Genetics
& Cell biology

◉ Sheet ○ Slides

Number

9

Done by:

Aseelwasfi

Corrected by:

Rana najada

Doctor

Diala

Today we are going to take a new subject regarding the components of the cytoskeleton (specifically microtubules and intermediate filaments)

Microtubule:

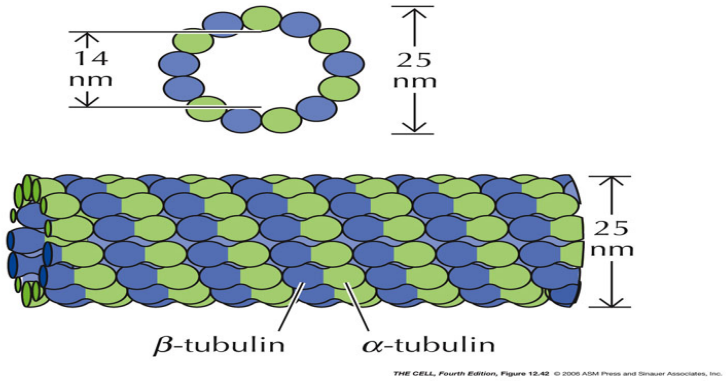
Structural component of the cytoskeleton, they are more rigid than the actin filaments.

1- Function：

a) Their main function is to give mechanical support.
b) They maintain the shape of the cell.
c) They contribute to movement, but in a different way than the actin filament.
   Actin filament: the cell will move as one unit.
   Microtubule: contribute in the movement of the internal structure, like the movement of the vesicles inside the cell.
d) In the separation of the chromosome , by spindle formation.

2- Structure：

a) The monomer is composed of 2 types of proteins (alpha , beta) tubulin.
   There is also (gamma) tubulin which is found in the centrosome, since it contributes in cell division, it's not found in the microtubule that is specialized in movement.
b) It has a hollow center.
c) It has 13 subunit.
   ***Note: In cilia and flagella their microtubules have a different structure (9 outer fiber, 2 central fiber).
d) The internal diameter is 14 nanometer, external diameter is 25 nanometer.
e) They have a very dynamic structure they get remodelled all the time (polarized and depolarized).
f) They are polar : The side that keeps growing is called (plus end/fast growing), and the side that keeps depolarizing is called (minus end/slow growing).
g) Polarity determines the direction of movement along microtubule.
h) To prevent rapid depolymerization the minus end is anchored to the microtubule's organizing center or centrosome .



******How can we distinguish between the two ends inside the cell?**

Through the binding of tubulin to GTP and GDP

The side which binds to GTP , favors polymerization, the side which binds to GDP

favors depolymerization

******The cell will enter one of these three states according to their needs:**

1- Treadmilling: continuing polarization and depolarization of the cell at the same time , like actin filament.
2- Growth: increase in the polarization rate.
3- Catastrophe : increase in the rate of depolarization

3. Drugs that work in the polymerization and the depolymerization process:

Remember that we said that the microtubules have a function in the division of cells by formation of spindles that separate the chromosomes, so we can use drugs that inhibit this step causing the cancer cells to not be able to divide.

a) Taxol: stabilization of microtubule (it doesn't grow or go back)
b) Vinblastine
c) Vincristine

B+C inhibit polarization process

d) Colchicine
e) Colcemid

D+E they are still experimental, they inhibit polymerization.

4- Microtubule accessory proteins (MAP) :

They help in stabilizing the structure of microtubules

a) Polymerases: help the microtubules in their growth.

b) Depolymerases : help the microtubules in catastrophe.
c) CLASB (MAP) : decreases depolymerization and increases polymerization

5- How are microtubules organized Inside neurons

Microtubules are found in all kind of cells, we will take neurons as an example, neurons need a lot of microtubules because their axons can reach more than one meter in length , in order to transport vesicles from the cell body to the axonal ends (synapse) and vice versa.

-Organization inside the axon:

a) The minus end is towards the cell body
b) The plus end is towards the axonal ends (synapse)

. Organization inside the dendrites:

Microtubules runs from the plus end to the minus end , and from the minus end to the plus end .

So vesicles will move in one direction, but actually in the axon there is movement in both directions.

