بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | Lecture 6

Peroxisomes & Cytoskeleton

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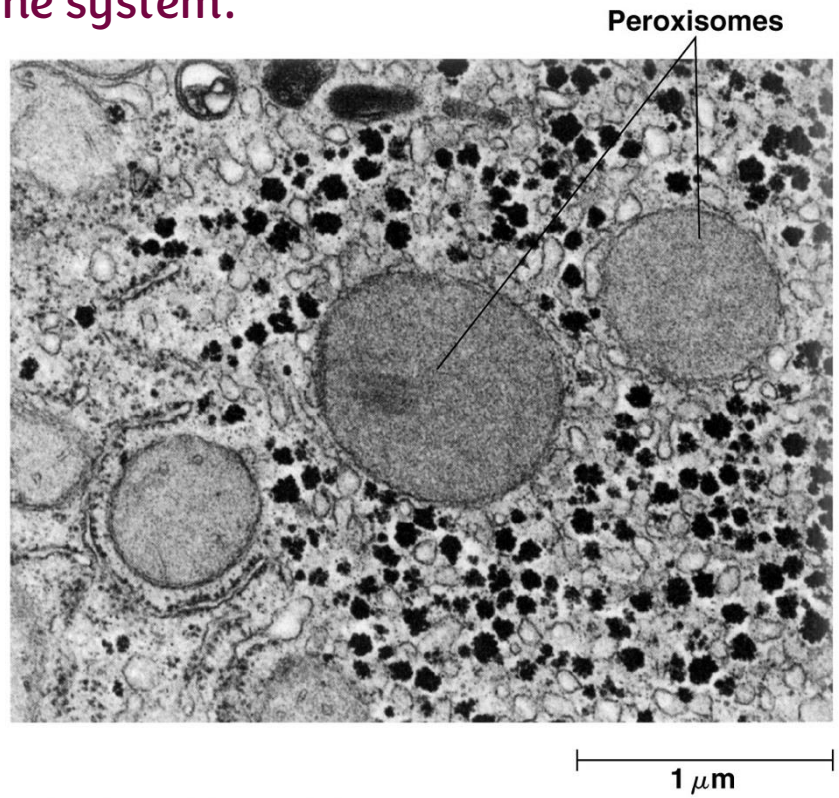
Peroxisomes

Structural features of peroxisomes

- Small, membrane-enclosed organelles
- They contain enzymes involved in a variety of metabolic reactions, including energy metabolism.
- They replicate by division.
- Most human cells contain 500 peroxisomes.

Peroxisomes look somewhat like lysosomes. They have a single membrane, unlike mitochondria, which have a double membrane system.

They can undergo fission just like mitochondria



Peroxisomes contain oxidoreductase enzymes that are important for metabolism. They are involved in the breakdown of very long-chain fatty acids (those with 22 or more carbon atoms), which is part of energy metabolism.

Peroxins

Peroxisomal proteins are called peroxins, abbreviated as PEX, and are numbered, for example, PEX1, PEX2, and PEX3. These proteins participate in the formation of peroxisomes. Peroxisomal membrane proteins are also considered PEX proteins, while the enzymes inside peroxisomes have their own names, such as catalase.

- Peroxisomal proteins are called peroxins (PEX).
- There are 85 genes that encode peroxins, most of which are metabolic enzymes.
- Internal proteins are synthesized on free ribosomes and then imported into peroxisomes.
- Other membrane proteins act as receptors for the import of internal proteins.

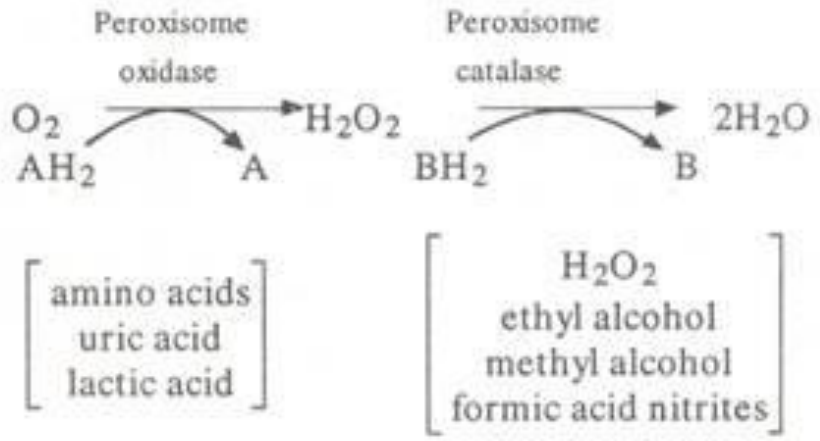
About 85 genes encode peroxins, and these genes are located in the nuclear DNA. **Internal proteins** (such as enzymes) are synthesized on free cytosolic ribosomes and then imported into the peroxisome. In contrast, **peroxisomal membrane proteins** are synthesized in the endoplasmic reticulum and then delivered directly to peroxisomes, without moving through the Golgi apparatus.

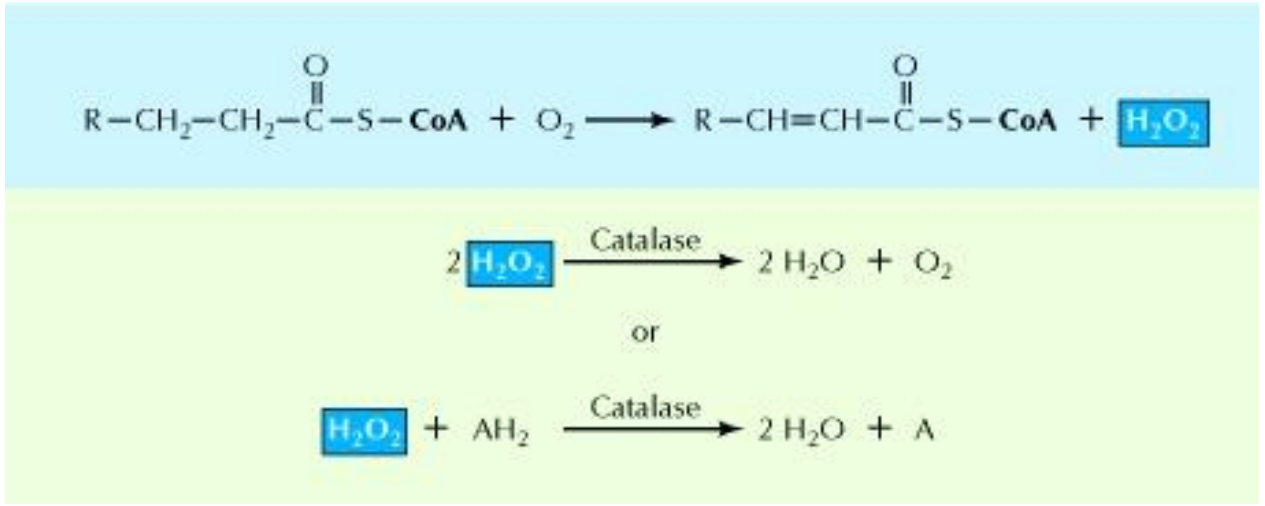
Function of peroxisomes

Peroxisomes carry out oxidation reactions -which produce hydrogen peroxide (H_2O_2) as a byproduct-and also remove harmful molecules, such as hydrogen peroxide (by converting it into water and oxygen using the enzyme catalase)

- Peroxisomes carry out oxidation reactions producing hydrogen peroxide, which is harmful to the cell
- But peroxisomes contain the enzyme catalase that converts it to water and oxygen
- Substrates like uric acid, amino acids, and fatty acids are broken down by oxidative reactions in peroxisomes.
  - Fatty acids are oxidized in both peroxisomes and mitochondria.

