CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

15

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The membrane enclosed organelles we discussed in the previous lectures are one level of the organizational structure of eukaryotic cells, a further level of organization is provided by the cytoskeleton. *What is the cytoskeleton?*

- **The cytoskeleton** is a **dynamic** network of protein filaments or tubules extending throughout the cytoplasm that perform different functions.

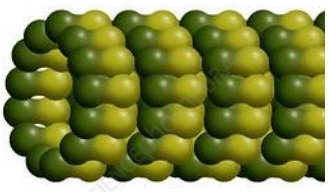
- **Functions of the cytoskeleton:**
  1- Provides support and a structural framework for cells.
  2- Creates pathways to regulate internal movement of organelles, vesicles and other structures such as mitotic chromosomes. *Note: we do not say movement of *molecules* because they do not move by themselves without the aid of certain transporters and vesicles.
  3- Determines cell shape and movement.
  4- Determines overall organization of cytoplasm.
  5- Determines positions of organelles (organelles are not just floating in aqueous solution, they are fixed and held in certain positions).

  As a result, we notice that organelles are organized as follows:
  - The ER is located near the nucleus and is followed by Golgi apparatus and vesicles.
  - The plasma membrane surrounds the cell.
  - The mitochondria is located in specific places and does not – *for example*- go near the nucleus.

*Sometimes organelles may need to be translocated in response to a signal or stress>>> they do not move randomly, but along the pathways of the cytoskeleton.

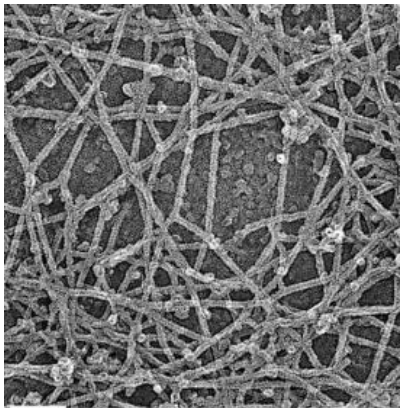
- **The cytoskeleton is a network of protein filaments which are 3 types depending on their composing proteins and properties:**

Actin microfilaments | Microtubules | Intermediate filaments


- **Actin filaments (microfilaments)**

  {Structure, functions and properties}



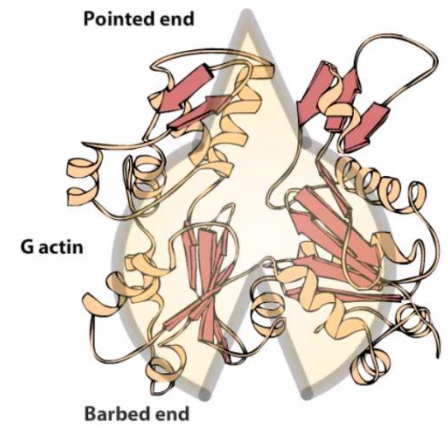
The photo shows a meshwork of actin filaments

  - They are thin, flexible fibers (*7nm* diameter and several µm length).
  - They are polymers composed of monomers. These polymers are then 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:
    1- Provides mechanical support.
    2- Determines cell shape.
    3- Allows cell movement, thereby enabling a cell to divide, engulf particles and migrate; e.g. by pseudopodium.

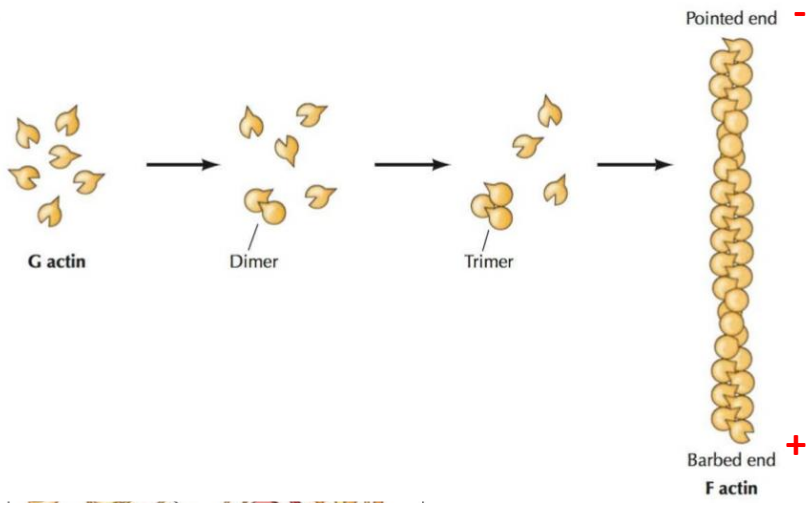
- **The actin protein.**
  - There are different types of actin encoded by different actin genes. In mammalians there are at least **6** distinct actin genes expressed in different locations in the body:
    - **Four** are expressed in different types of muscles.
    - **Two** are expressed in non-muscle cells.

