University of Jordan
Faculty of Medicine

GENETICS & Molecular Biology

Number: 22

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Subject: mitochondria, peroxisomes, and Cytoskeleton

Doctor: Diala Abu-Hassan

Price:

Date:

**** This sheet was written according to the record that belongs to pack 3. In addition, it was written according to the numbers of the slides in slide-6: "Mitochondria and peroxisomes" and slide-7: "The cytoskeleton and cell movement (actin filaments)".**

❖ Topics of this lecture:

Slide-6: "Mitochondria and peroxisomes".

*The last part about the mitochondria: mitochondrial phospholipids.

*Peroxisomes.

Slide-7: "The cytoskeleton and cell movement (actin filaments)"

*Cytoskeleton- Actin filaments.

❖ Mitochondria and Peroxisomes

For the first part of this sheet (pages 1-7): What has been written in italic was not mentioned by the doctor, but was mentioned in the slides.

Slide # 12: mitochondrial phospholipids

The mitochondrion is the energy factor of the cell. In the previous lecture, Dr. Diala talked about insertion of different types of membrane proteins into the mitochondrial membranes or soluble proteins into the inter-membranous space or the matrix of the mitochondria.

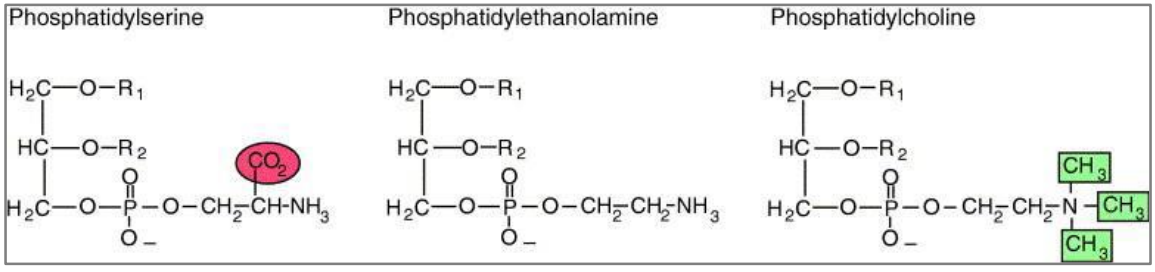
The last function to discuss about the mitochondria is their role in the production of phospholipids.

- In addition to being synthesized in the ER, phosphatidyl ethanolamine and phosphatidyl choline can be synthesized in the mitochondria.
- Furthermore, phosphatidyl serine can be synthesized from phosphatidyl ethanolamine.
  → Three types of phospholipids -that can be synthesized in the ER- can also be synthesized in the mitochondria.

Remember: The ER has a role in the production of certain types of phospholipids as well as sphingolipids precursor (ceramide).

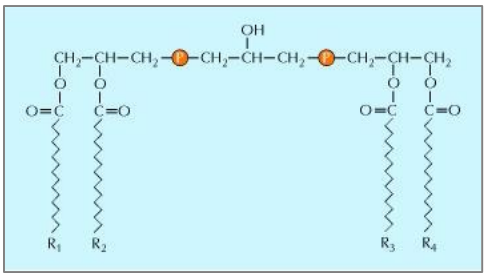
Types of phospholipids that can be produced in the ER:

Phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, and phosphatidyl choline.



- A specific type of phospholipids called "Cardiolipin" is exclusively present in the inner mitochondrial membrane.

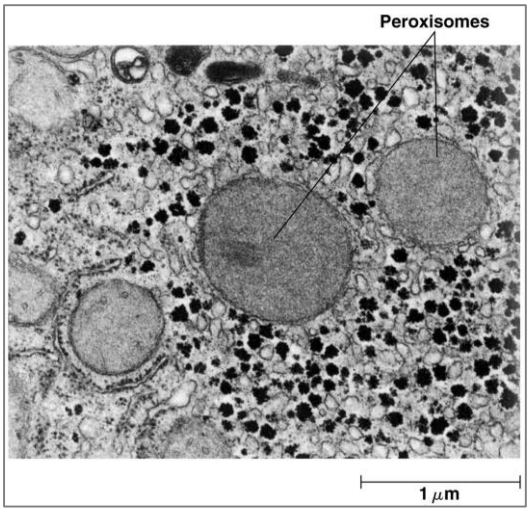
  Special characteristics about this type of phospholipids:

  - Presence of two phospholipid molecules that are joined to each other via a glycerol molecule that connects the two phosphate groups of the two phospholipid molecules.
  - *Cardiolipin is an unusual phospholipid that contains four fatty acid chains.*
  - *Cardiolipin is synthesized in the mitochondria.*
  - *Cardiolipin improves the efficiency of oxidative phosphorylation by restricting proton flow across the membrane.*

Slide # 13: peroxisomes

Structural features:



- Small, single membrane-enclosed organelles.
- Involved in oxidation reactions.