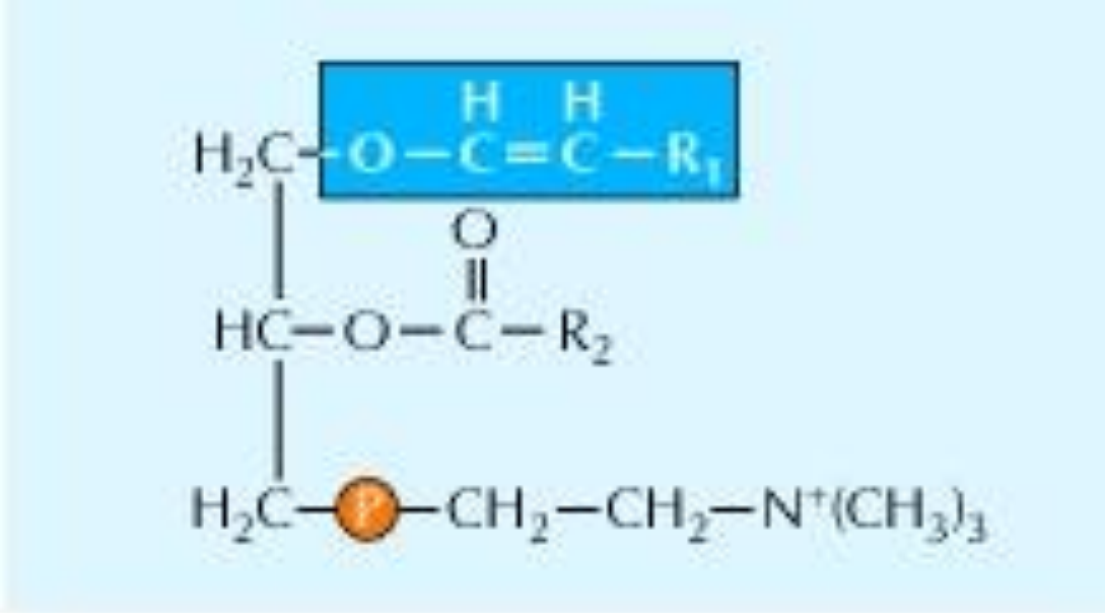
Specifically, very long chain fatty acids





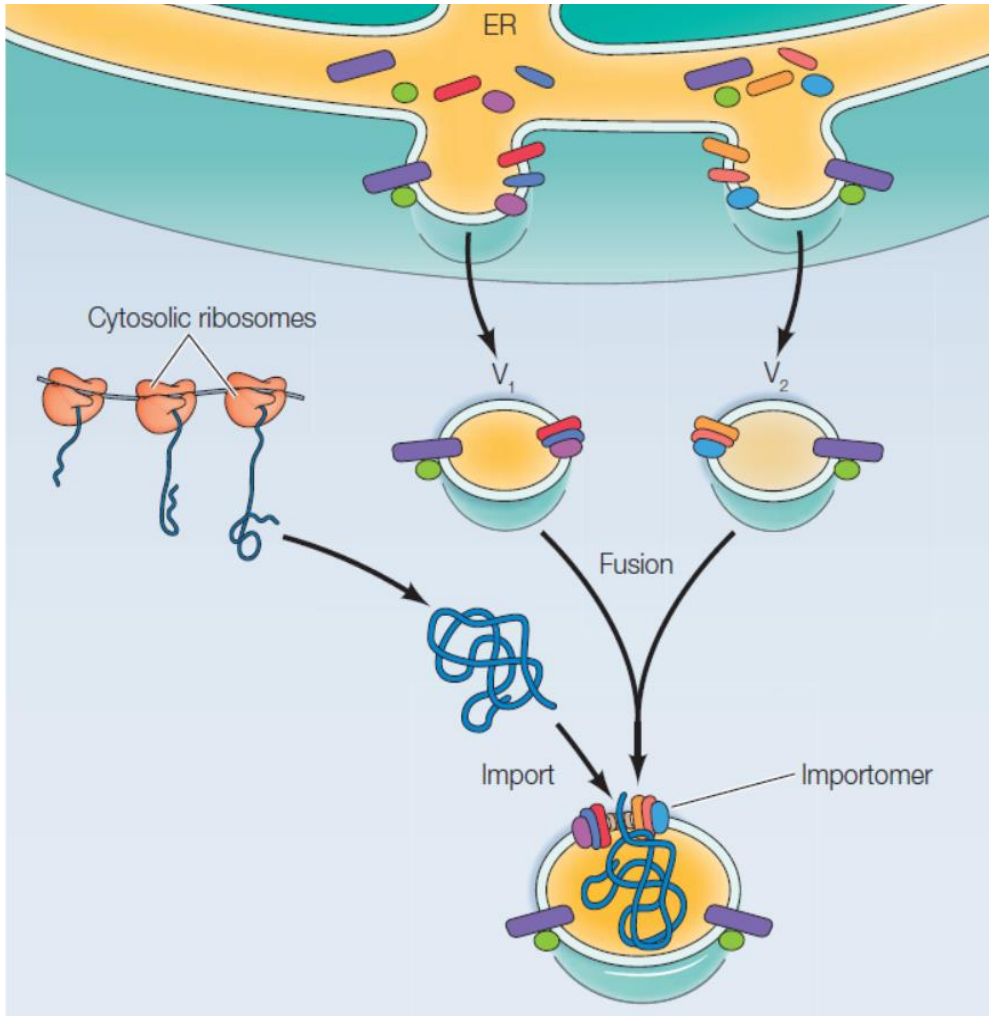
Synthesis in peroxisomes

- Cholesterol
- Bile acids (liver)
- Plasmalogens a type of glycerophospholipid
  - important in membranes of the heart and brain



Assembly of peroxisomes

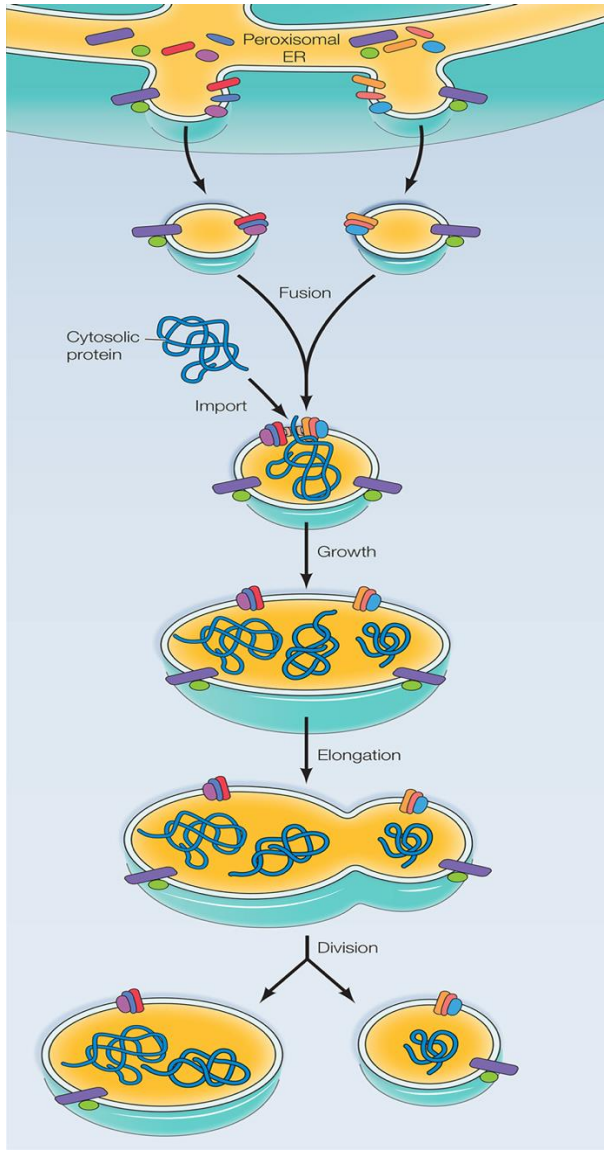
- Peroxisomal transmembrane proteins are derived from the ER.
- A functional peroxisome is formed from the fusion of two ER-derived vesicles carrying the peroxisomal-specific transmembrane proteins.
- The internal peroxisomal proteins synthesized by the cytosolic ribosomes can then be imported.
  - Peroxisomal matrix (lumenal) proteins contain a signal called peroxisome targeting signal (PTS).
- The membrane peroxins form a channel called importomer to translocate the newly synthesized (supposedly internal) peroxisomal proteins from the cytosol into the peroxisomes.



Explained in slide no.10

Another mechanism for the formation of new peroxisomes

- New peroxisomes can be formed by the growth and division of old ones.
  - Similar to the division of mitochondria.
- Division of peroxisomes is mediated by the GTPase Drp1, which also mediates mitochondrial fission.



