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GENETICS & Molecular Biology

Number: 23

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Subject: **The cytoskeleton and cell movement (Actin microfilaments)**

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❖ The Cytoskeleton :

Is a dynamic network of protein complexes on which different types of molecules , organelles and vesicles can move . This network provide the shape of the cell , structural and mechanical support as well as facilitating movement of a cell from one place to another (example : the leucocyte movement from blood vessel to site of injury is mediated by cytoskeleton)

- There are three major types of protein filaments :

I. **Microtubules**
II. **Intermediate**
III. **Actin(microfilaments)**

- ☞ Let's start with actin filament :
  - How does it look under the microscope? As network of fibers interacting and intersecting with each other.
  - This meshwork (actin) provides mechanical support , cell shape and allow cell movement (not organelle movement that is regulated by microtubules)
  - These filament are polymers composed of monomers of globular actin (G actin)
  - G actin molecules form dimers then trimmers then polymerized into a larger structure of actin filament which is filamentous actin (F actin)
  - The actin molecule is a polar molecule (polar means here that the molecule has two side , head and tail) .So when these molecules form a dimer , the tail of the first one interacts with the head of the second and so on to form F actin
  - Because of the polarity of the monomers we will expect polarity of the filaments, one side is the growing side or barbed end (+ end) and the other side is the pointed end or (- end)
- ✦ Polymerization and depolymerization occur on both ends but with different rate. polymerization is faster in growing end (+) and depolymerization is faster in the pointed end (-) , so we see growth in the (+) end and reduction in size in the (-) end , and that is important to push the membrane by polymerization on one side and depolymerization from the other side forming a podia.
- ☞ Now, how does the polymerization of actin happen?
  - Firstly, the filament can distinguish the time for assembly or disassembly by binding of ATP or ADP respectively that leads to conformational changes.
  - G actin molecule is found in two forms :

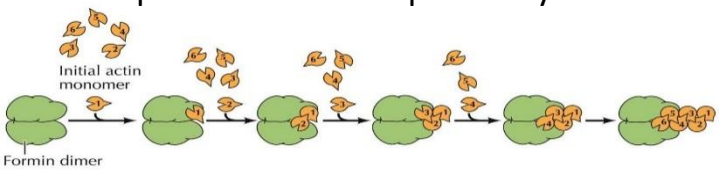
    1) Bounded to ATP which is going to fit well inside the 3D structure and provide a very stable F actin (+ end).

    2) Bounded to ADP which less interacts and accelerates depolymerization of F actin (- end) {more actin-ADP at –end, more actin-ATP at +end}

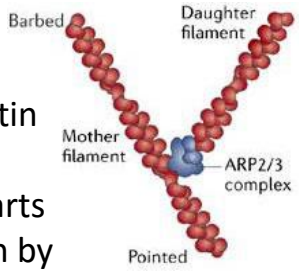
- To perform the function of providing shape we need to form collection of these filaments, and to provide more mechanical strength we need to connect these filaments to each other. This is mediated by actin binding proteins that play a role in assembly and disassembly too.

☞ Look at the table in the slide (that shows function of these proteins) ,as an example:

ᘓ ***Formin***, as its name indicates, initiates nucleation of G actin and formation of the filament (polymerization of F actin from G actin).These G actin molecules need a protein to carry them and put them in close proximity to each other so they interact and start forming the filaments.

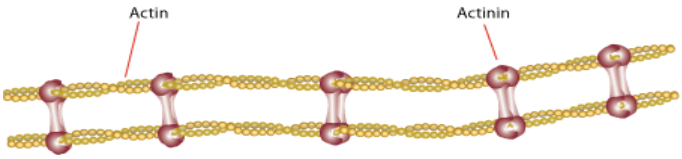


ᘓ ***Arp2/3*** ,which is a connecter at branching point , has a matching shape with G actin (as lock and key) , it is going to bind to G actin from one side , and the other side also matches G actin ,so Arp2/3 brings in another G actin , and starts buildup a new branches of this filament Stabilization by the action of tropomyosin which binds along the filament.



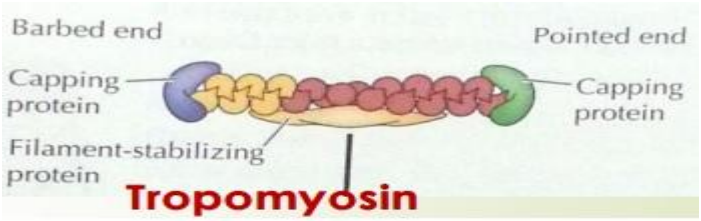
Actinin

ᘓ ***Alpha actinin***, crosslinks different filaments with each other to provide more mechanical strength.