- *Contain enzymes involved in a variety of metabolic reactions, including several aspects of energy metabolism.*
- *They replicate by division.*
- *Can rapidly regenerate even if entirely lost.*

- Their proteins are called Peroxins (Pex1, Pex2, etc.).

- Most human cells contain 500 peroxisomes. However, this number changes depending on the situation of the cell.

  - For example: in strokes, the biogenesis (formation) of peroxisomes increases because of their role in oxidation reactions. In ischemic strokes, there is much less blood supply reaching the neurons in a specific region in the brain (نقص تروية)
    →This will lead to neuronal death in this specific area.
    →Defect in function depending on the affected region.
  - How do surrounding cells (peripheral cells around the injury site) respond? They will increase the production of peroxisomes and their content of anti-oxidant enzymes like catalase.

Catalase converts hydrogen peroxide (H_2O_2) into water (H_2O).

Ischemia is a condition of oxidative stress for the injured cells and it will cause their death.

- That's why peripheral cells respond by increasing the biogenesis of peroxisomes with their content of enzymes to increase the capacity of anti-oxidants effect and try to repopulate the area and repair damage.
- That's why stroke patients might improve over time.

Slide # 14: peroxins

Peroxisomes have special types of proteins called Peroxins.

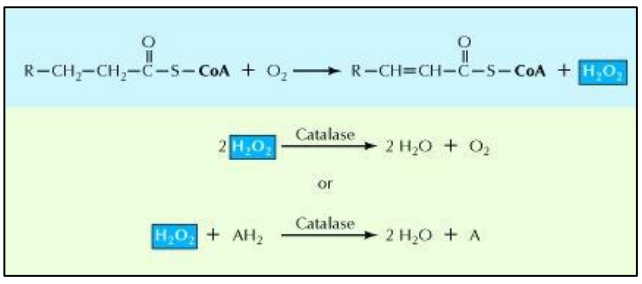
- There are many peroxins (Pex1, Pex2…).
- 85 genes encode peroxins.
- *Most peroxins are metabolic enzymes.*
- Internal proteins (soluble inside the lumen of the peroxisome) are synthesized on free ribosomes and then imported into peroxisomes.
- On the other hand, transmembrane peroxisomal proteins (membrane proteins) are synthesized on the ribosomes of the rough ER.
- *Other membrane proteins act as receptors for the import of internal proteins.*

Slide # 15: Function of peroxisomes

- How do these organelles function in metabolism and oxidation?
- *Peroxisomes from a single tissue contain at least 50 enzymes.*

A. One of the enzymes contained within peroxisomes is catalase that converts H_2O_2 molecules into water molecules, thus, removing the effect of H_2O_2 as one of the reactive oxygen species.

B. Another way of action is by reacting with certain substances like uric acid, purines, amino acids, or fatty acids to help in their oxidation (*substrates like uric acid, purines, amino acids, and fatty acids are broken down by oxidative reactions in peroxisomes to provide energy*). The oxidation of the previous substances may produce certain particles like H_2O_2 that will be taken up by catalase and converted into H_2O.



- Since peroxisomes contain the oxidase enzyme, they have a role in oxidation of fatty acids.
- *Fatty acids are oxidized in both peroxisomes and mitochondria*.

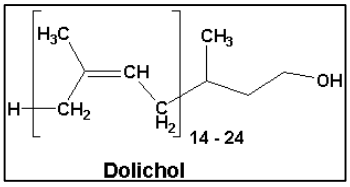
Slide # 16: Synthesis in peroxisomes

There are other functions of peroxisomes. For example, they are involved in certain synthesis processes.

