University of Jordan
Faculty of Medicine
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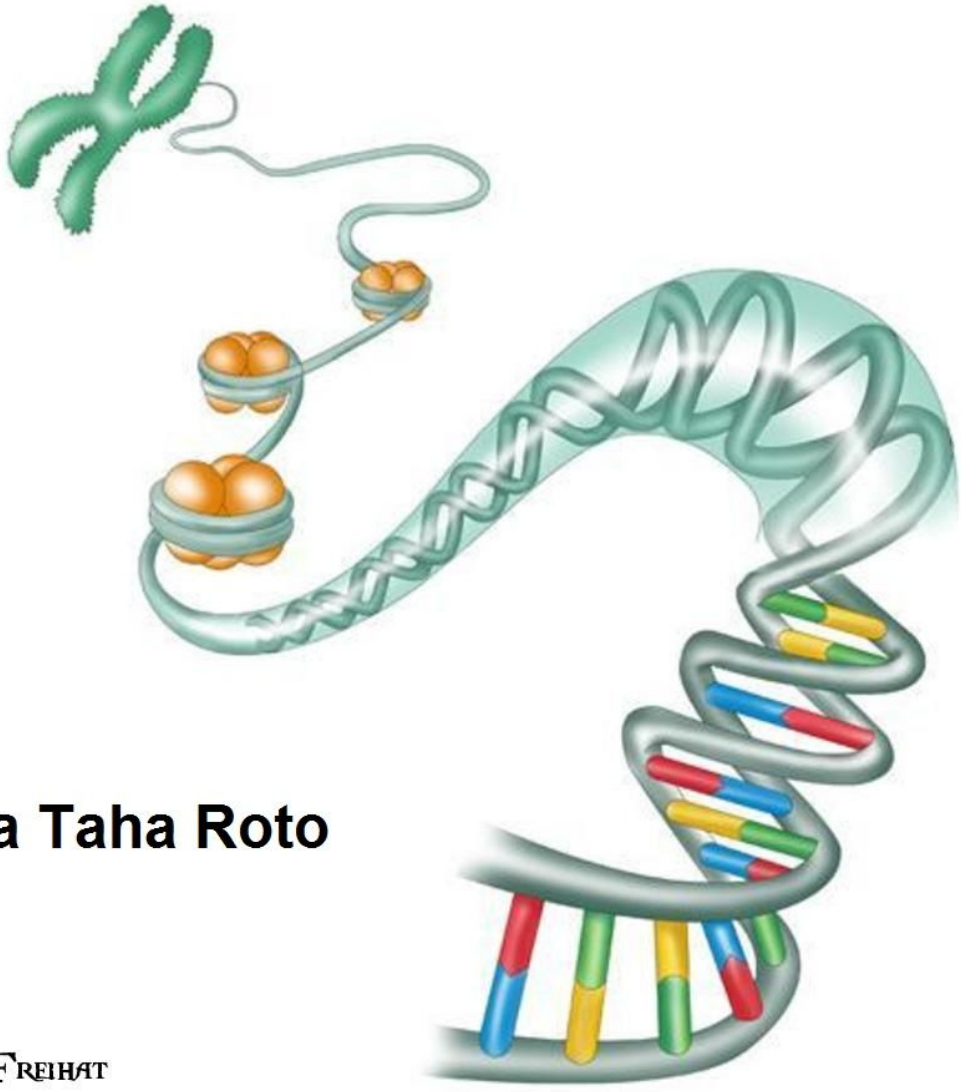
Medical Committee
The University of Jordan

Genetics & Molecular Biology

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



Lecture # 8
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RNA transport and The cytoskeleton

Recap:

We said that the steroid receptors' effect on DNA and gene expression is highly regulated. These receptors are found in the **cytosol** and bind to a **heat shock protein** which prevents their transport to the nucleus. Once the *small* and *lipophilic* steroid hormone diffuses through the plasma membrane and gets into the cytosol, it binds to these receptors and the HSP (heat shock protein) is released. Then, two of these receptors while they are bound to their ligand will form a **dimer** and enter the nucleus through the nuclear pore complex system and their DNA binding domains will bind to specific sequence on the DNA and regulate its expression (either inhibition or activation).

The nuclear receptors or the steroid receptors have three domains :

1- A DNA binding domain 2- A ligand binding domain

2-An activation function domain (AF) that regulates transcription *by binding to other proteins (once they bind to the DNA) that can affect gene expression like activators or suppressors of gene expression.*

***An important concept** to understand is that these domains are **independent** on each other .This means that the DNA binding domain is not affected by the ligand binding domain or the AF domain. For example : if you put the DNA binding domain of *estrogen* with the ligand binding domain of the *androgen* , each domain will still be active, functional and able to bind to its specific ligand:
1- The LBD of androgen binds to androgen
2- The DBD of estrogen binds to its specific sequence on DNA.