  - Actin filaments are composed of monomers called "**globular [G] actin**" which polymerize to form filaments called "**filamentous [F] actin**".

  - **"G actin"** is a globular protein made up of amino acids. Its shape is not symmetrically globular but instead it has some polarity *(it has 2 different ends -pointed and barbed ends- as shown in the picture).*



- An actin monomer **(Globular [G] actin)** is tightly bound to two other actin monomers having a head-to-tail interactions. The dimers and trimers that form grow by the addition of monomers to both ends forming a polymer.



- Polarity of the monomer affects <u>actin polymer assembly</u> since the resulting polymer will be polar too. It also affects <u>the direction of myosin movement relative to actin</u>. (*as will be discussed later in the chapter*)
  The two distinct ends are:
  1- Pointed end also called negative or minus end which is where the formation of the polymer started
  2- Barbed end also called positive or plus end. It is the growing side of the filament.

- **Formation of actin filaments** {in steps}

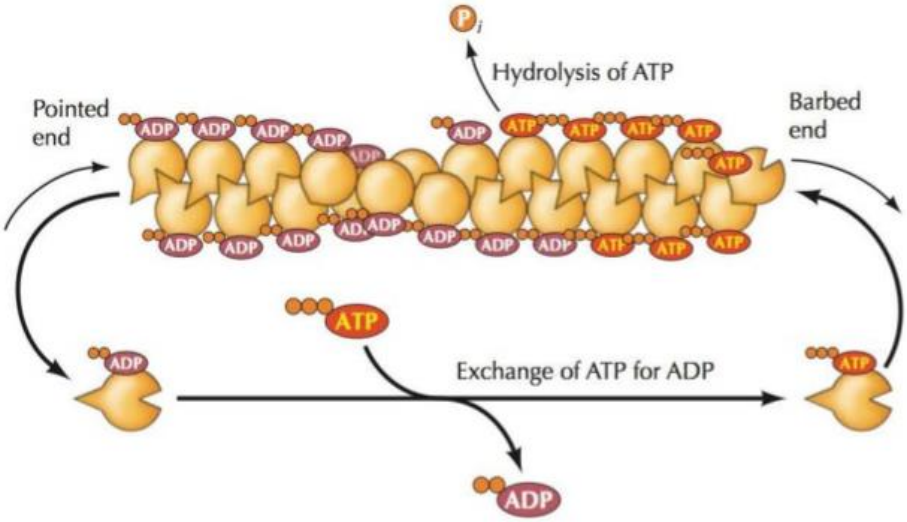
The process of actin filaments assembly is continuous especially when these filaments are performing a specific function, for example when a cell is moving.

Steps:

1- **Nucleation**: formation of an aggregate of **3** actin monomers (trimer).
2- Filament growth by adding additional monomers to both ends (barbed and pointed), but faster at barbed ends.
3- The monomers added are bound to ATP, which is not required for nucleation and is also hydrolyzed to ADP upon assembly. Despite this, ATP has some benefits in this process:
   a) Speeds polymerization
   b) Stabilizes binding.
4- Since ATP is hydrolyzed as soon as the monomer is added, we realize that the pointed end of the filament consists mostly of **ADP-actin** <u>not</u> **ATP-actin** and since the ADP-actin is <u>*less*</u> tightly bound to the polymer than ATP-actin, it's going to dissociate from the pointed end of the polymer.

5- The actin then exchanges the ADP for ATP (*not phosphorylation just exchanging*) and the ATP-actin monomer added to the barbed growing end of the polymer.

- The previous phenomenon is known as **treadmilling**, in which ATP-actin is added to the barbed end while ADP-actin dissociates from the pointed end of a filament. This results in the movement of the actin filament while it maintains a specific length **(dynamic behavior).**



- Actin filament depolymerization happens when all monomers become bound to ADP. >>starts from pointed end.