6- Motor (carrier) proteins: control the direction of movement inside the axon :

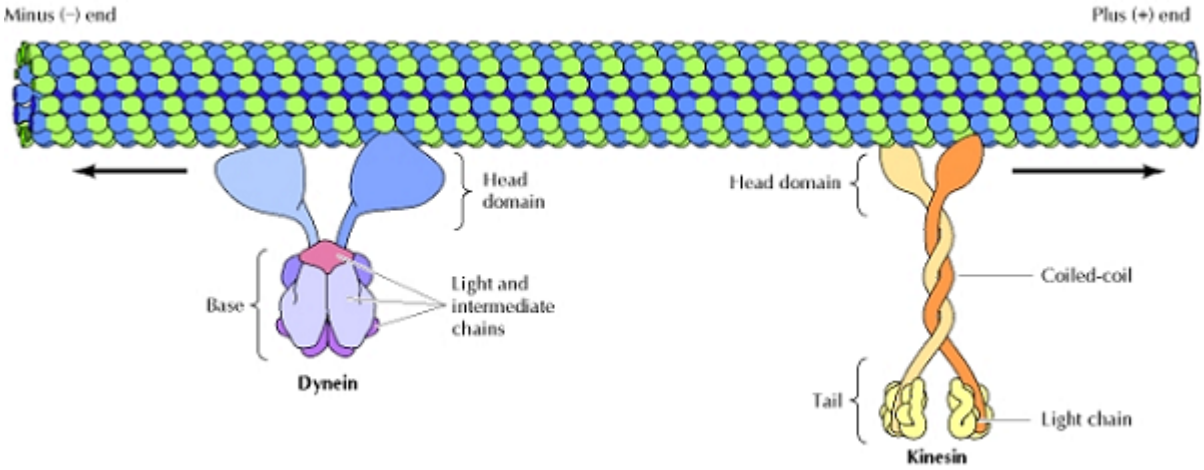
a) Dynein:

- moves from the plus end to the minus end.

- it has a head domain attached to the microtubule.

- it has a base domain which carries the vesicles.

b) Kinesin:
- moves from the minus end to the plus end.
- has a head domain attached to the microtubule.
- has a tail domain that carries the vesicles.



So ,they have a similar structure, but the difference is in the direction of movement and how they move.

Also both of the Dynein and Kinesin need to bind to ATP (in their head domain) in order for movement to happen because binding of ATP alter their conformation.

c) Myosin: is not attached to the microtubule, it's attached to actin filaments instead.

***Microtubules are distributed everywhere inside the cell, but actin filaments are distributed mainly in the cellular cortex so myosin works as a carrier in that place.

7- Labeling of different organelles or vesicles based on: how they move, and which protein carry them:

a) Kinesin：

- Pulls the ER towards the periphery, (it's part of the expansion process of the ER as a response to the stress)

- Carries the lysosomes away from the center of the cell.

- Moves the mitochondria from one place to another.

b) Dynein:

- Moves the Golgi apparatus to the center of the cell.

***Both kinesin an dynein transport selective mRNA molecules inside the cell.

****We mentioned an example earlier about the movement and how it can be stimulated by different stimuli like : (movement of keratin from the melanocyte toward the keratinocyte, and it's stimulated by light)

If there is light: Kinesin will work, Remember that kinesin moves organelles from the minus end to the plus end , and the microtubule extend toward the periphery (minus to plus), so it moves the melanin vesicles to the periphery of the cells and then they bud out to the keratinocytes.

Before the vesicles reach the membrane, they will meet actin filaments, so changing horses will occur and the vesicles will continue moving by myosin which walks in one direction.

If there is no light: dynein will work, remember that dynein moves organelles from the plus end to the minus end , so dynein moves Melanin toward the center of the melanocyte , and inhibits the vesicular transport of melanin and the budding process.

Student questions

1- Once the melanin is in the keratinocytes a change in color will occur, although the melanin is produced in more amounts in summer ,it won't produce a change in color unless the melanin vesicles move to the keratinocytes .
   a. Where the melanin pigment accumulate in the keratinocyte exactly, you should ask a histologist.
2- The differences in human skin color is related to genetic reasons.
3- Myosin can't change its structural feature, in experiments we change the feature of this protein to know its function.

Diseases related to motor protein：

1- Amyotrophic lateral sclerosis (ALS): is due to a mutation in kinesin protein , this reduces the ability of neurons to move organelles from the cell body to the axon leading to neurodegeneration.
2- Charcot Marie tooth disease: is due to mutation in kinesin that lead to peripheral neuropathy

You can read about this disease if you want to know more.

Intermediate filament:

Intermediate in size, intermediate in function, link between actin filaments and microtubules.

They provide mechanical strength to the cells and tissues .

They provide scaffold for localization of cellular processes .

Not involved directly in cell movement.

1- **Intermediate filaments are more variable than actin filaments:**
   a) Type 1,2:
      -Found mainly in epithelial cells .
      -It's protein component is composed of keratin (**Soft keratin**: cytoplasm of epithelial cell, doesn't form hard structure and **Hard keratin**: in nails and hair).

There is more than one kind of keratin that can make the intermediate filament, one type must be acidic, and the other must be basic or neutral .

   b) Type 3: its protein component is Vimentin which is found in fibroblast, smooth muscle cell, white blood cells and Desmin which is found in muscle cells.