****Note:** Yeast two-hybrid system slide is not required

Let's begin the new lecture:

RNA transport:

RNAs are synthesized in the nucleus and are exported out of the nucleus .As you know, there are different types of RNAs (tRNA, rRNA, snRNA, mRNA,), and so we have different mechanisms of transporting them to the cytoplasm.

Note 1: All types of RNA molecules are exported as *ribonucleoproteins* (RNPs). A ribonucleoprotein is a complex composed of RNA and proteins, so RNAs don't leave the nucleus alone, they need a friend, which is the protein.

Note 2: All RNAs (except mRNA) are exported by a mechanism that is Ran and Exportins DEPENDENT, similar to the mechanism of transporting proteins outside the nucleus.

For the exception mRNA, now this one is exported by a mechanism that is Ran and exportins INDEPENDENT. There is a set of proteins (about 20) that form a complex, and that complex binds to the mRNA once it is processed completely and transports it to the cytosol.

How do we make sure that mRNA doesn't go back to the nucleus?

The key point is a protein called *helicase* that is bound to the nuclear pore complex on its cytosolic side. It is waiting for the mRNA that is trying to go back again. This helicase separates the mRNA from the protein complex and thus prevents it from re-entering the nucleus.

So, helicase regulates the directionality of transport of mRNAs, which happens in a one way direction (to the outside).

snRNAs:

There is a subtype of RNAs known as small nuclear RNAs, and they function in *regulating RNA processing and splicing of the pre-mRNA* .So they are synthesized in the nucleus and work in it, but they can't function by themselves, they have to bind to certain proteins (i.e. their active form is the

ribonucleoprotein form which is called snRNP). In other words, the functional snRNA is the one that is bound to proteins that are synthesized in the cytoplasm, so we need to export this snRNA out of the nucleus to make it bind to its proteins and then import it back to the nucleus as snRNP (bond to proteins) that is functional .Therefor we need the help of exportins, importins and Ran in the transport mechanism.

So, snRNA transport is Ran, exportins and importins DEPENDENT.

To conclude; mechanisms of RNA transport are:

- Ran, exportin dependent (for tRNA, rRNA…)
- Ran, importin, exportin dependent (for snRNA)
- Ran independent (for mRNA)

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Internal organization of the nucleus:

-The Nucleus is not a homogenous distribution of chromatin. As the nucleus performs different functions, it has specific regions that are specialized in specific functions, like the concept of compartmentalization of the cytoplasm, but here the organelles are not membrane enclosed :P .

Examples: (Just know the names and the functions)

- Nucleolus: it forms a large portion of the nucleus and it is responsible for rRNA synthesis and processing.
- PML bodies: named PML as they were first identified in cancer cells of leukemia. They interact with chromatin and are a site of accumulation of proteins such as transcription factors, chromatin-modifying proteins, and DNA repair enzymes. So they are like storage place for certain molecules that can be released to other parts of the nucleus.
- Cajal bodies: site for snRNP assembly. Note that we don't mean the assembly of the snRNA with its proteins to form the snRNP which occur in the cytoplasm, but we mean the assembly of the snRNPs with the mRNA.
- Nuclear speckles: RNA splicing.

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The Cytoskeleton and cell movement

It is a Dynamic network of proteins that provides the structural framework of the cell.

There are three **types** of these proteins:

- Microtubules (made from tubulin protein)
- Intermediate filaments (made mainly from keratin)
- Microfilaments (made from Actin monomers)

Functions:

- Structural framework of cells
- Determines cell shape :
  So because of the cytoskeleton, there are cells that are tubular, cuboidal, squamous or spherical.
- cell movement :
  For example when macrophages move through endothelial cells to the site of inflammation, they squeeze themselves with the help of the cytoskeleton, or when a macrophage wants to engulf bacteria, it extends pseudopodia using the cytoskeletal filaments)
- Determines positions of organelles: so you have Golgi located to the periphery and the ER is close to the Nucleus. Moreover, in the epithelial cells of the intestine, cytoskeletal proteins cause the nucleus to be located at the basolateral aspect of the cell. When these cells become cancerous, the nucleus goes to the apex for some reason, probably due to disturbance of the cytoskeletal network.
- Determines overall organization of cytoplasm
- Regulates internal movement of organelles :
  Vesicles that move from ER to Golgi, endosomes and lysosomes don't swim in the cytoplasm; rather, they attach themselves to the microtubules and move along them to reach their destinations.