Explained in slide no.10

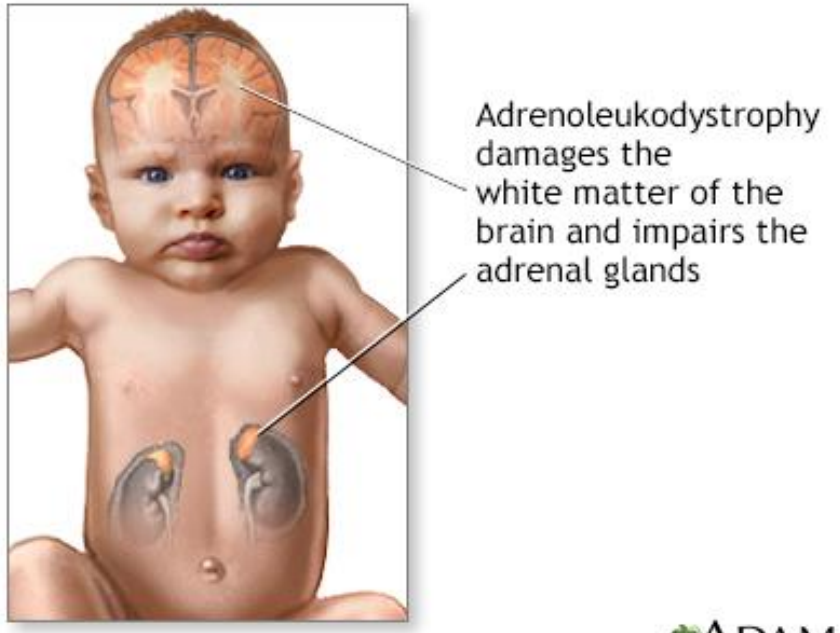
How does the generation or formation of peroxisomes happen?

- Two vesicles (V1 and V2 here) pinch off from the ER's membrane, each carrying different combinations of membrane proteins.
- Then these two vesicles fuse together, forming one large vesicle, which is the peroxisome.
- Lastly, the membrane proteins form a channel, which allows the internal peroxisomal proteins (the ones synthesized in the cytosol) to be carried inside.
- Once the internal proteins are inside, a mature peroxisome is formed.

There's another way to form new peroxisomes, and that is fission. Just like mitochondria, this process involves a protein called Drp1, which mediates peroxisome division. A single peroxisome grows larger and then divides into two peroxisomes.

Peroxisomal diseases

- Single peroxisomal enzyme deficiencies
  - Defective specific peroxisomal enzymes
- Peroxisomal biogenesis disorders (PBDs).
  - Mutations of PEX genes leading to deficiencies of multiple peroxisomal enzymes
- Example: Zellweger syndrome
  - Lethal
  - Due to mutations in at least 10 genes
- X-linked adrenoleukodystrophy (XALD).
  - Defective transport of very long-chain fatty acid (VLCFA) across the peroxisomal membrane.



Peroxisomal diseases are disorders associated with defects in peroxisomal enzymes or proteins.
For example, If the enzyme catalase is defective, a specific type of peroxisomal disease will occur. Similarly, If the enzyme responsible for the breakdown of very long chain fatty acids is defective, these fatty acids will accumulate inside peroxisomes and cell leading to clinical consequences.
Also, if we had mutation in any of the PEX genes (which encode peroxins), a certain type of disease will result depending on the function of the affected protein.

One important example is: **Zellweger syndrome**,
which is a lethal disease caused by mutations in at least 10 genes.
In this condition, the fusion of ER-derived vesicles and the formation of membrane proteins channels composed of PEX proteins are affected. If any of these PEX proteins (e.g., PEX1, PEX2, PEX3, PEX5) are defective, the channel that imports internal proteins becomes defective. As a result, internal proteins remain in the cytosol and cannot enter the peroxisome.
Mutations in different PEX genes can cause the same general disease, but the severity varies (spectrum) depending on which protein is affected and the nature of the mutation. Thus, two individuals may have Zellweger syndrome, but one may present with a more severe form if the mutation causes greater loss of function.

Another disease is: **X-linked adrenoleukodystrophy (XALD)**
caused by a defect in the transport of very long-chain fatty acids into peroxisomes. As a result, these fatty acids accumulate in the cytosol, as they cannot be properly metabolized within the peroxisome.

Lecture 4: the cytoskeleton and cell movement (Actin microfilaments)

Prof. Mamoun Ahram

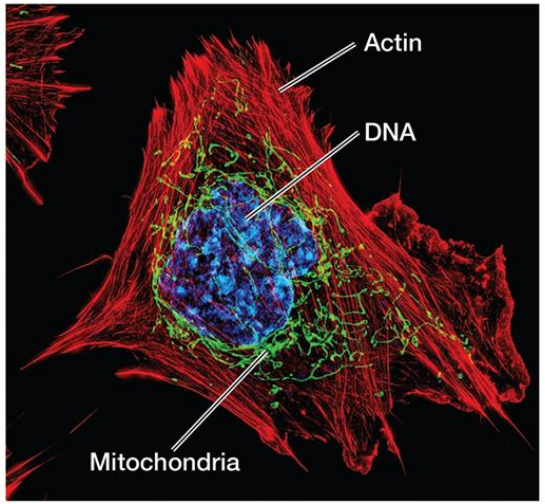
School of Medicine

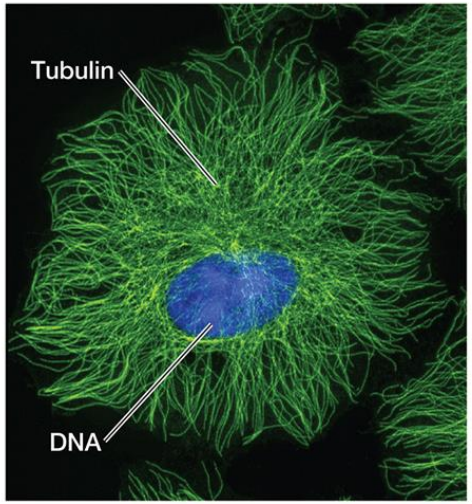
The Cytoskeleton refers to a network of filaments/ fibers that perform certain functions within the cell.
There are three main types of filaments, which together make up the cell body, Each type has distinct functions depending on its location and role in the cell.
Actin filaments (microfilaments) are important for cell movement and muscle cell contraction.
Microtubules are essential for vesicular transport, as vesicles move along these filaments to reach their destinations within the cell.
Intermediate filaments have a structural function inside the cell, as they help determine and maintain its shape.

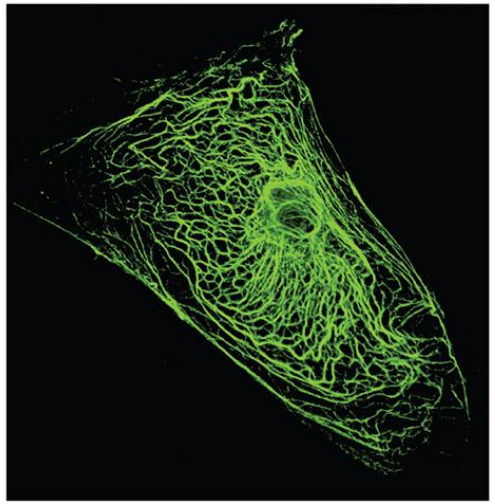
What is the cytoskeleton?

- What is it?
  - A dynamic network of protein filaments extending throughout the cytoplasm
  - Three types: actin microfilaments, microtubules, intermediate filaments
- Functions:
  - A structural framework of cells
  - A determinant of the overall organization of cytoplasm
  - A regulator of the internal movement of organelles
  - A determinant of cell shape and movement
  - A determinant of positions of organelles

Here we have the three cytoskeletal proteins visualized using immunofluorescence, with each color indicating a different structure.





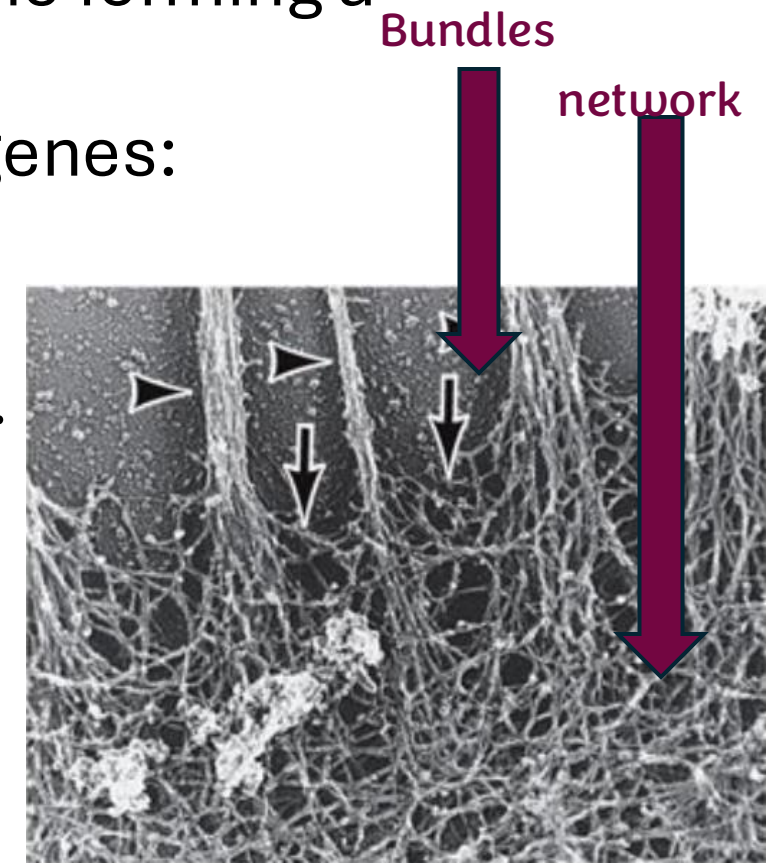


The position of organelles: for example, the endoplasmic reticulum (ER), which is always found near the nuclear membrane, and the Golgi apparatus, which is located close to the ER—is determined by the cytoskeleton. As we mentioned before, mitochondria are found in large amounts at synapses in nerve cells, and their positioning is also determined by cytoskeletal proteins.