ᘓ Forming ***end caps*** to stabilize the structure and prevent its degradation.

ᘓ ***Tropomyosin,*** is long molecule binds on the side of filament so it will stabilize the structure and prevent it from degradation.



ᘓ ***Cofilin***, activates binding to ADP and that will accelerate depolymerization of actin. (mostly at – end)

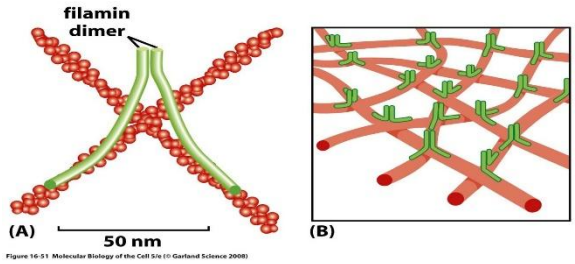
ᘓ ***Profilin***, activates polymerization by activating the binding of ATP to G actin (Accelerate and enhance monomers binding).(mostly at + end)

ᘓ Linkage to other proteins in cytosol or other proteins in plasma membrane (and these proteins connected to ECM) or to other proteins in other cells surfaces to give more strength and rigidity.

❖ Now, how these filaments are organized relative to each other?

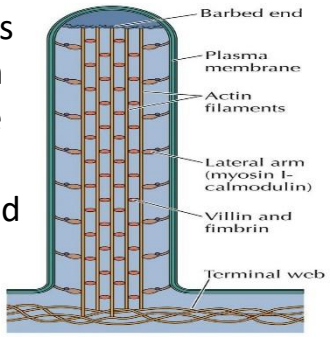
There are two ways:

1) ***Parallel structure*** (as the picture of alpha actinin) to form actin bundle, the bundle is rigid and its filaments connected by cross linkage protein such as alpha actinin to provide more rigidity.
2) ***network structure*** , several filaments that intersect at certain points to form A meshwork like structure, and there are also actin binding proteins in the intersecting points. The network is flexible.



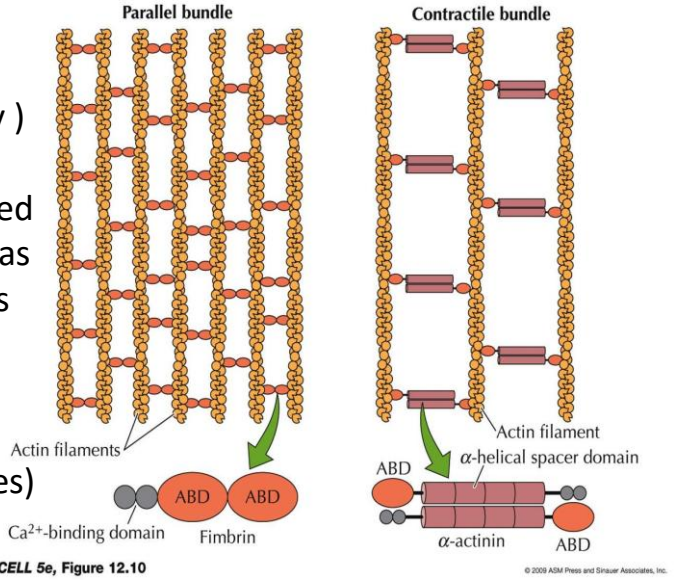
✸ Why the bundle is rigid but the network is flexible? because there are much more spaces in the network , so I expect to have much loose structure , in addition to that , the actin binding proteins of network (filamin dimer) is much larger and they have two arms can move more and provide more flexibility .

✦ The bundles themselves are also organized in different ways depend on different types of cross-linkers which depend on the cell or tissue that these bundles present in, for example inside intestinal microvilli they have to be rigid in structure so the filaments are organized in parallel way and connected by fimbrin cross linker, fimbrin is composed of two actin binding domains as well as calcium binding domains, that helps in contraction and movement of these projections.