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Actin filaments or actin cytoskeleton:

They are the microfilaments. They are thin and flexible.

They are made from actin monomers which polymerize to form actin filaments , and these filaments can further form different higher ordered structures; they can form either **Actin Bundles** (which have linear structure), or **3D Networks**.

They are flexible and not rigid, so they form a semisolid gel and give the cell it shape and flexibility in its shape.

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:

There are 6 genes that code for actin protein:

1-Four are expressed in different types of muscle

2-Two are expressed in non-muscle cells.

Let's return back to the idea of homology:

There is a homology between yeast's actin and mammalian actin (90% of the amino acid sequence is identical); this homology tells you something about the function of that protein.

Good point:

Whenever you have a protein that is highly conserved, meaning that its amino acid composition is very similar in many different species, with the presence of some small differences that indicates that this protein is really important.

Actin filament structure:

Actin monomers are globular proteins, so the *monomer* is called G Actin.

These monomers polymerize to form an actin microfilament, so we call the polymer of G Actin, which is the filament (F Actin) or (filamentous Actin).

So the G Actin is the monomer & the F Actin is the polymer.

This filamentous actin has a distinct polarity, meaning that its two ends are different from each other; one of them is called the minus end (or the pointed end), the other one is the plus end of the (barbed end).

Why do we call these ends **minus** and **plus?**

At the **plus** end there is **assembly** and addition of new actin monomers, so the filament gets longer at the plus end.

At the **minus** end there is **disassembly** of the G actin monomers, so the filament gets shorter here.

*** Formation of actin:**

The first step which is also the rate limiting step is **Nucleation**.

Nucleation is the combination of 3 actin monomers that form the nucleus on which the whole long filament will be synthesized, the addition of further G actin monomers is on that nucleus.

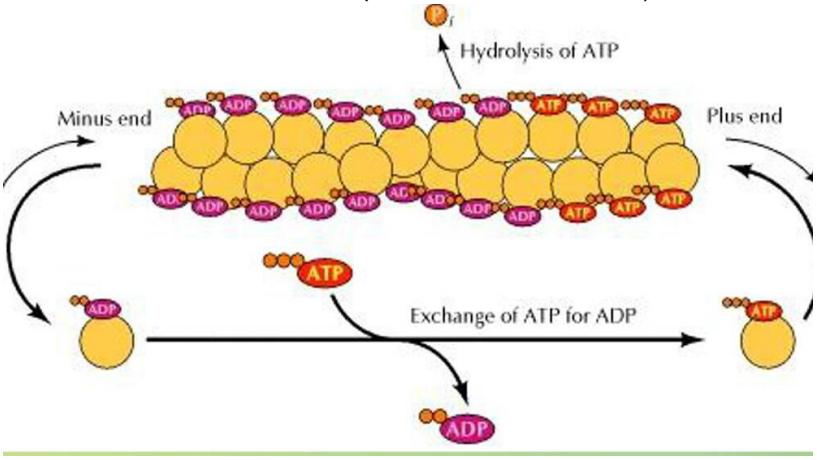
Note that:

- G actin that is added at the barbed end is bound to ATP.
- G actin that is removed from the pointed end is bound to ADP.

***Considering ATP role in F actin formation:**

By now you know that ATP is bound to the actin monomer that is added to the filament, but this doesn't mean that ATP is important or a determinant of the actin filament formation. **The whole idea is about stability**, meaning that actin is more stable when it is bound to ATP and less stable when ADP is bound to it. So, Actin-ATP is added to the plus end and the filament gets longer and longer. At the other end, ATP is hydrolyzed to ADP, so that end becomes less stable and the G Actin-ADP (Actin monomer) is released.

***Note:** the disassembly (dissociation) of G Actin-ADP from pointed end is called **Treadmilling.**



Actin-binding proteins:

Actin microfilaments are bound to proteins that regulate their assembly, disassembly, stabilization…etc.
We will talk about some of them, so you are required to memorize just the ones that we mention in details, which are the marked ones.
**Don't memorize them now, after finishing the sheet it will be easier ☺

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

Let's talk about some Examples:

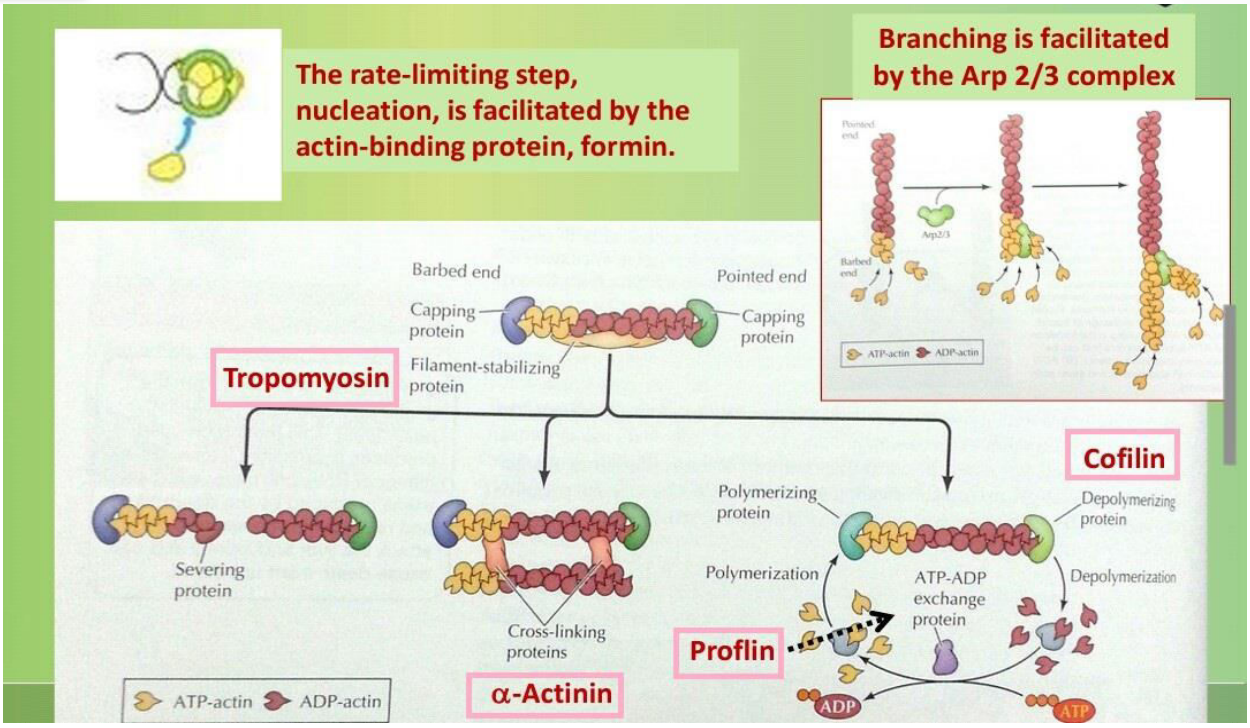
1-Formin: as the name implies, it is responsible for the formation of the nucleus of F-Actin, which is the main block on which other blocks are added to form the bigger structure.

2- α actinin: it crosses links different actin filaments to each other.

3-Tropomyosin: is the filament stabilizing protein.

Now: 4- cofilin and 5- profilin:

- Cofilin: it induces depolarization and disassembly of actin microfilament.
- Profilin has two jobs :
    1) It releases the actin monomer from cofilin, so the monomer is recycled and can be used again.
    2) It promotes the exchange of ADP that is bound to G Actin with ATP to regenerate an active actin monomer that can bind the filament with high stability.

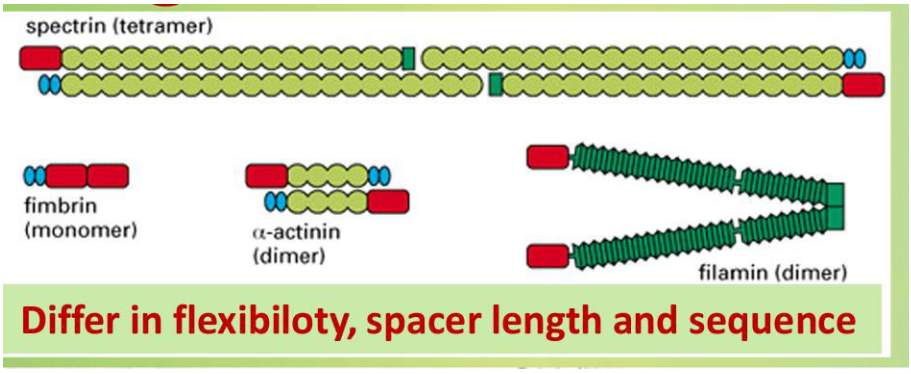


Organization of actin filaments:

There are 4 actin binding proteins that mediate the interaction of actin **microfilaments** to form higher structures (i.e. the bundles and the 3D network)

Notice the structures of these proteins:

- spectrin (the largest one and is a tetramer)
- α actinin (small dimer)
- filamin (large dimer)
- fimbrin (monomer)



These proteins differ in their function and the length of the spacer (the one colored in green)
All of them have a calcium binding domain, so they depend on calcium to interact with actin.