The actin (micro)filaments

Bundles: collections of filaments or fibers that are tightly packed together.
Networks: web-like arrangement of filaments

- Thin, flexible fibers that can be organized into bundles or networks.
- They form semisolid gels. Jell-O-like
- They are bound to and regulated by various actin-binding proteins.
- They are abundant beneath the plasma membrane forming a network for cellular function.
- Mammalian cells have at least six distinct actin genes:
  - Four are expressed in different types of muscle.
  - Two are expressed in non-muscle cells.
- The actin proteins are conserved among species.
  - 90% similarity of amino acid sequence between yeast and human cells



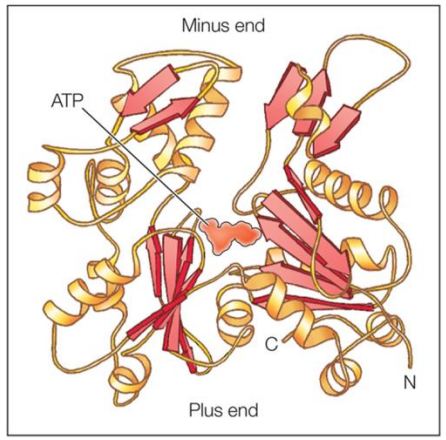
Actin-binding proteins:

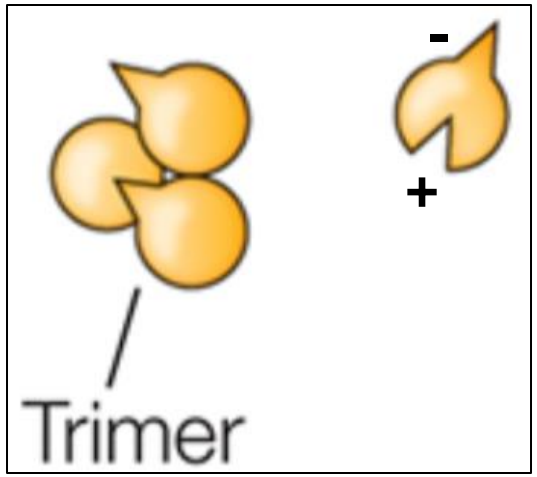
1- Determine the structure of actin, either forming bundles or networks depending on the specific proteins involved.

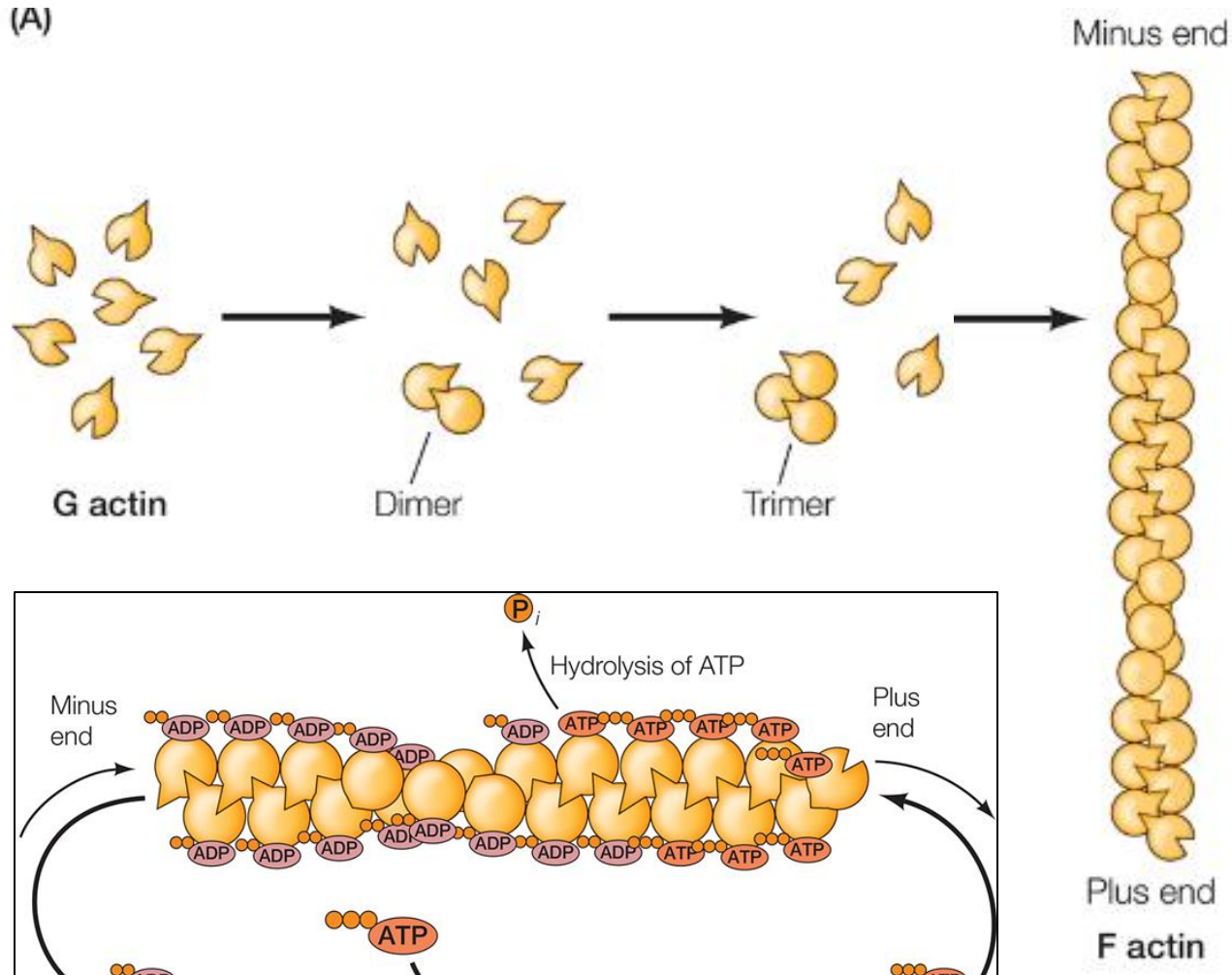
2- Allows actin filaments to interact with the plasma membrane and other organelles -helps in determining the shape and function of the plasma membrane.

The actin polymerization

- **Nucleation** is the start of actin polymerization at both ends when a trimer of G-actin protein is formed. The rate limiting step of the formation of F actin is the formation of a trimer .
  - Actin filaments then grow by adding monomers to both ends.
  - The ends are polar (the plus and minus ends). Different ends (in function and structure) due to the polarity.







https://www.youtube.com/watch?v=n-b7Zz-sfBk

The dynamic (de)polymerization of the actin filaments

In order for the G Actin to form filament, it has to be ATP bound.