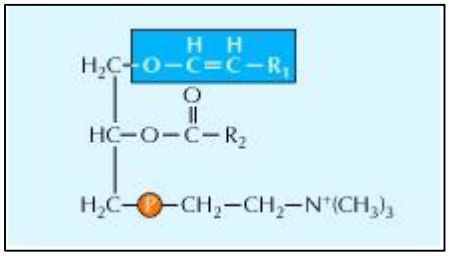
Examples on substrates and compounds produced by peroxisomes:

- Lysine amino acid.
- Certain lipid molecules:
  - Cholesterol
  - Bile acids that are derived from cholesterol
  - Dolichol
  - Plasmalogens (the figure below)
    These are specific types of phospholipids with one **ether bond** (R – O – R).
    Plasmalogens are important in membranes of heart and brain cells.

Dolichol is a lipid carrier that carries the sugar portion that will later be added to certain polypeptide chains during N-linked glycosylation.



Dolichol



The structure of a normal phospholipid:

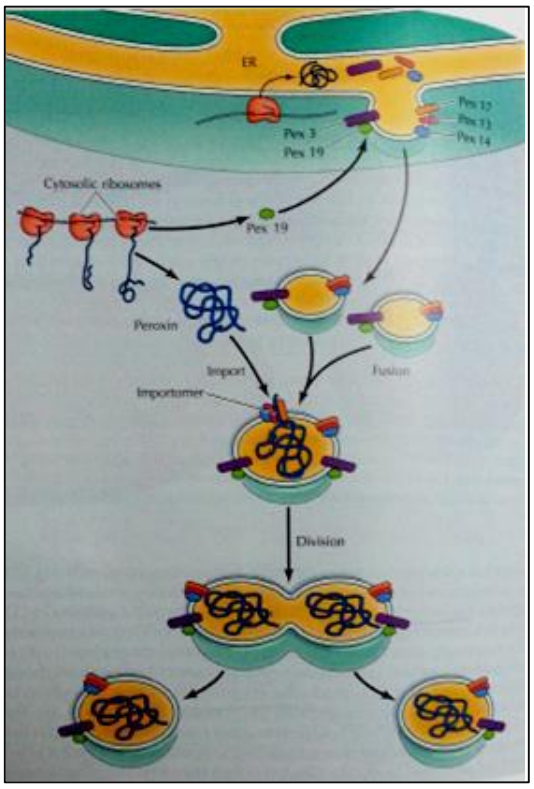
- Glycerol molecule connected to 2 fatty acid chains and {phosphate group + different head group to result in different subclasses of phospholipid}.
- The bond between the glycerol molecule and each fatty acid is an ester bond
  (R – O - carbonyl group)

Slide # 17: Peroxisomal Assembly (formation)

- A peroxisome looks like a vesicle.
  → It is expected that a peroxisome forms through budding from a membrane.
- Peroxisomes originate from ER membranes.
- How does the ER membrane know that a certain vesicle will become a peroxisome?
  - Depending on the type of membrane proteins in that specefic area of the ER membrane. (there would be specific peroxisomal membrane proteins expressed in that region).

Remember: membrane proteins are expressed on ribosomes of the rough ER, then, they will be inserted into the ER membrane.

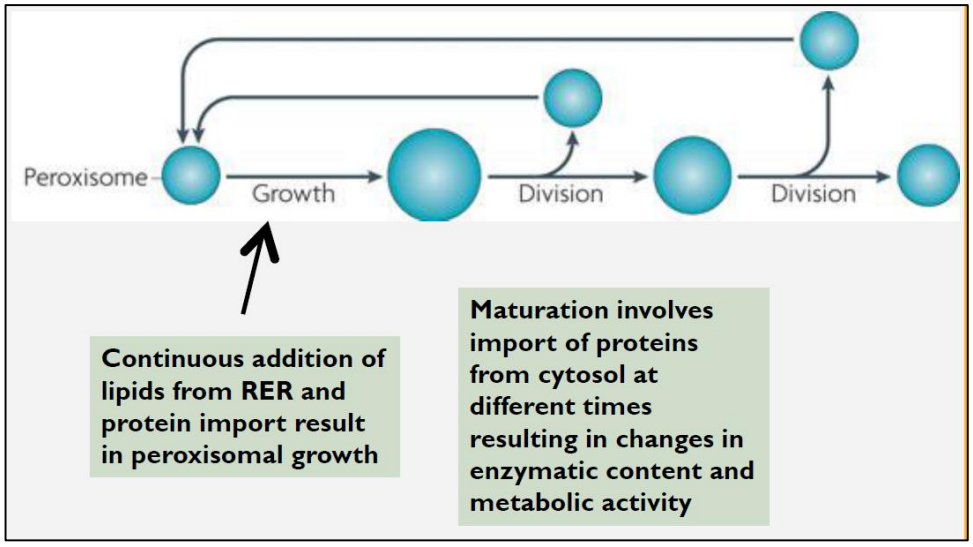
- One of these peroxisomal membrane proteins is called **Pex 3** (see the figure to the right and notice the presence of the rod-like transmembrane protein Pex 3).
- This will give a signal that the budding vesicle is going to become a peroxisome.