*As we said, actin filaments form **two types of higher structures**:

1- *Bundles*: these are straight filaments that are parallel to each other and crossed linked by proteins and these filaments are rigid.

2- *Network:* it is more flexible.

Bundles

Now, **we have two types of actin bundles:**

1-Bundles that are very close to each other and they are more rigid.

-These are called also the *parallel bundles* as they run in the same direction (the plus ends at one pole and the minus ends are at the other pole).

- The protein connecting them is the **fimbrin**, which is a **monomer**.

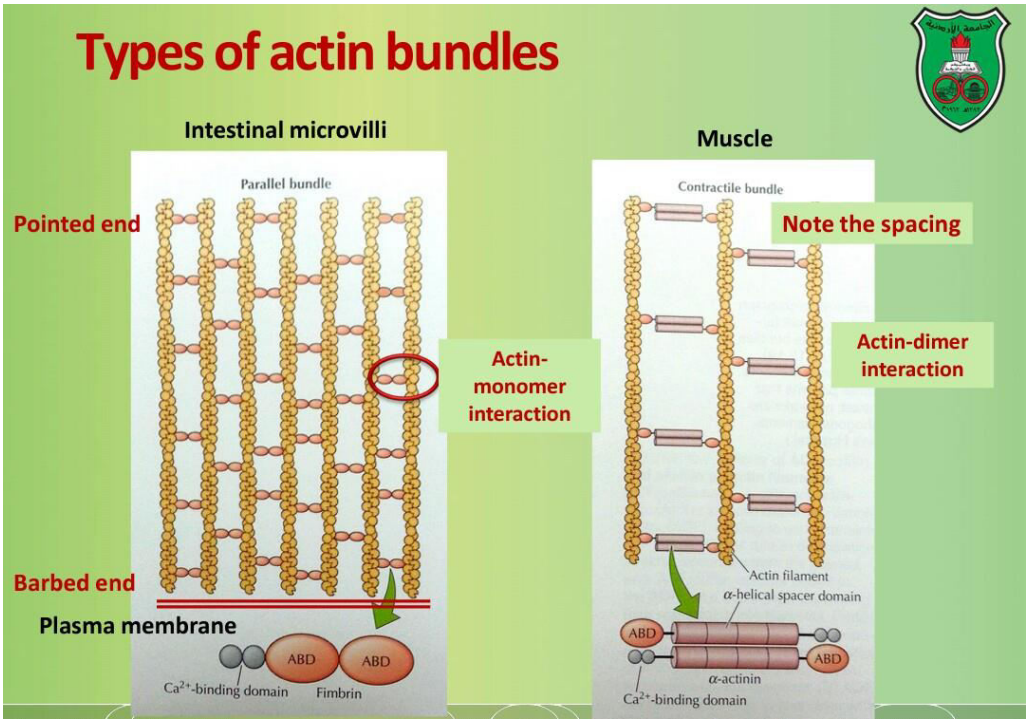
- One monomer is needed to crosslink the actin filaments, so we have actin-monomer interaction

2-Bundles that have a larger spaces between them and they are more flexible.

- These are found in muscle cells and known as *contractile bundles.*

- The protein connecting them is α actinin, which is larger than fimbrin so we have more space and more flexibility.

- α actinin must be a dimer to cross link the actin microfilaments, so in this case we have actin – dimer interaction.



Actin Network:

The crosslinking is by the protein filamin.
Filamin is a dimer and has a longer spacer, providing more flexibility.
These networks are usually found beneath the plasma membrane, making the membrane fluidic and flexible, changing its shape easily.
So again, if we look to the entire cell and try to figure out where the bundles and networks are located, we will see that the **networks** are located **all over the cytoplasm** and **beneath the plasma membrane**, but wherever there is an **extension**, it will be lined by the **actin bundles**, and the synthesis of these bundles is active in these extensions.

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Cell cortex:

-It is the **Network** (not bundles) of actin filaments and associated with actin-binding proteins.
- it was studies in RBC because:
1- **No other cytoskeletal structures**, meaning that the **only** cytoskeletal proteins found in RBCs are the **Actin filaments.** (it doesn't have microtubules or intermediate filaments)
2- No organelles, so this network will be free from contamination with other types of proteins.
3-The cytoskeleton is uniform with no specialized regions like in other cells, so there is no extension containing actin bundles (there is a pure network)

After analyzing the network, one of the major proteins that were found was spectrin.