• Actin binding proteins

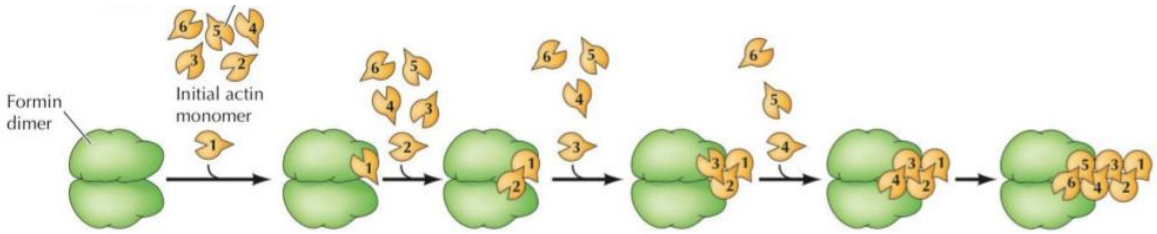
- Actin filaments cannot perform their functions alone, they need other proteins called **actin-binding proteins**. These proteins regulate actin assembly and disassembly as well as stability of actin cytoskeleton.
  - Some examples of actin-binding proteins and their functions:

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

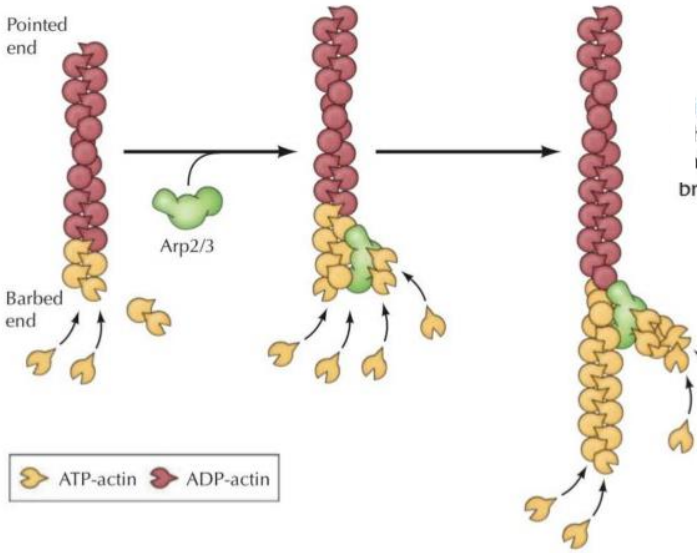
Only the proteins that are surrounded by red rectangle are required.

Notes on the information the table:

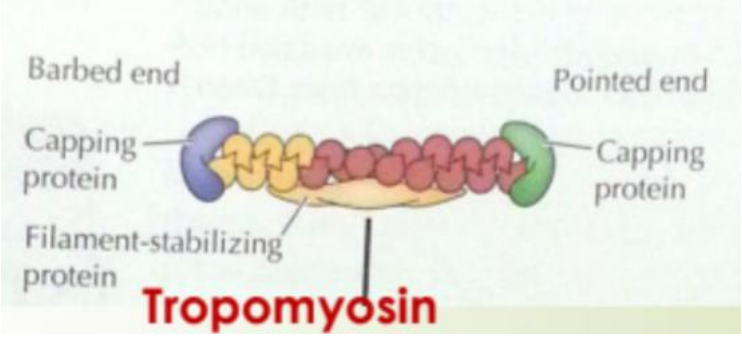
- Filament initiation: the rate-limiting step of actin formation is **nucleation** which requires the correct alignment of the first three actin monomers. *In other words, nucleation is the step that determines the rate at which the actin polymers are synthesized*. Consequently, this step must be facilitated by a specific protein (e.g. **formin**) which initiates the assembly of the filament by forming the required trimer.



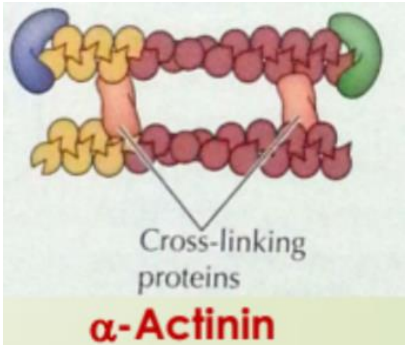
- The protein **Arp2/3** is especially used for branching resulting in a meshwork of actin by providing an initiation point for the formation of a branch. The original filament continues growing too.