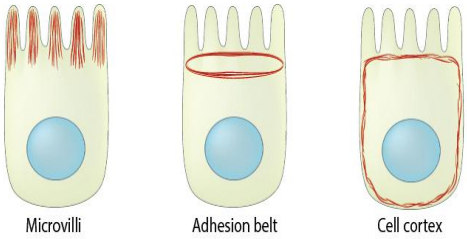


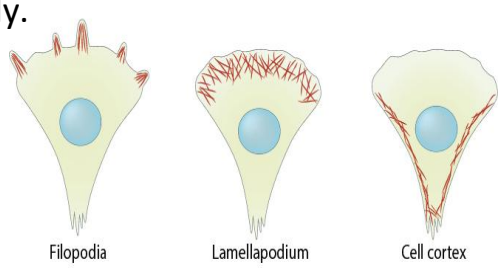
✦ Another example, inside the muscle cells there are larger spaces within the bundle, this structure is little more flexible than the one inside the intestinal microvilli, why are the spaces larger? To accommodate the function (which requires strength with some flexibility) and the cross linker protein which is alpha actinin , alpha actinin is composed of two domain ; actin binding domain as well as calcium binding domain ,so this molecule is much larger makes the spaces between the filaments larger .

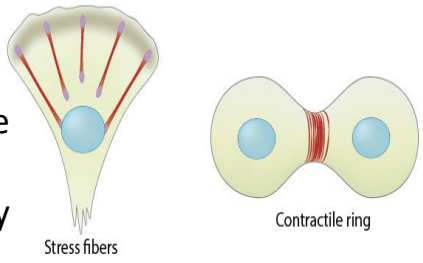
(left: as in microvilli, right: as in muscles)



☞ Now, where do we find these structures?







1) the <u>brush border</u> (microvilli)
2) <u>Adhesion belt</u>, is a way to connect the cells together, it surrounds the circumflex of the cell and is going to interact with adhesion belt of other cells that is important in epithelium which acts as a barrier so the cells hold together firmly.
3) <u>Cell cortex (or cortical cytoskeleton)</u>, is a meshwork of actin filaments underneath the plasma membrane to support the membrane and contribute to the cell shape.
4) <u>Filopodia and lamellipodium</u>, they help in cellular movement and phagocytosis.
5) <u>stress fibers</u> , are collection of actin filaments contribute to rigidity and structure of the cell by supporting cytosol ,(in some diseases there are activation of stress fibers formation , this provide more rigidity , and some cells need some flexibility such as muscle cells , example of these diseases is a problem in *trabecular meshwork cells* inside the eye : when there a high pressure inside the eye , the cells start to contract to provide more space to the aqueous humor to go out of the eye decreasing the pressure , but if we have these cells rigid then they wouldn't contract and wouldn't provide spaces to the fluid to exit ,so the pressure is going to buildup) .
6) <u>contractile ring during cell division</u>

☞ Let's talk about some of them in details :

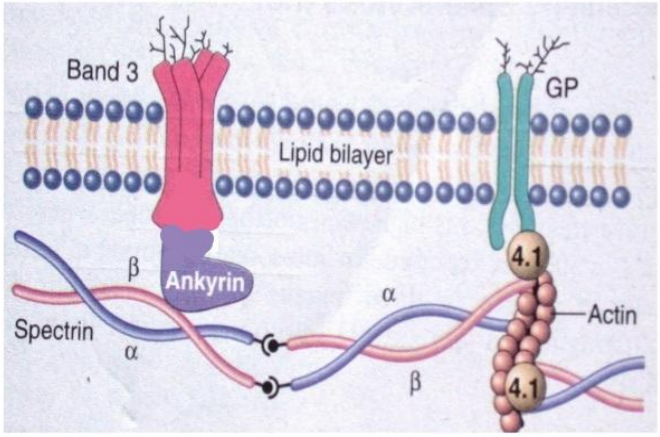
ꕥ ***cortical cytoskeleton*** : as we said is a collection of fibers beneath the membrane to support it and provide the shape of the cell , and have to be connected to the membrane proteins to provide more stability and also interact with other molecules to connect them together and an example of this is spectrin molecules.

- Why did scientists choose ***<u>RBCs</u>*** to study the cortical cytoskeleton?

1) Many organelles are not found in RBCs.
2) It is the only cytoskeleton type found in RBCs so they conclude that the shape is contributed or mediated by it.

✦ Spectrin is composed of two alpha subunits and two beta subunits. Alpha chain has a calcium binding domain, and the beta chain has an actin binding domain which binds to the filament.