Spectrin:

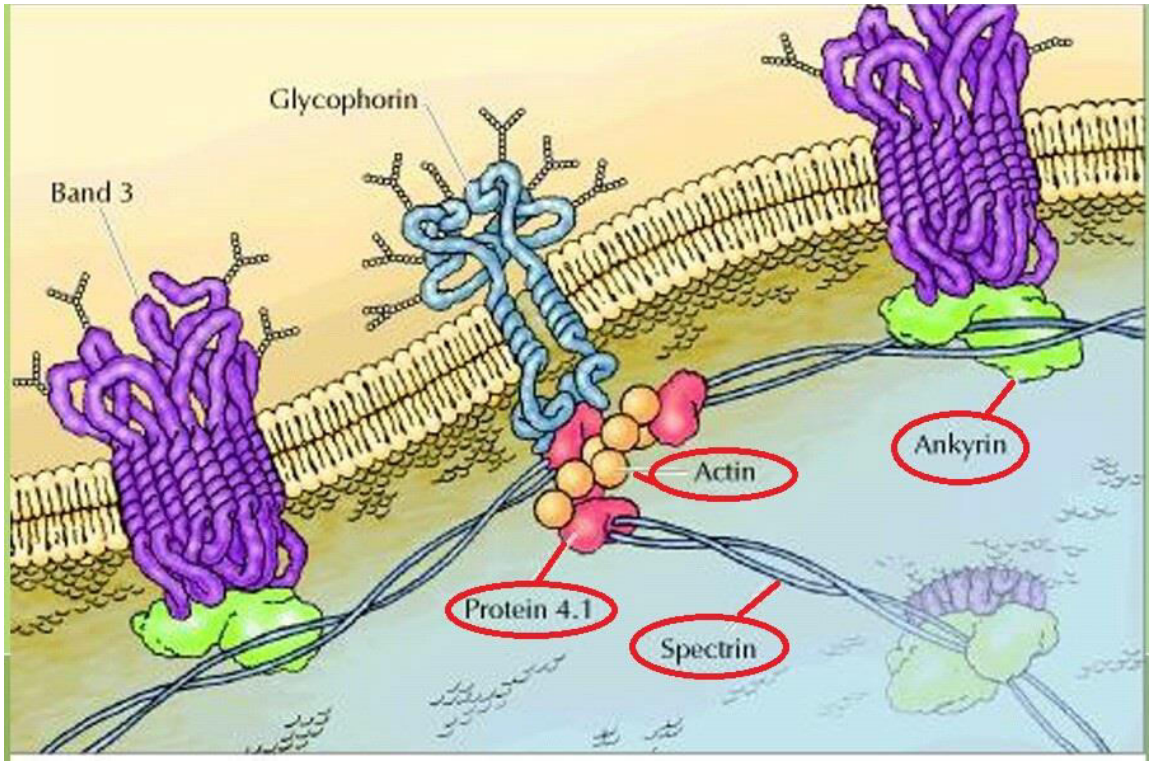
1- It is a very large protein and has two similar ends, with actin binding domain and calcium binding domain.
2- A tetramer of two α and β polypeptides with the α chain having two Ca2+ binding domains at its carboxy terminus and the β chain having the actin binding domain . the dr. said that it's a dimer which is the opposite of what's written in the slides and what we can see in the pic.

So, what is the function of this big spectrin?

- It binds to actin , forming spectrin-actin complex , then spectrin molecules bind to any of two proteins that link them to the membrane via the transmembrane proteins , these two proteins are :

1- **Ankyrin**, which links **spectrin** to the abundant transmembrane protein **band 3**.

2- **protein 4.1**, which binds to **glycophorin**.



By this organization, any change in the structure of transmembrane proteins due to signals or any other effector outside the cell can be sensed by the cytoskeleton due to this network and its proteins' interactions.

***This is for the erythrocyte, what about other cells, how do they connect the cytoskeleton with the plasma membrane?**

Different cells have different linkages, for example:

1- **Platelets** have **filamin** (spectrin related) that links the F actin to the plasma membrane.

2- **ERM** proteins (protein 4.1-related) link actin filaments to the plasma membranes of **different kinds of cells**.

3- **Dystrophin** (the most important one)**:**

It is a very large protein and it is **spectrin related**, and is found in many cells, especially **muscle cells**. This protein is able to interact with the actin filaments and the plasma membrane proteins as well, and the transmembrane proteins link the cytoskeleton to the extracellular matrix. *These connections maintain cell stability during muscle contraction.*

Dystrophin and muscular dystrophy: *important*

There are two forms of muscular dystrophy, both of them are due to mutations in dystrophin protein, differences are summaries in this table.