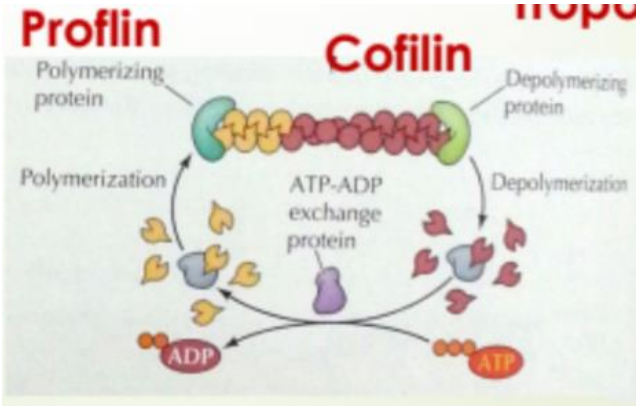
- **Filament stabilization**: making the filament stronger and more rigid by covering it with a protein like **tropomyosin** *-a longitudinal protein-* which makes the filament inaccessible to destabilizing and depolymerizing agents.



- **Filament cross-linking**: when actin filaments are organized in bundles (*parallel lines*), they can be strengthened by linking them cross-like by certain types of interactions that differ from interactions between monomers in a single filament. Strength is needed because actin filaments are used in support and providing cell-shape.



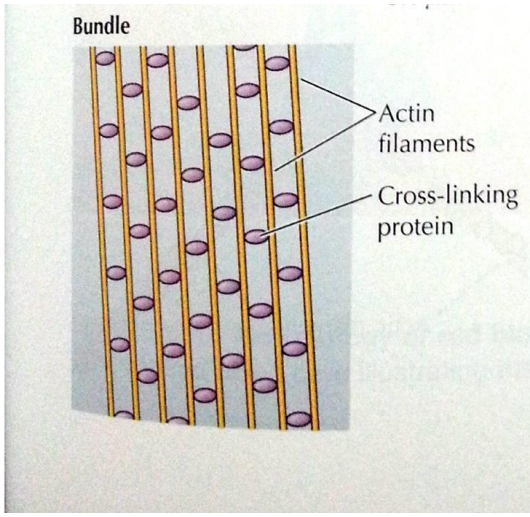
- ***Proflin***: monomer binding (polymerization)
  Cofilin: depolymerization.



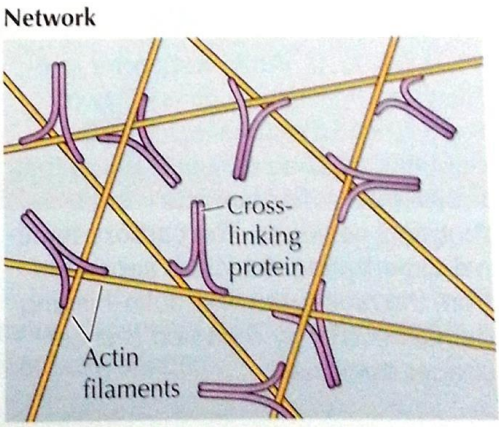
- **Organization of actin filaments**

  Individual actin filaments are assembled into two general types of structure called:

1-Actin bundles



2- Actin networks



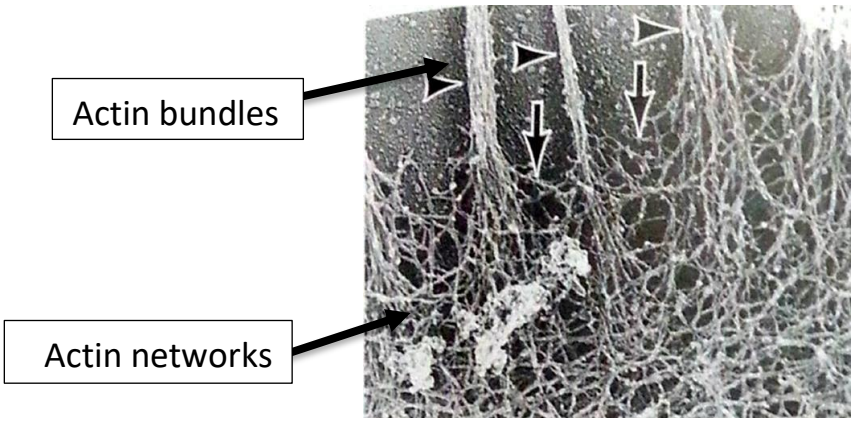
- Actin bundles and networks differ in many aspects as shown in the following table:

Side of comparison	Actin bundles	Actin networks
Structure	Actin filaments are cross-linked into closely packed *parallel* arrays.	Actin filaments are cross-linked in *orthogonal* (perpendicular) arrays.
Types of the proteins that cross-link the actin filaments	Small, rigid proteins that force the filament to align with one another. Such as: fimbrin, alpha-actinin.	Large, flexible proteins that can cross-link perpendicular filaments. Such as: filamin*.
Distance between the adjacent filaments (spaces between the actin filaments are determined by the size and shape of the cross-linking proteins)	Smaller distance. So that the number and chances of non-covalent interactions are ***larger***, making the filaments more rigid.	Larger distance. Number and chances for non-covalent interactions are **smaller**, thus making the filaments more flexible.
Flexibility	Rigid	Flexible (loose)

- **Function of actin networks**:

  Underlie the plasma membrane and support the surface of the cell.

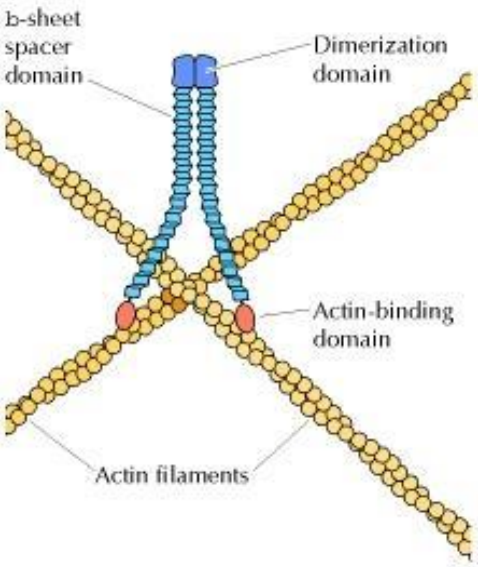
- Different types of actin filaments under electron microscope:



***Filamin structure:**

- The actin filaments in networks are held together by large actin-binding proteins, such as **filamin**, which binds actin as a flexible dimer (dimer= 2 subunits).

1. The **actin-binding** domains (ABD) and **dimerization** domains are at opposite ends of each subunit, so the filamin dimer is a flexible V-shaped molecule with actin-binding domain at the ends of each arm.
2. The *carboxyl-terminal* dimerization domain is separated from the *amino-terminal* actin-binding domain (ABD)by repeated **beta-sheet** spacer domains.