- Pex 3 recruits and interacts with other sets of proteins including Pex 19 (Pex 19 is a soluble –not membrane- protein. It's synthesized on free ribosomes. (See the figure)).
- The previous protein-protein interactions will encourage the budding of the vesicle that will later become a peroxisome.
- After budding, we have a complete vesicle with peroxisomal membrane and soluble proteins attached to its outer surface.
- The next step would be maturation and growth of this vesicle. How does this occur?
  - Because we still need more proteins like the enzymes active in metabolism and oxidation, more proteins need to be synthesized by free ribosomes -if they are soluble proteins.
  - Free ribosomes will synthesize these proteins that will then be transported into the lumen of the vesicle (or the peroxisome).
  - After being synthesized by free ribosomes, how are these soluble proteins going to be identified and recognized by the initial structure of the peroxisome that has Pex 3 and Pex 19?
    By certain receptors *(certain membrane proteins)* that would identify the soluble proteins *(inetrnal matrix proteins)* through **signal sequences**.
  - The internal soluble proteins have two signal sequences; peroxisome targeting signals 1 and 2 (PTS 1 and PTS2). One of these signals is in the C-terminus and the other signal is in the N-terminus.
  - Thse sequences can be recognized *by cytosolic receptors* to allow the translocation of these soluble peroxisomal proteins into the lumen of the peroxisome *via a channel (importomer).*
- As a step of maturation and growth of these organelles, the newly formed peroxisome can either **fuse** with another new peroxisome or it might fuse with an old peroxisome.
  → This also helps in the formation and assembly of peroxisomes.
- The whole set of proteins and enzymes inside the lumen of the organelle as well as membrane proteins = the functional unit of peroxisomes.
- This functional unit can **divide** (like the mitochondria).

Slide # 18: Peroxisomal Maturation and Division

- During the development and assembly of peroxisomes, these structures can grow by fusion with old or new peroxisomes.
- There are also other mechanisms involved in the growth of peroxisomes. For example, addition of more lipids and membrane proteins will cause an increase in the size of these organelles.
- After the peroxisome reaches its full size, it begins to divide. This division is considered a step of peroxisomal maturation.



- Maturation also involves other steps like **preferential expression of some proteins.**
  - In a certain cell, and under certain conditions, a situation of oxidative stress might occur. The cells would preferentially express proteins and enzymes needed to overcome this stress.
  - Protein components (as well as their amounts and concentrations) inside the lumen and on the membranes of peroxisomes are not constant (these will be different from one peroxisome to another depending on the situation in the cells and the needed metabolic and oxidative activity of the organelle). This is one way by which peroxisomes can mature into their final structures.

Slide # 19: Peroxisomal Diseases

- Deficiency in any of the peroxisomal enzymes will cause a problem.
  - Single peroxisomal enzyme deficiency → that's a problem.
  - Multiple peroxisomal enzyme deficiencies → that's a bigger problem.

- *Peroxisomal biogenesis disorders (PBDs) that involve multiple peroxisomal enzyme deficiencies due to failure of import → e.g. Zellwegar syndrome.*

- Single peroxisomal enzyme deficiency
  - Lesser effect compared to multiple peroxisomal enzyme deficiencies

- Multiple peroxisomal enzymes deficiencies
  Example: Zellwegar syndrome
  - Lethal syndrome
  - Multiple peroxisomal enzymes are deficient in these patients *(the disease happens due to mutations in at least 10 genes such as the receptor of PTS 1).*

- X-linked adrenoleukodystrophy (XALD)
  - Defective transport of very long chain fatty acid (VLCFA) across the peroxisomal membrane.