Polymerization

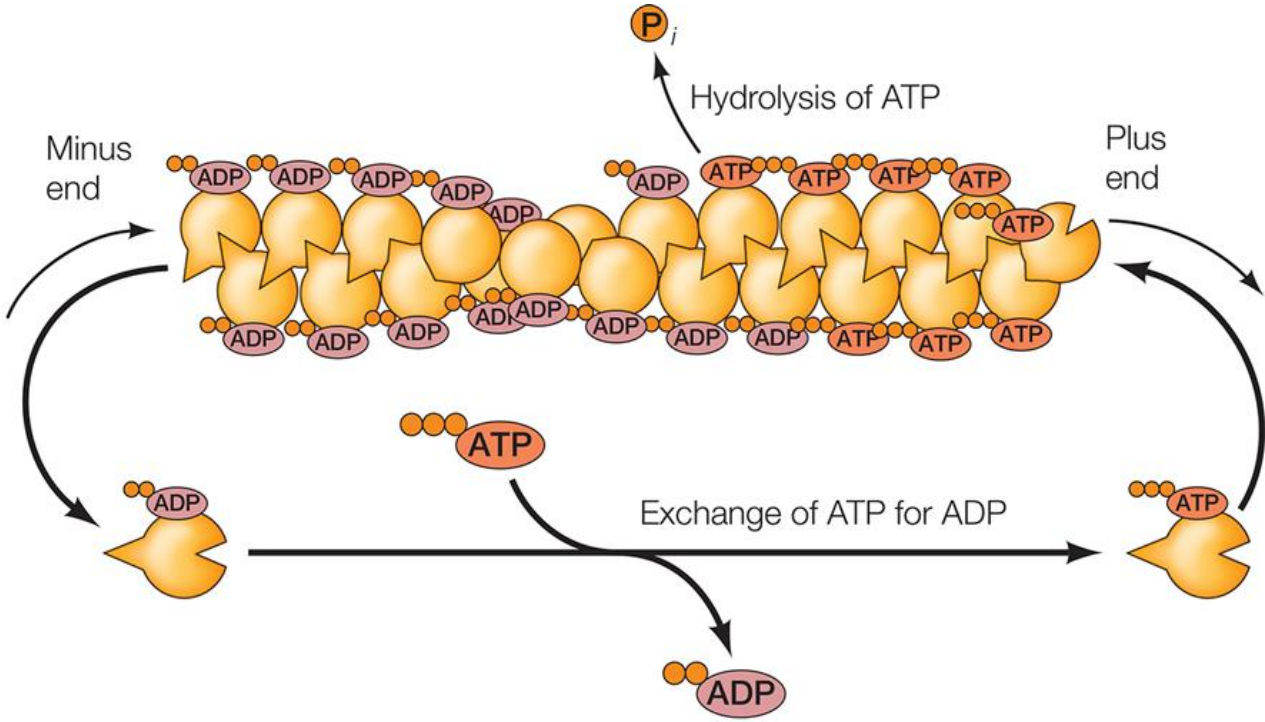
- The monomers are bound to ATP.(It helps)
- ATP is not required for nucleation but:
  - It is hydrolyzed into ADP following assembly,
  - It speeds up polymerization,
  - It stabilizes binding.

ADP bound G-actin is less probable and ATP is more preferable since it helps in making a stable trimmer which helps in the filament growth

Note that both ends can polymerize and depolymerize.

Treadmilling

- The filaments dissociate rapidly from
  - The ADP-actin
  - The minus end

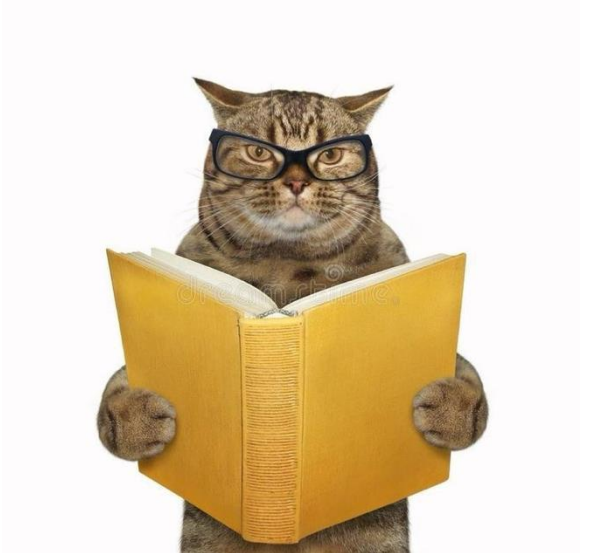


Depolarization (breaking up a polymer) and polymerization (formation of a polymer) can occur at both ends.

At the minus end, there is more removal than association and at the plus end there's more association than removal, which indicates that both ends are different in the way they behave.

Note that both ends can polymerize and depolymerize.

This is a sign of the dynamic nature of the actin and filament by growing on one side and shrinking on the other, this process is called (treadmilling)
what happens here is that once you have the association of a G-actin protein on the plus end eventually the ATP will get hydrolyzed into ADP which will cause destabilization of G-actin binding to the filament and lead to depolarization.



Actin-binding proteins

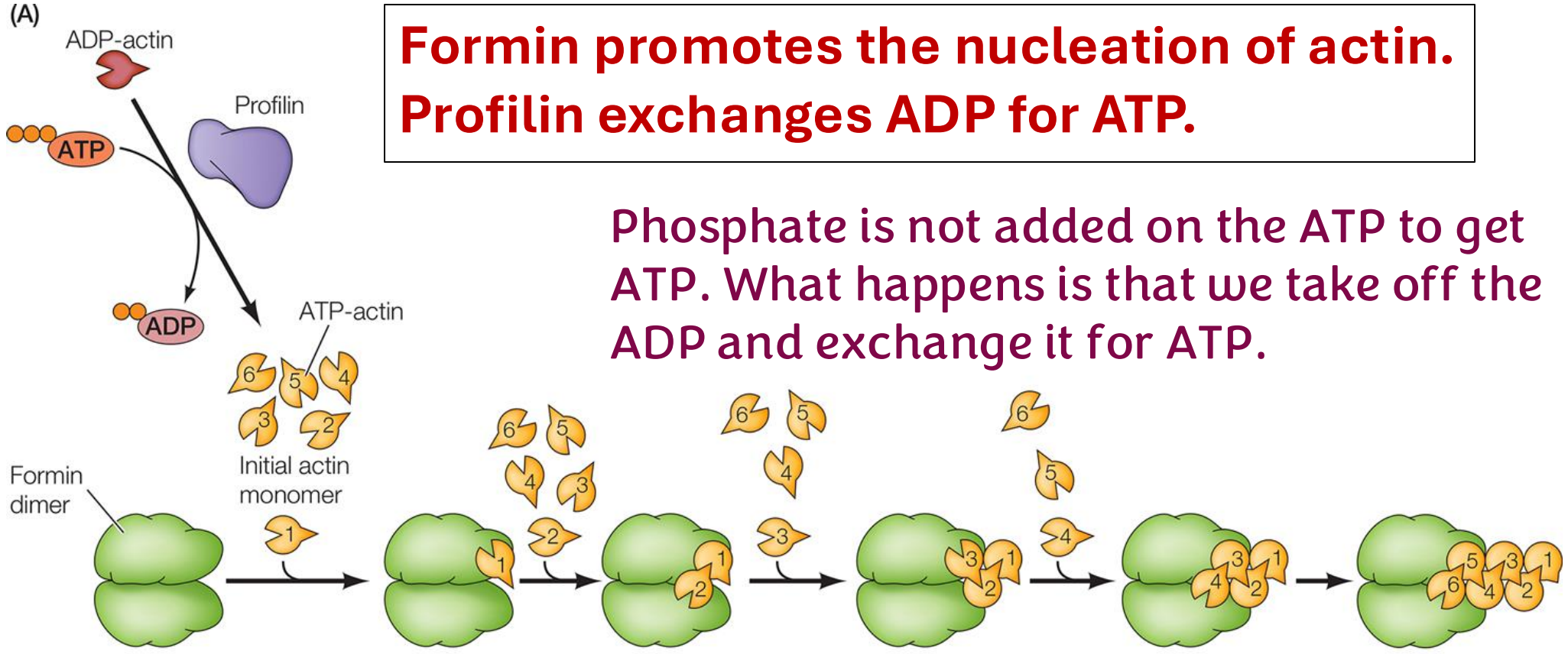
The (dis)assembly of actin filaments is regulated by actin-binding proteins.

Note that not all of them are required, the doc only mentioned the highlighted ones.