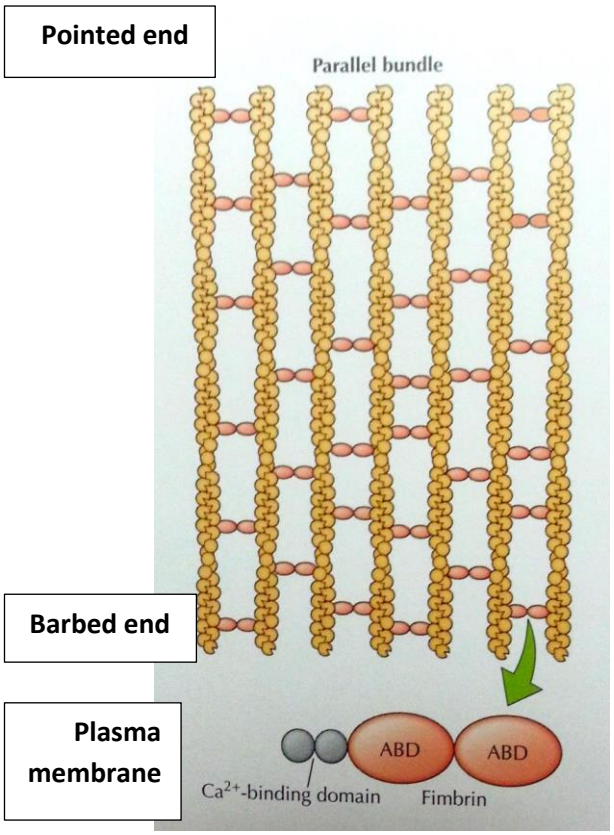


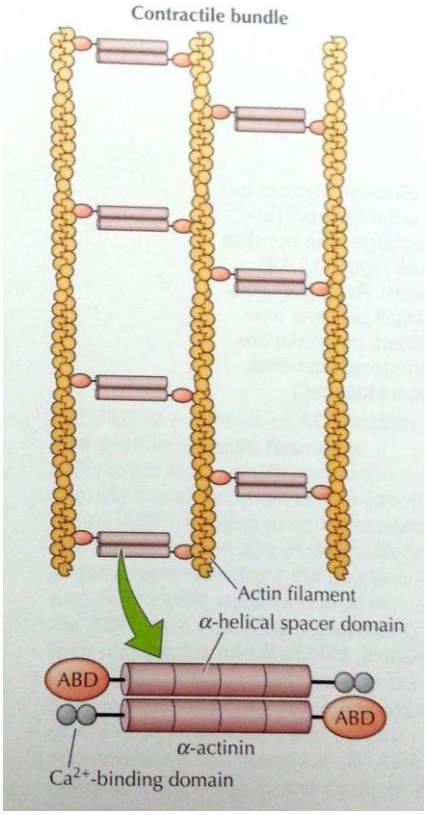
- Filamin forms cross-links between orthogonal actin filaments, creating a loose three-dimensional meshwork.

- **Actin bundles have two structurally and functionally distinct types depending on the location in the body**

Side of comparison	Parallel bundles	Contractile bundles
Places	Actin bundles in intestinal microvilli	Actin bundles in muscles
Structure	Closely spaced actin filaments aligned in a parallel way.	Filaments are more widely spaced, allowing the bundle to contract. *The wide spacing between filaments (open structure) allows the motor protein myosin to interact with the actin filaments of these bundles, which enables the bundles to contract.
Bundling protein	Fimbrin *Type of interaction*: Actin-monomer	Alpha-actinin *Type of interaction*: Actin-dimer interaction.
Flexibility	More rigid	Less rigid (more flexible)

- Note:
  Flexibility of the types of actin bundles depends on the spaces between the adjacent filaments and these spaces depend on the size of the bundling protein.
  (If the bundling protein is larger, the spaces between the adjacent filaments will be larger and therefore the filaments will be more flexible).





Structure of **fimbrin**:

1- 2 adjacent actin-binding domain.

2- Ca^{2+}-binding domain.

Structure of **alpha-actinin**:

Consists of two subunits each with:

1- Single actin-binding domain.

2- Ca^{2+}-binding domain

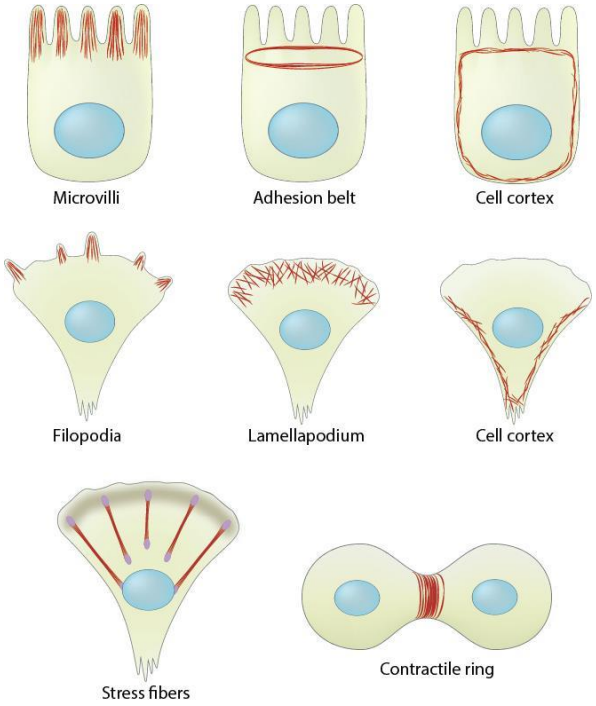
- **Location of actin fibers within cells:**

1- **Microvilli** (projections that increase surface area *e.g. in intestines*).

2- **Adhesion belts** which are responsible for adhesion between cells, especially in epithelial tissue cells.

