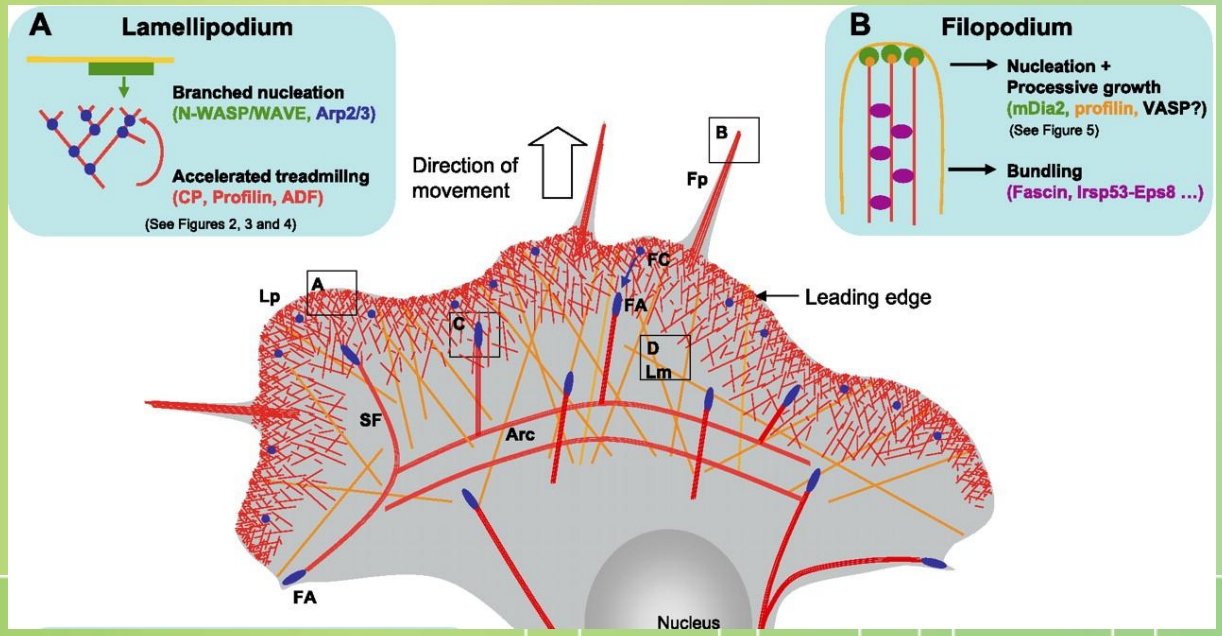
- Filament bundles are linked by villin (major) and fimbrin.
- Attachment to plasma membrane is mediated by calmodulin and myosin I.
- Filaments are linked to the cortex at the base via a spectrin-rich region called the terminal web.



Other protrusions

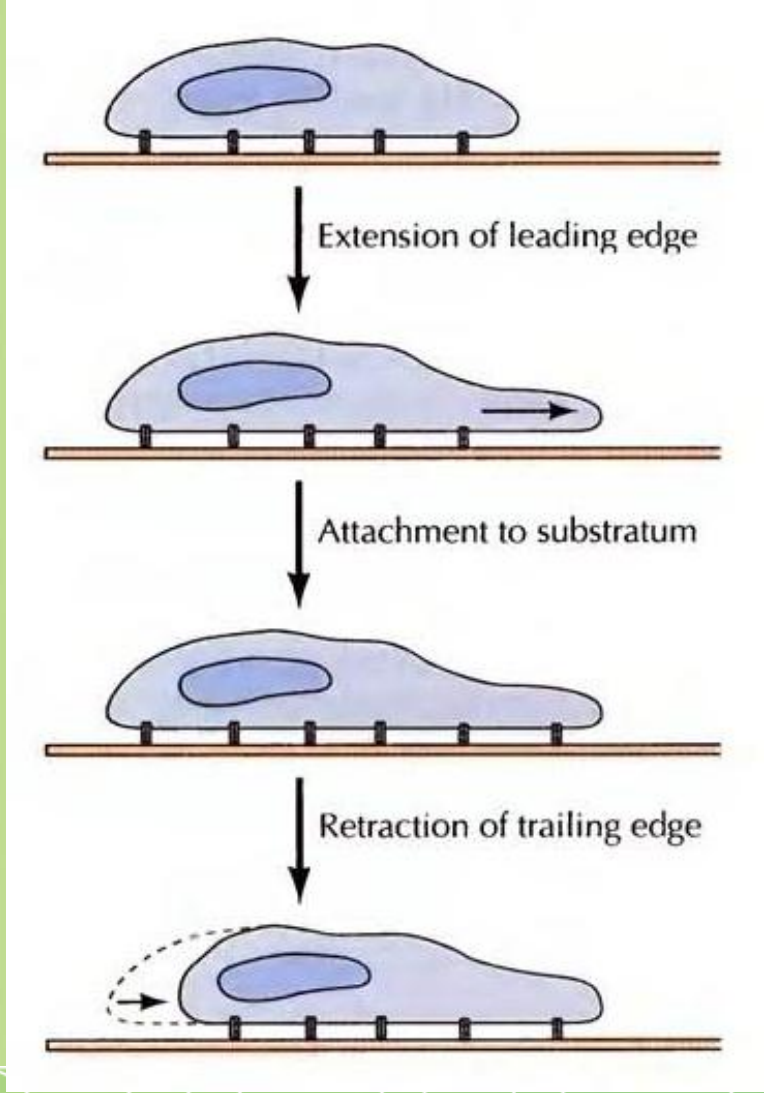
- Psuedopodia: phagocytosis
- Lamellipodia: broad, sheet-like networks of actin leading edge of moving fibroblasts
- Filopodia: thin projections extending from lamellipodia