☞ Let's see the interaction of spectrin to actin ,membrane proteins and phospholipids :



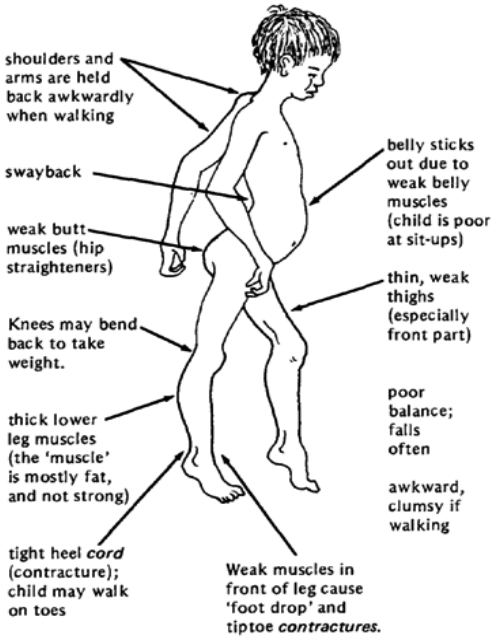
1) Actin will be anchored and connected to plasma membrane proteins via spectrin molecules that interact with one type of peripheral protein called ankyrin in one side and with actin in the other side. Ankyrin is connected to a transmembrane protein called band 3. So by this way we connect actin through spectrin to a plasma membrane protein via ankyrin.
2) Another way is through protein 4.1 which is going to interact with another transmembrane protein called glycophorin .
3) Third way is by interaction between spectrin and phospholipid

Note: within the muscle cells, the spectrin is not present and we have another protein instead of it called dystrophin. In platelets we have filamin. In fibroblast we have ERM protein. Both dystrophin and filamin are spectrin related while ERM proteins are protein 4.1 related.

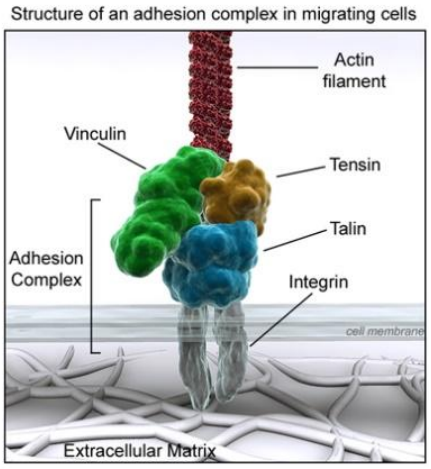
✸ Clinical application: muscular dystrophies.

- Mutated dystrophin protein will result in a type of muscular dystrophy, the most severe form is Duchene, the moderate form is Becker, and that depend on the type of mutation which is a large deletion in Becker, but in the case of Duchene there are both deletion and frameshift leading to a more severe situation.

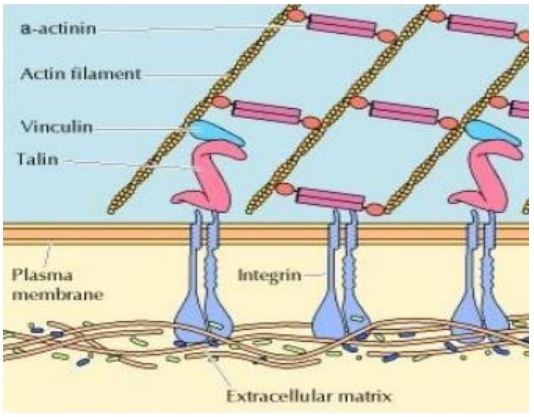
☞ Notice in the figure that the abdomen is protruded and the extremities are bent. Patients die early mainly due to weak muscles as the heart and diaphragm.