The disease	Becker's	Duchenne'
Mutation type	Large deletion but there is no frame shift; this mutation will not produce a totally non-functional protein as the change in the protein is minimal, that's why this form of disease is less sever.	As simple as single point mutation: Small deletion but causes Frame shift, resulting in a completely nonfunctional protein. (i.e. deletion of one base/nucleotide which results in changing the whole DNA sequence and so changing the whole protein structure and having a completely different protein that is not functional)
Severity	Less sever (it is moderate)	Very severe
Features	Progressive degeneration of skeletal muscles	There is a progressive degeneration of skeletal muscles, but here the patients usually die in their teens or early twenties
	Both are inherited and are X linked	

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Now we'll talk about some structures related to *actin bundles*, RBCs don't have these structures as they have no bundles.

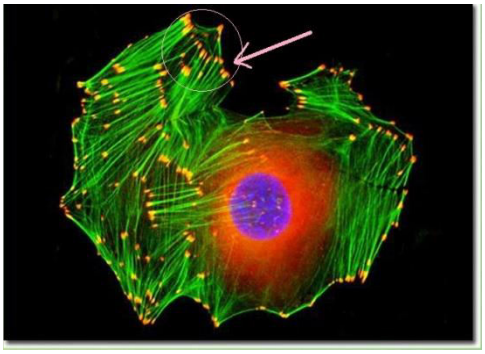
*focal adhesion
*adhesion junctions
*protrusions from cell surface.

Let's start

- **Focal adhesion:**

The actin filaments that form bundles interact with the cell surface (membrane) at certain regions , these regions are called **focal adhesions** as there is a focus of filaments and concentration of the actin binding proteins in that region.

If you look at the picture to the right, it is an immunofluorescence image of a cell. The lines in green are actin microfilaments, note that at the end of each filament, there is a focal point/adhesion (red in color) which has a focus of actin binding proteins.



Usually these focal adhesions are at the bottom Of the cell, so *they serve as the legs that the cell uses to attach itself to the substratum (the surface on which the cell sets) and they also connect the cytoskeleton to the ECM.*

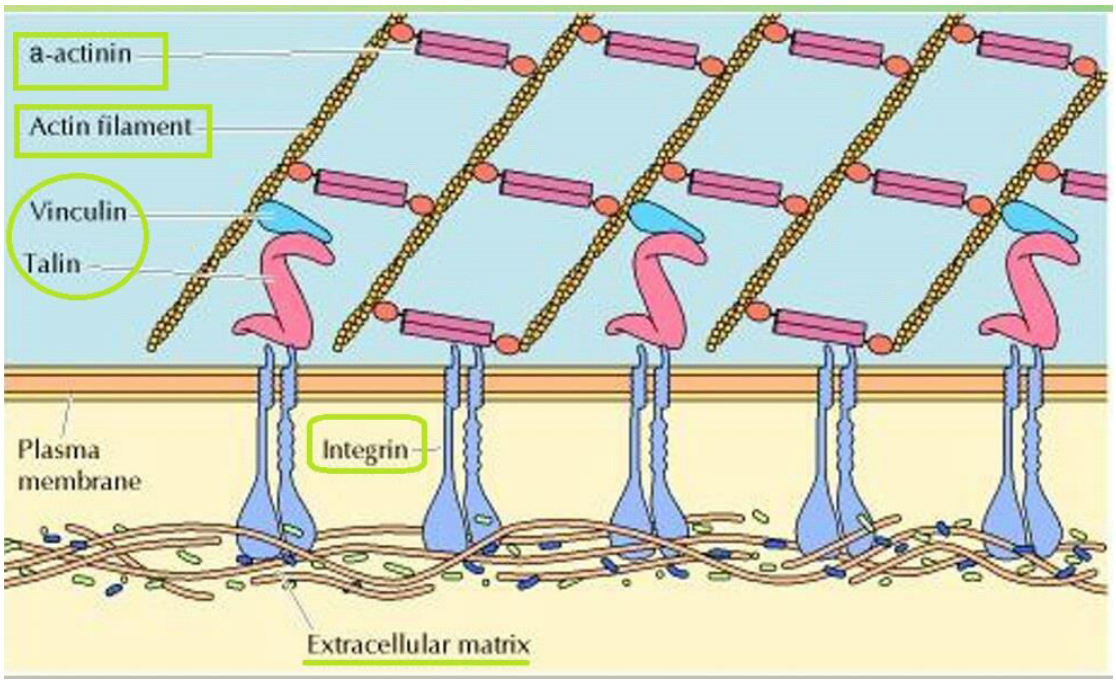
Substratum: the surface that a cell attaches to, especially when the cell is growing or moving

* These bundles are also called **stress fibers** as they exist when the cell is stressed and want to attach itself to the surface.