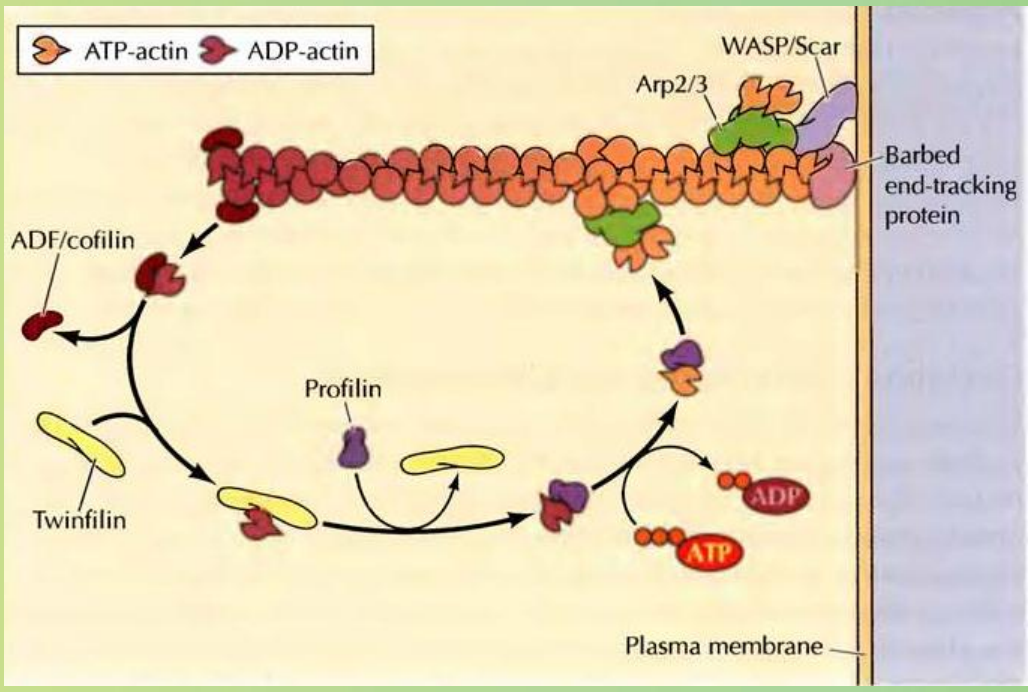
Cell migration

1. Develop polarity via specialization of the plasma membrane or the cell cortex.
2. Extend protrusions (lamellipodia, filopodia, psuedoppodia) at the leading edge cia pbranching and polymerization of actin filaments.
3. Attach to substratum (e.g. focal adhesions)
4. Dissociate trailing edge.



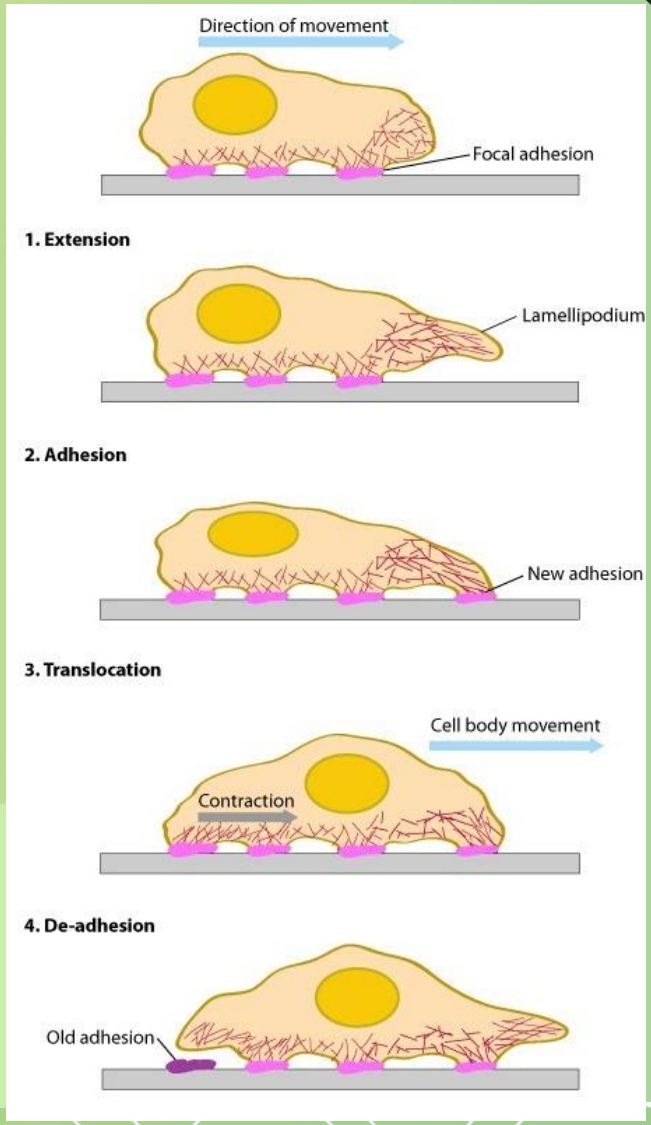
Dynamics of actin filament

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



Modification of focal adhesions

- Cell-substratum attachment is initiated via transporting actin-bundling proteins (like what?) 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 relatively slow moving cells like fibroblasts and epithelial cells, but rapidly moving cells like macrophages have loose attachments.