3- **Cell cortex** which are actin filaments underneath the plasma membrane that support its shape (all cells have cell cortex)

4- **Lamellipodia and filopodia** which are used in cell movement (lamellipodia are larger in structure and filopodia are projections from lamellipodium)



5- **Stress fibers**: cross-linked actin filaments that appear in different cells under stress conditions. Example: In normal situations, certain cells in the human eye regulate eye pressure (**flexible** therefore able to feel high pressure). However, eye cells in patients with glaucoma disease contain a lot of stress fibers making the cells more rigid and not having the maximum effectiveness in feeling the eye's high pressure. (*The increased pressure, called intraocular pressure, can damage the optic nerve, which transmits images to your brain. If the damage continues, glaucoma can lead to permanent vision loss. Without treatment, glaucoma can cause total permanent blindness within a few years.*)

6- **Contractile ring**: strong structure that is needed in cell division (to divide the plasma membrane).

- **Actin filaments and 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.

- **Red blood cells have proven particularly useful for studies of both the plasma membrane and the cortical cytoskeleton because of the following reasons:**
  1- They do *not* have other cytoskeletal structures.
  2- They do *not* have nucleus or organelles. So, their plasma membrane and associated proteins can be easily isolated without contamination by the various internal membranes of organelles that are abundant in other cell types.
  3- The cytoskeleton is uniform. RBC do *not* have specialized regions as in other cells. In other words, the entire actin portion in RBC is part of cortical cytoskeleton and no other structures like filopodia.

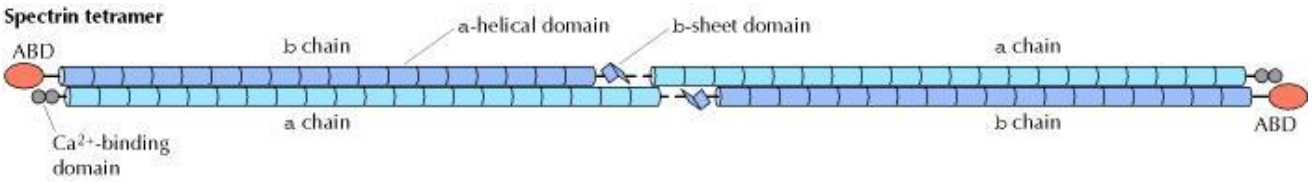
- **Actin filaments-plasma membrane Interactions**

Spectrin as a structural component of cortical cytoskeleton

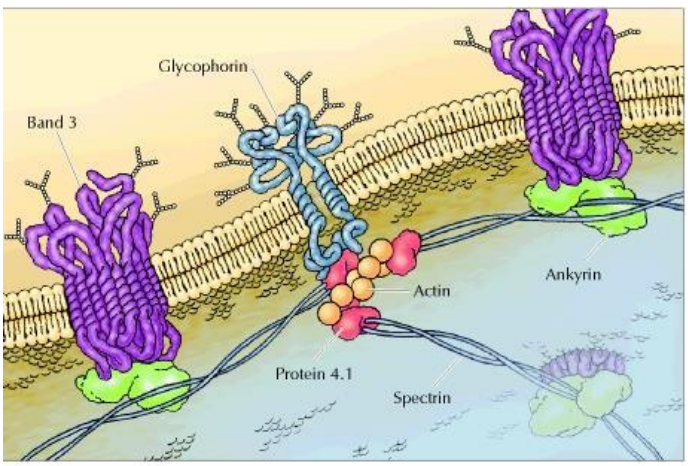
Spectrin is a major protein (actin binding protein) that provides the structural basis for the cortical cytoskeleton in erythrocytes (RBC).

- **Spectrin Structure:**

A **tetramer** of *two chains* (alpha and beta polypeptides) with the alpha chain having two Ca^{2+} binding domains at its C-terminus and the beta chain having the actin-binding domain at its amino terminus, both the alpha and beta chains contain multiple repeated regions of alpha-helical spacer domains, which separate the two actin-binding domains of the tetramer.



- The ends of the spectrin tetramers then associate with short actin filaments, resulting in the *spectrin-actin network* that forms the cortical cytoskeleton.
- <u>The spectrin-actin network is linked to the plasma membrane by</u>:
  1. **Ankyrin**, which binds to both *spectrin* and the abundant transmembrane protein *band 3*.
  2. **Protein 4.1** that binds to glycophorin (another abundant transmembrane protein).
  3. **Phospholipids**.



Videos recommended by Dr. Diala:

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

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

“It does not matter how slowly you go as long as you do not stop.”