Now, if we look to the molecules found in the focal adhesion, notice the following:

- There are actin filaments that are cross linked by ***α actinin***.

- There is actin binding proteins (***vinculin*** and ***talin)*** that are bound to actin filaments, as well as to ***integrins*** which are transmembrane proteins (receptors) that are connected to the ECM proteins.



Any changes will be sensed by the integrin which can change the structure of actin microfilament as a result.

Adherens junctions:

They are regions where we have cell-cell adhesion, interaction and communication. This adhesion is mediated by actin microfilaments and actin binding proteins.

So if anything happens to a cell, it will be sensed by the adjacent cell via changes in the cytoskeletal actin filaments.

Proteins involved:

Actin bundles in the cytosol attach to *catenins*, **which** are cytosolic actin binding proteins, these catenins connect Actin bundles to receptors called *cadherin*s coming from the two adjacent cells, and these cadherins connect the cells.

Note:

Cadherins are lost in cancer cells.

To conclude: *actin bundles* and their binding proteins mediate:

- Cell- surface adhesion by focal adhesion.
- Cell- cell adhesion by adhesion junctions.

- **Protrusions of the cell surface:**

These are Extensions (as a finger or a leg) from cell surface that are filled with actin bundles, and these actin bundles are inserted into the actin network beneath the membrane. These extensions perform certain functions according the cell type, for example:

- Cell movement: the cell wants to extend a leg to walk and attach to the surface, the leg must be rigid and strong, that's why these legs have bundles rather than a network which is less rigid. Also , the leg must attach to the surface, so it needs focal adhesions, then it must detach, so it is a continuous process (involving assembly and disassembly of actin filaments)
- Phagocytosis :

For example, macrophages send extensions full of *actin bundles* to engulf bacteria.

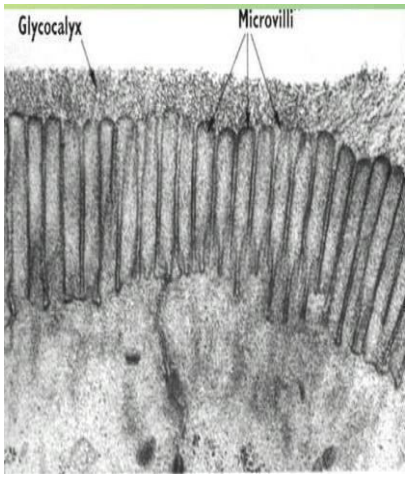
- Absorption.

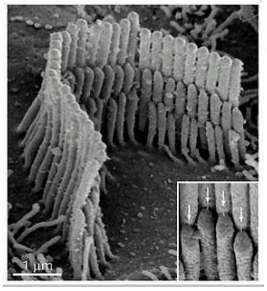
Now let's take some examples of these protrusions:

- **Microvilli**:

-Extensions from the plasma membrane of intestinal epithelia cells to increase the surface area of absorption and they are filled with the bundles and beneath it is the network. The microvilli are covered with glycocalyx.

- actin binding proteins connect the actin bundles to the network, and we have *calmodulin* and *myosin* which are regulatory proteins.





- **Stereocilia**: specialized forms of microvilli on the surface of auditory hair cells which are responsible for hearing by detecting sound vibrations.
- **Psuedopodia:** phagocytosis, and they are used by macrophages and neutrophils.
- **Lamellipodia**: broad, sheet-like networks of actin leading edge of moving fibroblast. For wide movement causing a change in the shape of the cell.
- **Filopodia:** thin projections extending from lamellipodia and they are thinner than microvilli and pseudopodia.

Cell migration:

How do cells move? The cell attaches to the substratum, and there is directionality in how actin filaments are formed. So the growth and elongation of actin microfilaments by adding new monomers and the branching of these filaments will push the plasma membrane forward. At the same time as the cell extends, there is a formation of focal adhesions that represents new attachment points with the substratum, and they contain vinculin and talin. Now at the same time, the other end of the cell must detach to be pulled to the new place and form new attachment, so there is disassembly of actin filaments at that end. By this mechanism we notice the *polarity* and *directionality* of cell movement. **Remember** that cell movement mechanism is a result of the dynamics of actin filaments.

Proteins involved in this process:

1-Arp2/3: it is important in nucleation and branching of actin filaments. (this is important)
2-profilin: in reassembly
3-cofilin: in disassembly

Now memorize this:

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

** the last slide is self-study ☹

I'm really sorry for this long and boring sheet, wish you all the best

يلا معلش ^_^ ، إن الله مع الصابرين وما تنسوني من صالح دعائكم

Written by: Alaa Taha Roto

إهداء إلى أختي وصديقتي بشرى ماجد المعاقبة