- As we said earlier, strokes are diseases associated with peroxisomes –not the enzymes they contain-. Differences in expression, maturation, and biogenesis of peroxisome structures within cells that surround the stroke site affect the healing ability.
  - There is a certain enzyme called "**soluble epoxide hydrolase**". This enzyme aids in reducing the oxidative stress in the site of stroke. In addition, it helps in repair.

❖ The cytoskeleton and cell movement (actin filaments)

For the second part of this sheet (pages 8-14): please refer to the slides because these pages contain only what Dr. Diala have mentioned in the lecture.

Slide # 1 : Introduction

- We ended discussion about organelles. Now we move to other cellular components like the cytoskeleton.
  - Organization of organelles and parts of the cytoskeleton inside the cell.
  - Movement of vesicles and organelles in relation to the cytoskeleton.
  - Are the previous processes regulated or random?

Slide # 2: What is the cytoskeleton?

- The cytoskeleton: dynamic network of proteins that are involved in providing structural support as well as organization of the organelles inside the cytoplasm.

- The cell membrane is a gel-like structure –and organelles are also enclosed in membranes-. The cell is like a sac filled with fluid. If left like that, the cell would lack structural stability.
- There are many cells that are involved in the function of mechanical support or they really have to be strong in order to resist trauma and mechanical stress.
  → That's why cells must be enforced by the presence of the strong components of the cytoskeleton.

- The cytoskeleton is composed of three types of protein complexes (filaments):
  1) Microtubules
  2) Intermediate filaments
  3) Actin filaments

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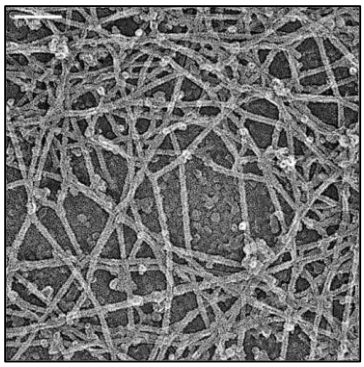
(1) Actin Filaments

Slide # 3: The actin filaments (microfilaments)



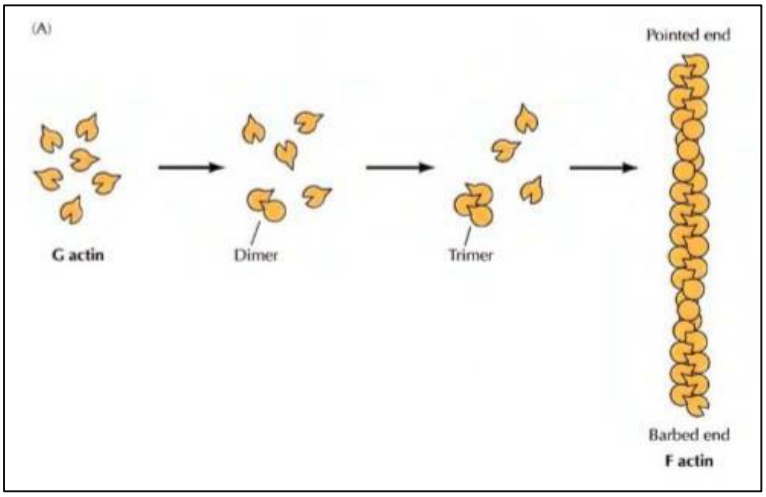
- The figure to the right: this is how actin filaments look like under the microscope → multiple filaments forming different structures like networks (meshworks) or bundles. (these filaments can be arranged in different ways).

- The main functions of actin filaments:
  - Mechanical support
  - Determining the cell shape
  - Cell movement
  - Actin, together with myosin → important in muscle contraction.

Slide # 4: The actin protein

Components of actin filaments:

- An actin filament is a polymer of proteins.
- The monomer of this protein complex is called **G-actin**.
  (G stands for Globulin)→ G-actin is a globular (ball-shaped) protein.
- G-actin molecules will first dimerize head to tail (the end (tail) of the first molecule with the beginning (head) of the second one).
- G-actin molecules can also form trimers. (three molecules can interact with each other head to tail to form a trimer).
- The trimer is still not a polymer. Trimers will be organized into a much more complex structure as you can see in the figure. The formed structure is a rigid strong filament-like structure that can perform the structural support function associated with this type of filaments. The formed structure is called **F-actin** (actin filament).