Cellular Role	Representative Proteins
Filament initiation and polymerization	Arp2/3, formin
Filament stabilization (By coating of filament)	Nebulin, tropomyosin
Filament cross-linking	α-actinin, filamin, fimbrin, villin
End-capping	CapZ, tropomodulin
Filament severing/depolymerization	ADF/cofilin, gelsolin, thymosin
Monomer binding (Nucleation)	Profilin, twinfilin
Actin filament linkage to other proteins	α-catenin, dystrophin, spectrin, talin, vinculin

Formin and profilin

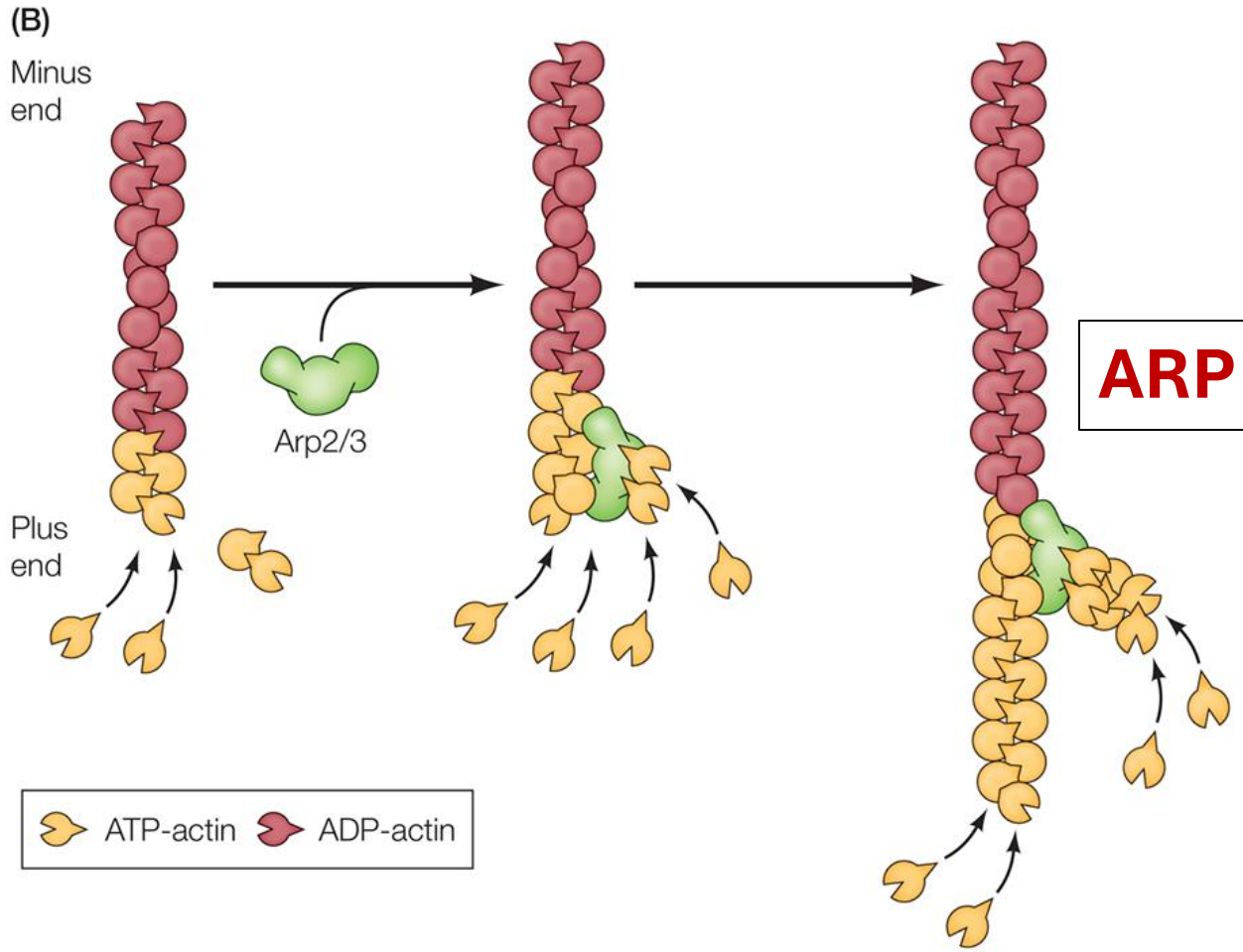
Formin is involved in the formation of a trimmer (the nucleation process)



Formin promotes the nucleation of actin.
Profilin exchanges ADP for ATP.

Phosphate is not added on the ATP to get ATP. What happens is that we take off the ADP and exchange it for ATP.

Branching by ARP 2/3

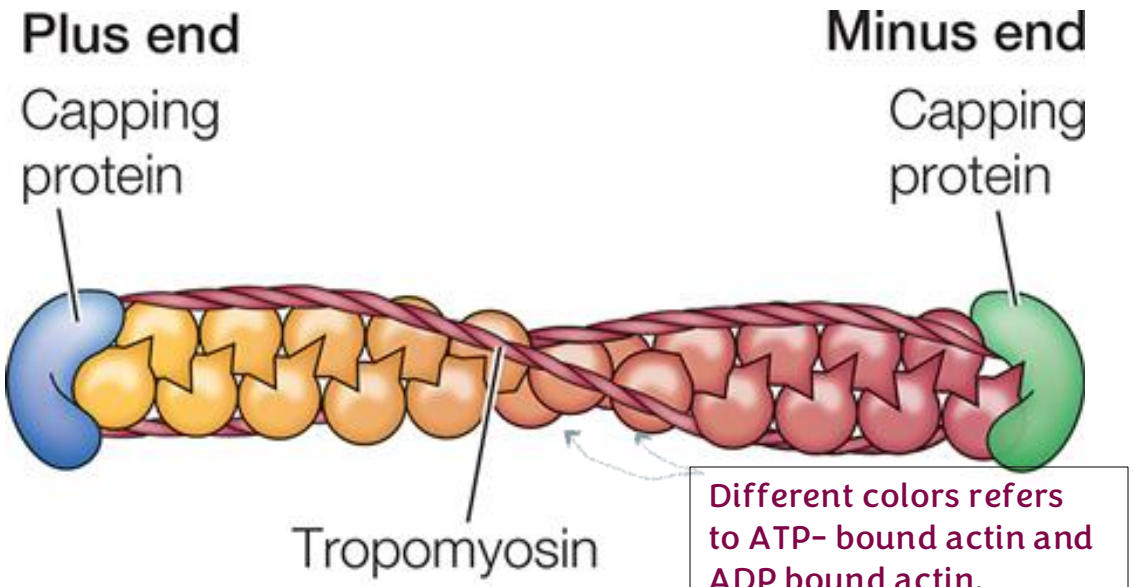


ARP 2/3 initiates branching.

Coating the filament like a rope to stabilize it.

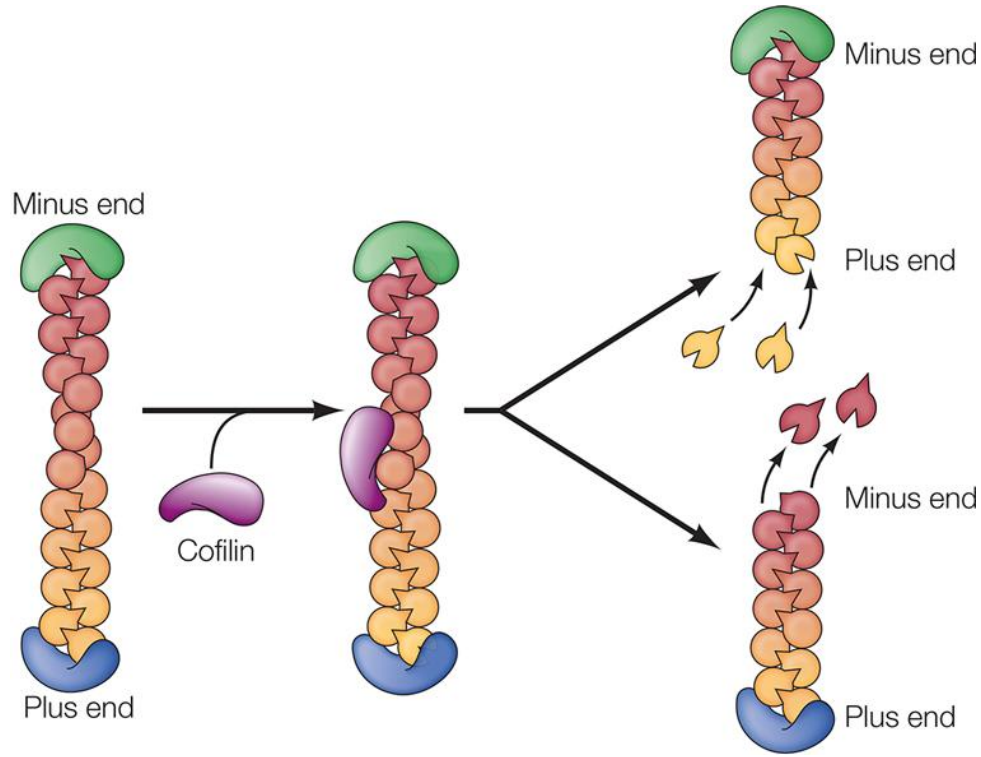
Tropomyosin and cofilin

Tropomyosin stabilizes the ends by binding to capping proteins at both ends of the actin filament.



Different colors refers to ATP- bound actin and ADP bound actin.