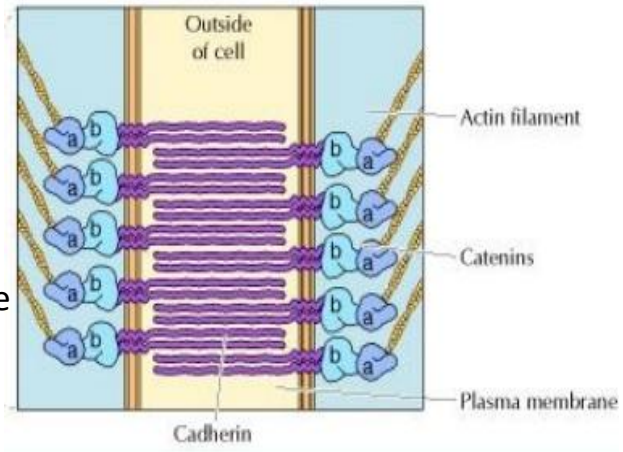
- Now, how do actin filaments contribute to different types of junctions between cells?
- ***Focal adhesion***, as its name indicates, is localized in specific region rather than surrounding the circumflex of the cell as the adhesion belt which is stronger than the focal.
- How do the fibers contribute to focal adhesion?
- The focal adhesion can link one cell to another or can stabilize this cell in its substratum or the surface where it is found in.
- in focal adhesion(at a specific region) , integrin will interact from one side to the fibers present in the ECM or with another integrin in another cell surface, from the other side (cytosolic side) it will interact with bundle of actin , this interaction is mediated by a focal adhesion proteins (talin and vinculin), talin and vinculin mediate this interaction by binding to actin from one side and to integrin from the other side so by this we will connect filaments inside with ECM outside.



- Also these focal adhesion proteins contribute to the presence of stress fibers which provide rigidity to the cell.



- there is another type of junctions which is a belt-like structure surrounding the cells , connecting them together much strongly than focal adhesion , is called ***adherens junction.***
- Adherens junctions are composed of a set of proteins that connect actin filaments of one cell to actin filaments of another , so actin filaments are connected by proteins (alpha and beta catenin) to cadherins (membrane proteins) , then cadherins will extend to intercellular space and interact with cadherins of adjacent cell .(note: this type of junctions still have some sort of permeability.)



✸ How does protrusion of a cellular surface happen?

ᘓ Starting with ***microvilli***, it is not for movement, they form brush border on the apical surface of the small intestine to increase the surface area for more efficient absorption. They are composed of actin bundle structure for integrity and support.

- There is a specialized form of this microvilli known as stereocilia, they are found in auditory cells, move with the sound waves and generate an action potential that can be analyzed as a voice (responsible for hearing by detecting sound vibrations).

☞ How the actin filaments are organized inside the microvilli? (look at page 3) As we said ,there are bundles and these bundles are arranged longitudinally inside the finger projection) , they are cross-linked and connected by actin binding proteins (specifically villin and fimbrin) **mainly villin and fimbrin to a lesser extent** , and they are anchored to a terminal web (which is part of cortical cytoskeleton) , and connected to the membrane of the projection in both sides by myosin 1 and calmodulin (myosin is a motor protein , it contributes to movement and contraction of the projection to adapt with the pressure on its surface)

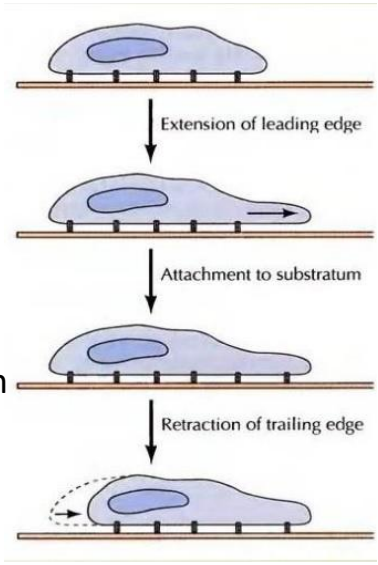
ᘓ Other types of protrusions are ***pseudopodia*** that in case of phagocytosis extend to engulf the material and foreign bodies, its width is intermediate between the width of lamellipodia and filopodia.

ᘓ The ***lamellipodia***: are very wide sheet-like structures (flat) that help in movement.

ᘓ ***Filopodia:*** are thin small extensions come out from lamellipodia.

☞ Now, how does cell migration occur?

1) the cell is going to polarize : define which side of the cell is going to move (leading edge) and which side is going to follow (trailing edge)
2) extend a protrusion from leading edge
3) establish connection by focal adhesion to the substratum
4) Detach trailing edge and retract it.



- ✸ How does this occur at the molecular level?
- ➢ Protrusion occurs by assembly of more actin filaments that start pushing the membrane to form leading edge . this becomes more efficient when there are more filaments to push the membrane that is why recruitment of arp2/3 that induces syntheses and branching will result in more protrusions and pushing of the membrane. In the other side depolymerization will occur and that will provide some G actin used in polymerization in (+) end
- ☞ Depolymerization is mediated by cofilin (by binding of G actin to ADP), then <u>ADP-actin monomers are carried to leading edge by ***twinfilin***</u> that transfer the complex to profilin then forming ATP-actin complexes.