Monomer → G-actin
Polymer → F-actin

- The G-actin molecule has a certain characterestic which is polarity. The two ends of the protein are distinct, not similar to each other (the molecule has a head and a tail).
  →We expect the growing polymer to have polarity as well. (to have two distinct ends).
  - one end is called the **positive** end (or Barbed end) of F-actin.
  - The other end is called the **negative** end (or the pointed end) of F-actin.

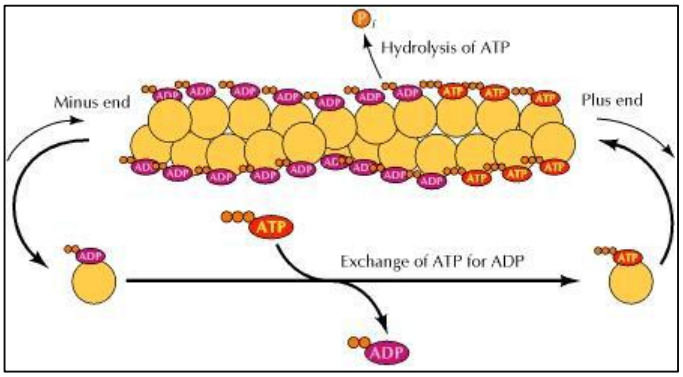
- The positivity and negativity here indicate polarity, not positive and negative charges.
  - The addition of G-actin trimers is more active on the positive end. On the other hand, addition of trimers on the negative end is less.
  - These molecules are dynamic (assembly and disassembly). These molecules change over time depending on the function they perform. For example, if they are involved in movement as in the case of Pseudopodia formation, the pseudopodium needs to extend, which means that more trimers must be added to elongate the actin filament in one direction. Accordingly, there will be disassembly in the other direction (other end).
  - It appears as if the filament moves, but this is not the case. It's going to assemble on one side to become elongated and disassemble from the other side.

- *Note: assembly and disassembly can occur on both sides. However, assembly is much more active (or much faster) on the positive end while disassembly is more active on the negative side.*
  - →As a result, there is more assembly on the positive side of the molecule and more disassembly on the negative side.

- Actin has a role in muscle contraction. Its role involves its **movement**.

Slide # 5: Formation of filaments *(it's important to study this slide)*

- In the figure to the right, notice the positive and negative ends.
- The units of F-actin polymers are added in the form of trimers.
- These molecules are ATP bound proteins (ATP molecules bind to them).



- What is the role of ATP in assembly and disassembly?
  - Exchange and hydrolysis processes are important in order for the cell to know which process (assembly or disassembly) it should carry out.
  - What would be the difference between an actin molecule bound to ATP and another actin molecule bound to ADP?
    Conformational difference. Actin bound to ATP has a different conformation than that bound to ADP.

➔ ATP-bound actin can activate polymerization (assembly). On the other hand, ADP-bound actin misses a phosphate group which will cause conformational changes that will encourage dissociation (disassembly) of actin molecules.

Slide # 6: Actin-binding proteins

- Formation of actin filaments is a very active process;
  - Assembly and disassembly
  - Branching might be needed

➔ We expect the presence of the help of other proteins to perform these fuctions.

➔ We need different sets of proteins called "**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

- Actin binding proteins are categorized into several groups depending on their functions. For example, some of these proteins:
  - initiate polymerization and elongate the chain → formin and Arp 2/3 (Formin: formation)
  - have a role in stablization of the filament at a certain length or in a certain situation → Tropomyosin
  - Have a role in cross-linking the fibers → α- actinin
    When the fibers are-arranged as longitudinal bundles, they need to be cross-linked together.
  - End-capping
    If we want to prevent loss or prevent assembly or disassembly at one side, we need to cap it with proteins.

From molecular biology part: mRNA is capped in order to prevent the degradation of its end.

- Filaments severing (depolymerization) → Cofilin

- Monomer binding proteins → Profilin
  Carry monomers for polymerization steps.

- Actin filament linkage to other proteins (because the cytoskeleton might interact with membrane proteins or other cellular proteins)
  → α-catenin

The cytoskeleton components -specifically, **microtubules**- interact with proteins of the vesicles that are going to move on these microtubules.