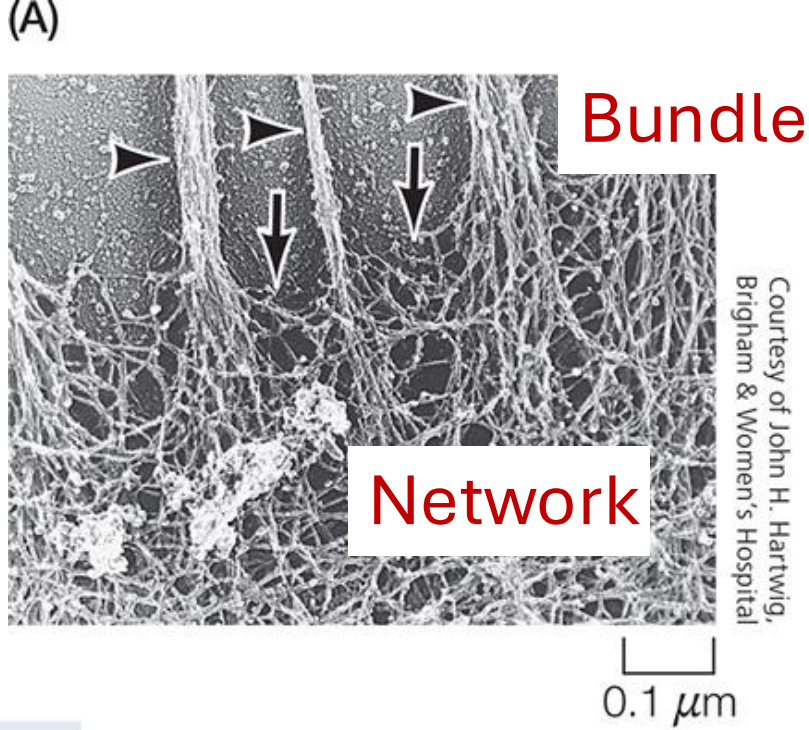
Cofilin breaks up the actin filament.

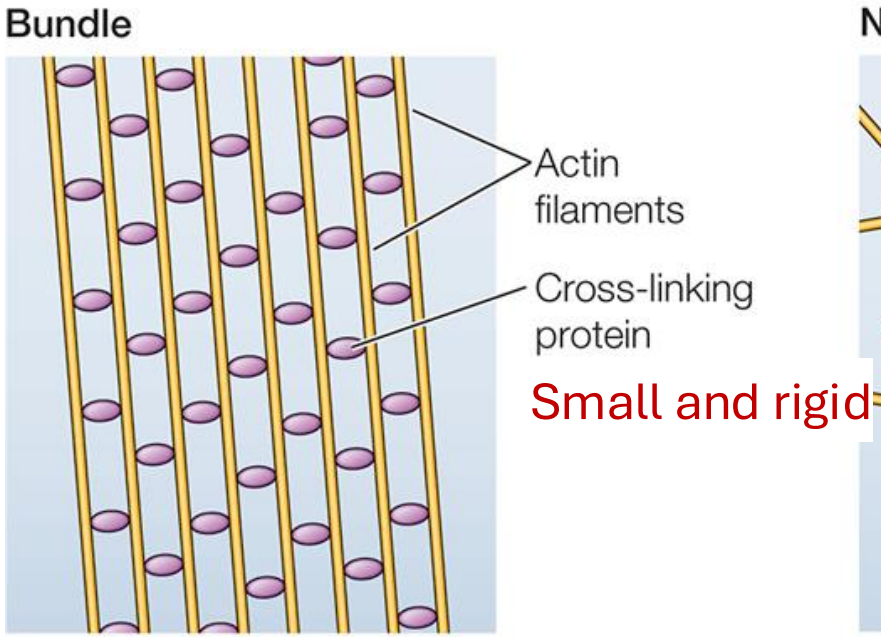


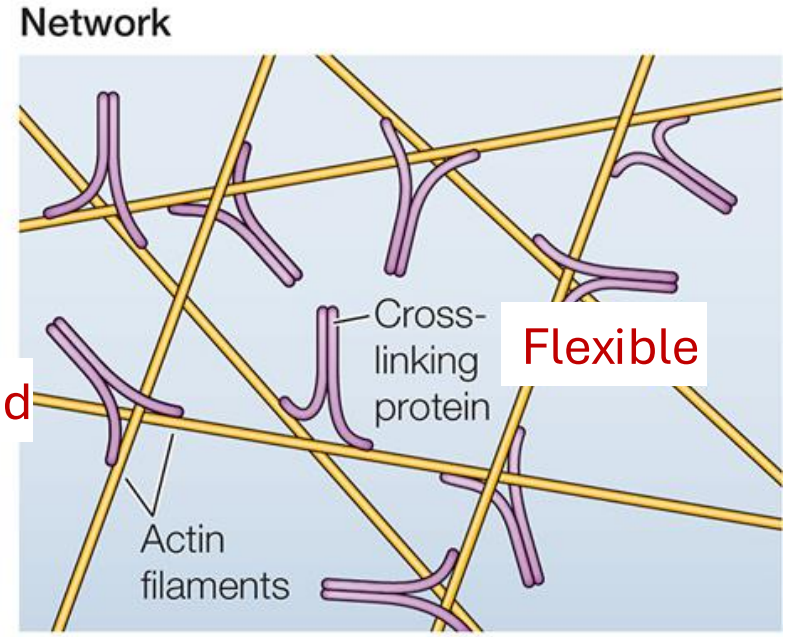
The cells cortex

Bundles and networks

The cell cortex is made of an actin cytoskeleton that is organized as a three-dimensional network beneath the plasma membrane via actin-binding proteins determining cell shape and cellular activities.







(This picture is from an intestinal cell) these fingers are bundles of actin filaments , and theses bundles are connected to the network underneath.

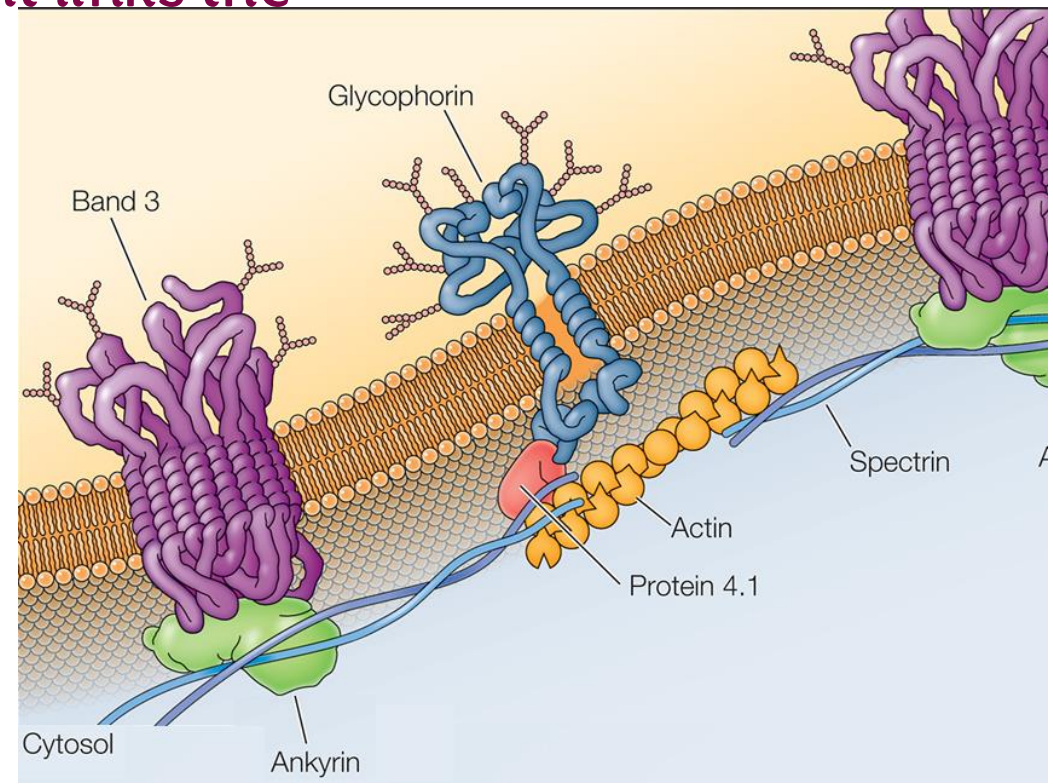
The actin binding proteins of bundles are small and rigid which makes the actin filaments more packed. In networks, they have to be flexible to give a flexible structure inside the cell.