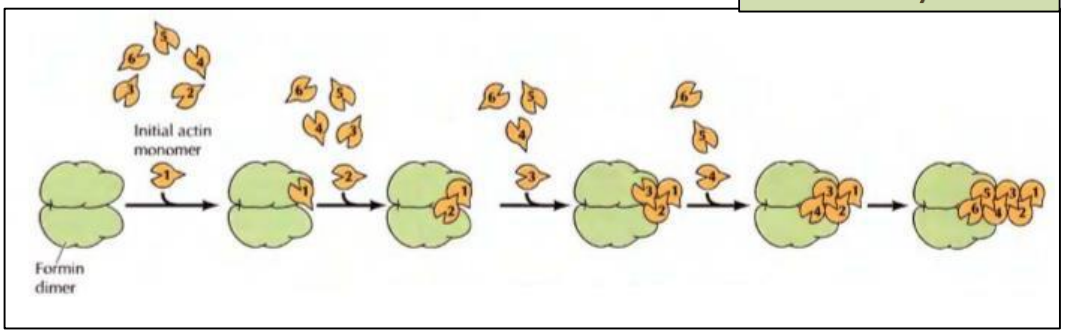
Slide # 7: Actin-binding proteins

There are many actin binding proteins, the doctor discussed some of them in details:

1) Formin

- An actin binding protein that is related to initiation of polymerization.
- It's a dimer.

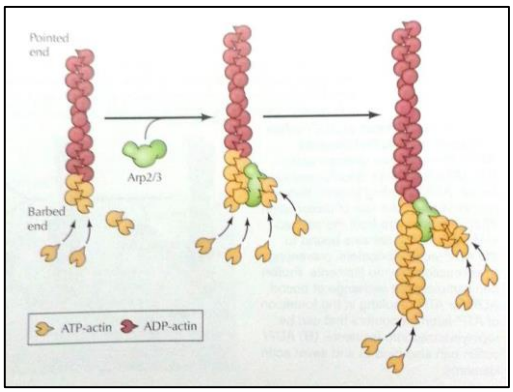
The rate-limiting step of actin formation, nucleation, is facilitated by formin.



- Formin binds to actin monomers and starts joining them to form trimers and initiate polymerization steps.
  Note: no further details are required but it's important to know that actin molecules can't initiate polymerization by themselves. They need the guidance of another molecule which is formin.
  →Formin will arrange the monomers in the right direction and bind them together to assemble actin filaments.

2) Arp 2/3

- Its main function is related to branching.
- The figure to the right: if we have a growing chain of an actin filament and we need a branch to emerge from a certain point, what will happen?



- Arp 2/3 complex will bind at a specific region in the polymer.
- This will bring more molecules to bind Arp at this point.
- The result will be activation of the process of building a new filament (strand) from this point. → "Branching".

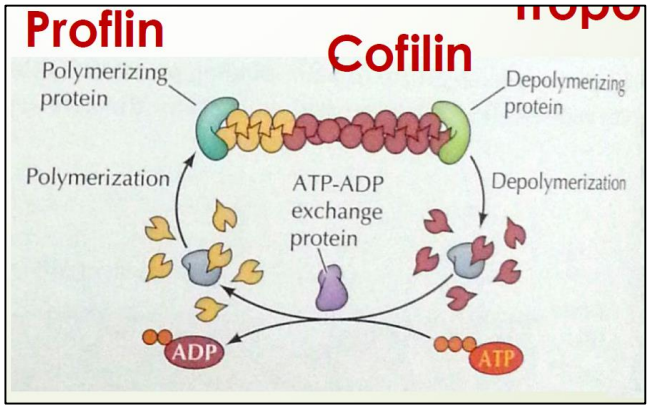
- We can say that Arp 2/3 acts as a scaffold, it binds at a certain point and then monomers start binding to it leading to branching at this point.

3) Profilin

- Helps in polymerization by holding actin molecules and adding them to the growing end of the filament.