The red blood cells (*actin-membrane connection*)

no nucleus, no organelles, no microtubules, no intermediate filaments

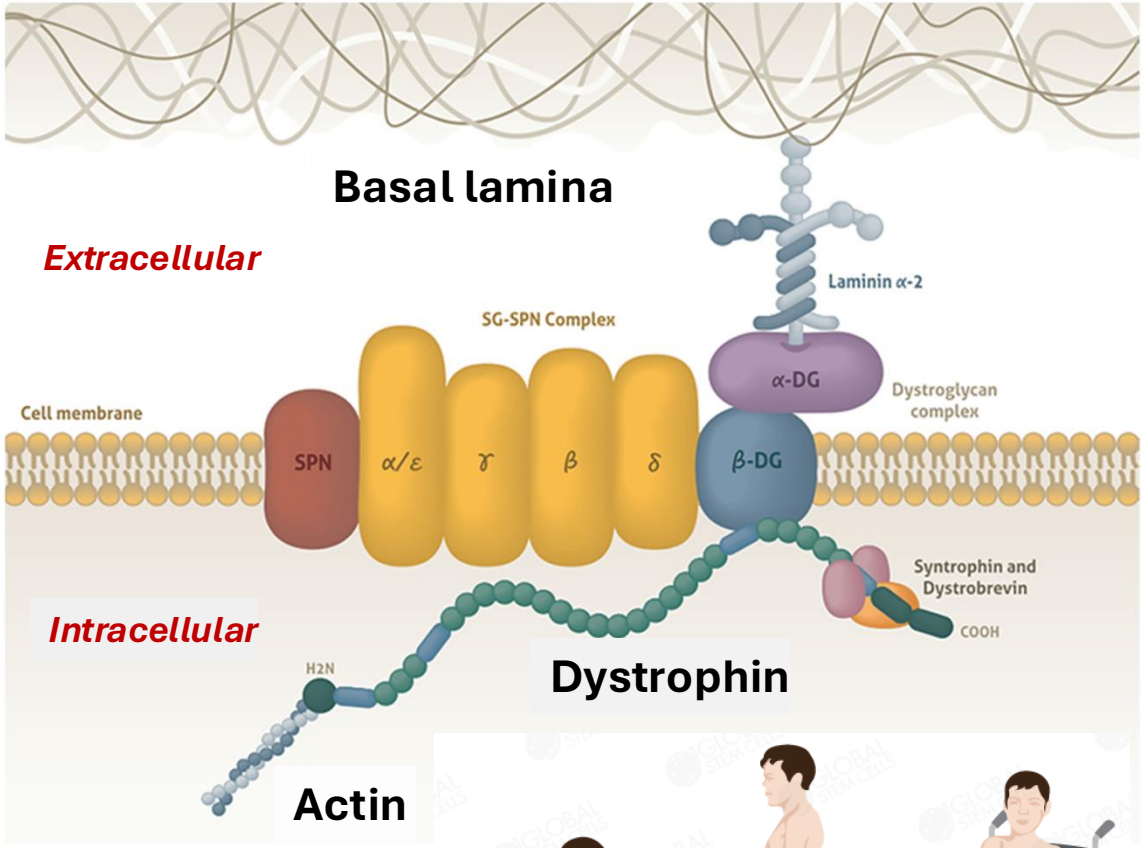
- The major actin-binding protein that provides the structural basis of RBCs is **spectrin**. A long actin binding protein , it links the actin to the plasma membrane.

- Spectin links actin filaments to transmembrane proteins of RBC's plasma membrane via:
  - Ankyrin
  - Protein 4.1



RBCs are gelatinous, flexible and can squeeze between the blood vessels and cells due to the cytoskeleton –actin- inside them, which make them sort of mushy.

Muscle cells and dystrophin



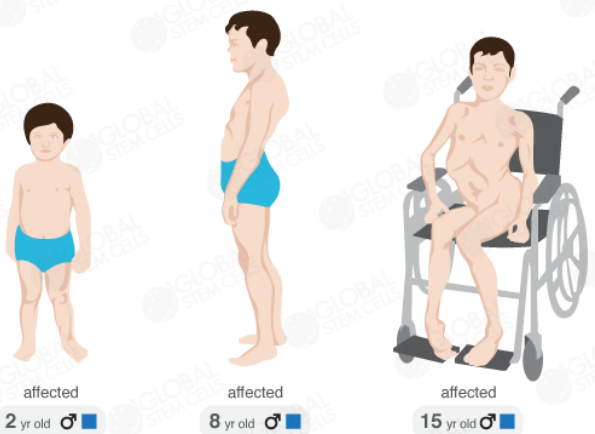
- Dystrophin is a large protein that links the actin filaments to transmembrane proteins of muscle cells.
- These linkages are important in maintaining cell stability during muscle contraction.
- Defective dystrophin is responsible for two X-linked, inherited, progressive, degenerative muscle diseases:
  - Duchenne muscular dystrophy
    - Absent protein; severe disease
  - Becker muscular dystrophy
    - Defective protein, less severe

The gene exists but it gives defective protein (it's as if the protein doesn't exist)

Part of the gene got deleted.

When it exists but it's still somewhat functional

Becomes more progressive (it gets worse with time and sever by teen and the twenties), it also can be fetal.



Additional Resources:

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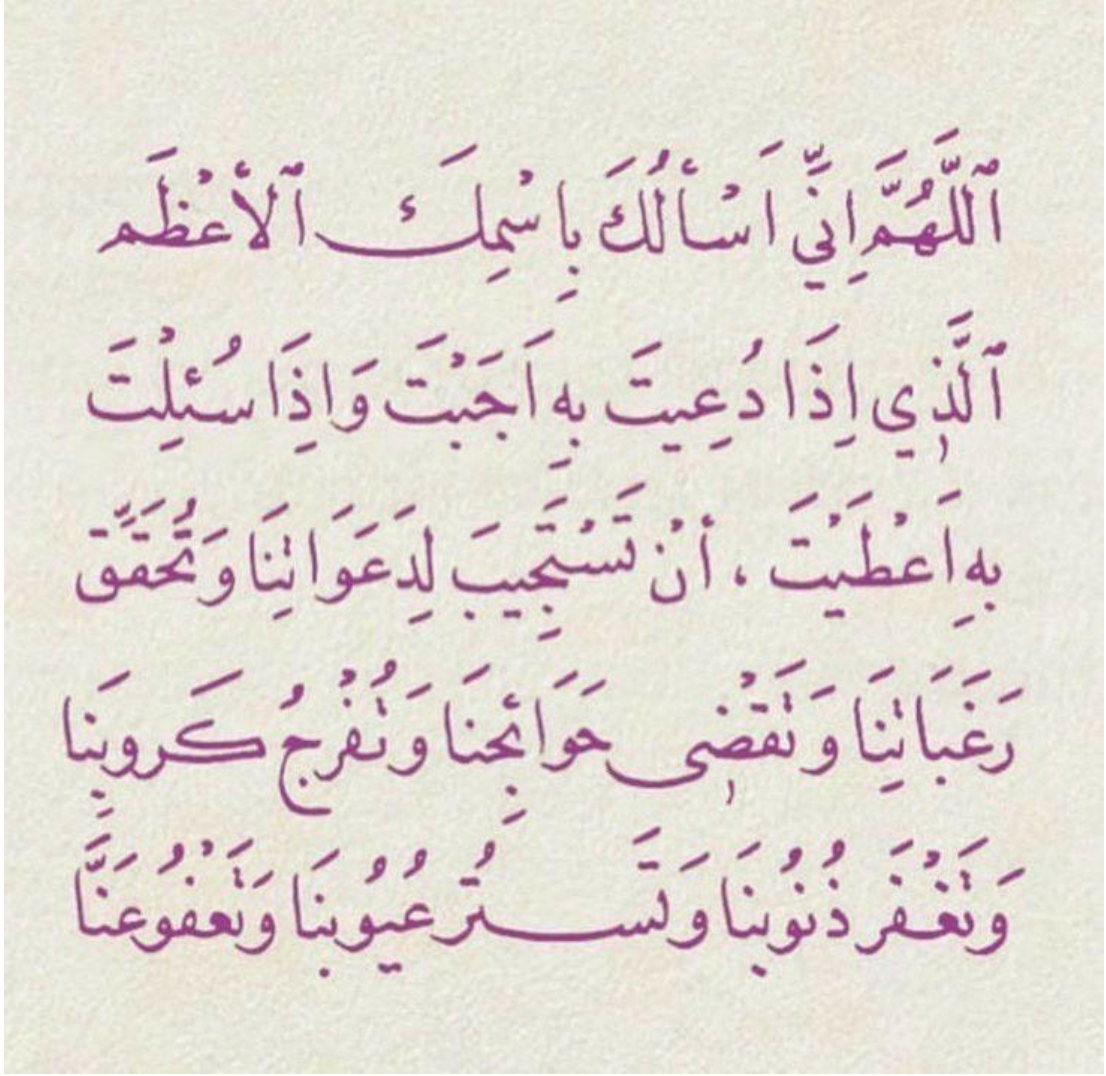

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Corrections from previous versions:

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