4) Cofilin

- Activates depolymerization

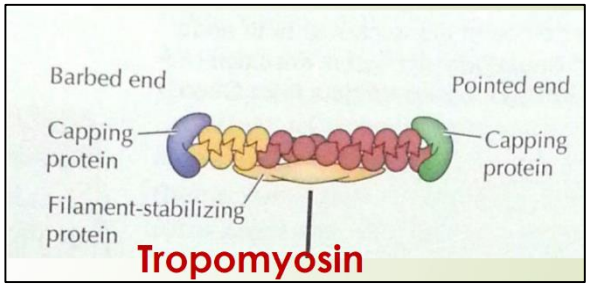


Note: polymerization and depolymerization can also happen by changing the ATP-ADP status:

- If the monomers of F-actin are bound to ADP, this will activate their disassembly.
- If the monomers of F-actin are bound to ATP, this will activate their assembly.

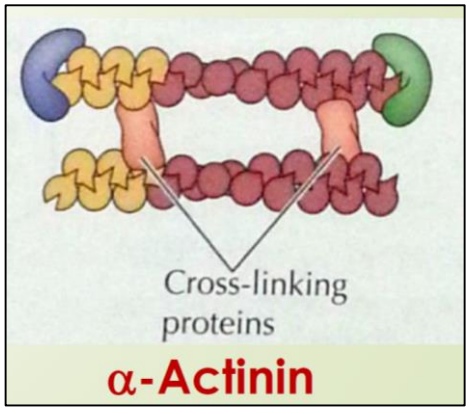
5) Tropomyosin

- You can see from the figure to the right that tropomyosin binds to the chain (filament) from the side. This will stablize and protect the structure.



6) α- actinin

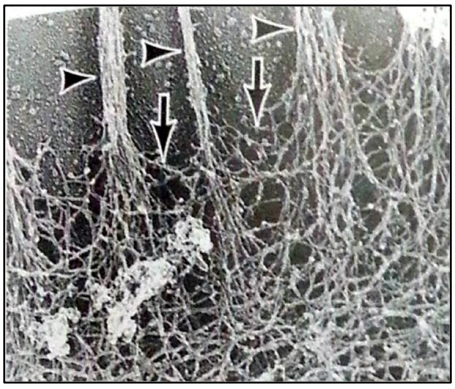
- joins one strand (filament) with another strand. → 'Cross Linking"



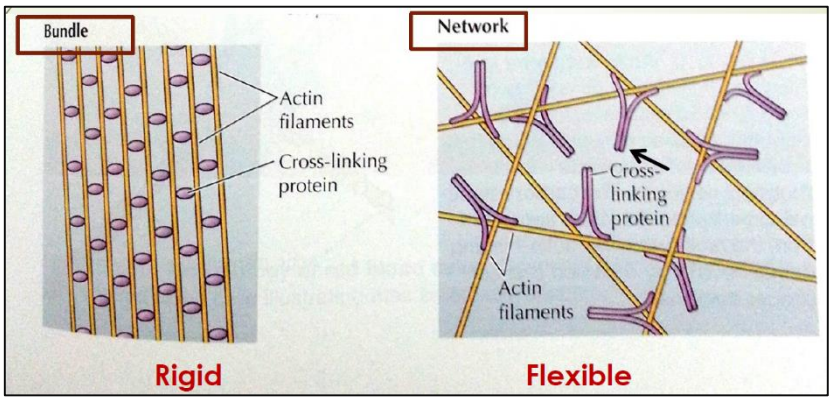
Slide # 8: Organization of actin filaments

- How are actin filaments organized within the cell? Are these filaments scattered or arranged parallel to each other?
- The filaments can be organized into **bundles** or **networks**.

  - Bundles: actin filaments are parallel to each other and held by actin binding proteins that cross-link these bundles.
  - Networks (meshworks): less organized form compared to bundles. specific actin binding proteins join the filaments to each other forming an actin network.



- Which form (bundles or networks) is stronger?
  Bundles, because the arrangement of filaments in this form reduces the distances between the filaments.
  - ➔ Within a unit area, there will be more actin filaments in that region. On the other hand, you can notice from the figure that the spaces between the actin filaments in the network form are larger.
  - ➔ More flexible structure in the case of the actin network compared to the actin bundle.
- *Note:* cross-linking joins filaments together in both cases (bundles and networks).
- *Note 2*: Arp2/3 is responsible for branching, but the formed branches are not perpendicular to the filament (the angle is not 90^{o}).



I apologize for any mistake I may have made.

Special thanks goes to Joud Al-Majali.

Wish you all best of luck **:D**