The type of protein which makes up the intermediate filaments depends on the cell kind.

   c) Type 4: neurofilaments (found in mature neurons and the axon of motor neuron)

Nistin (found in stem cells, especially stem cells that is going to give rise to neural tissue)

   d) Nuclear lamins: found inside the nucleus, underneath the nuclear envelope.

2- **Assembly of the intermediate filament:**

Although the intermediate filaments are variable, but there are similar properties between them.

a) The protein (monomer) has a head, tail, and a central rod domain (polar molecule).

b) Dimer formation: 2 molecules of this monomer winded around each other in the same direction (head to head, tail to tail)
c) Tetramer formation: 2 dimers will arrange in opposite fashion, 2 heads with each other beside them 2 tails of another dimer .

The two dimers run in an antiparallel direction, but they are not aligned exactly, because the head is larger than the tail, so interference will occur, Steric Henderson will cause displacement.

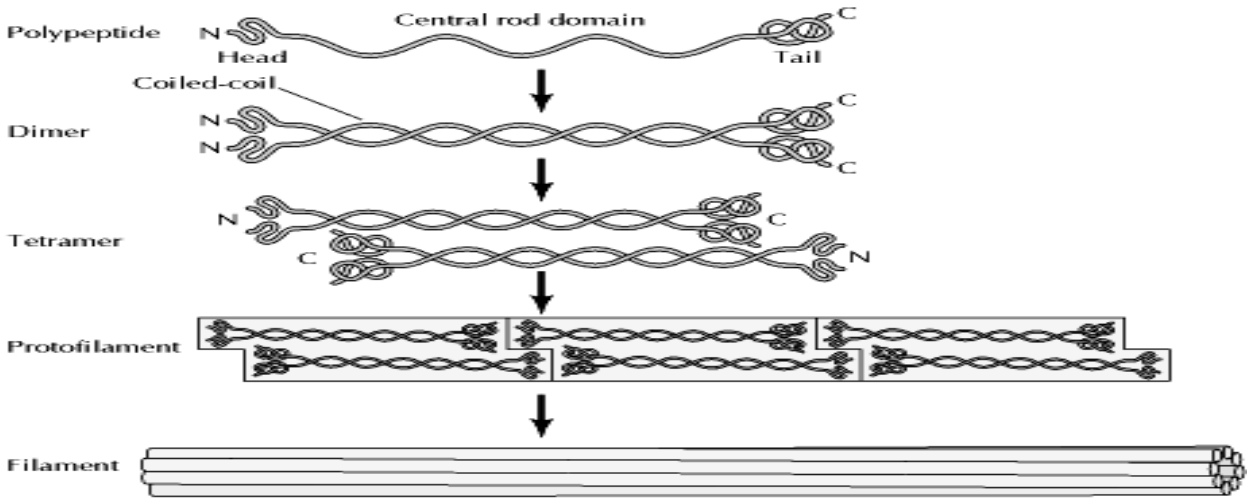
Tetramer is composed of 4 monomers arranged in the same direction.

So when I look at this protofilament each end has 2 heads and 2 tails, so it's non polar.

We started with a polar arrangement but the final arrangement is non polar.

The difference between the intermediate filaments and other cytoskeletons component is that the intermediate filaments are non polar, while the others are polar.

d) 8 tetramers will be arranged in rod like structure , hollow from the inside similar to microtubules but with a smaller diameter .



3- Comparison between assembly and disassembly in the intermediate filament and other cytoskeleton components:

Previously we said that microtubules and actin filaments assembly and disassembly depend on binding to ATP, GTP (assembly), ADP,GDP disassembly.

In the intermediate filament: the disassembly happens through phosphorylation, the addition of a phosphate group that has a negative charge and is relatively a big molecule will cause the dimers to separate from each other .

Intermediate filaments are not dynamic structures , the disassembly and assembly is not a continuous process (they are much more stable in comparison to actin filament and microtubules)

4- The interaction between intermediate filaments and the other cytoskeleton components

a) Keratin filaments are always assembled from heterodimers containing one type 1 and one type 2 polypeptide
b) The type 3 protein can assemble into filaments containing only a single polypeptide (vimentin) or consisting of two different type 3 proteins (vimentin plus desmin).
c) The type 3 proteins do not form copolymers with the keratins.
d) Alpha internexin, type 4 protein, can assemble into filaments by itself, but the NFs (neurofilaments) copolymerize to form heteropolymers.
e) Phosphorylation affects the assembly and disassembly of Ifs.

As we know that the intermediate filaments link between the actin filaments and the microtubules, so they have some flexibility.

We will see some examples about this interaction:

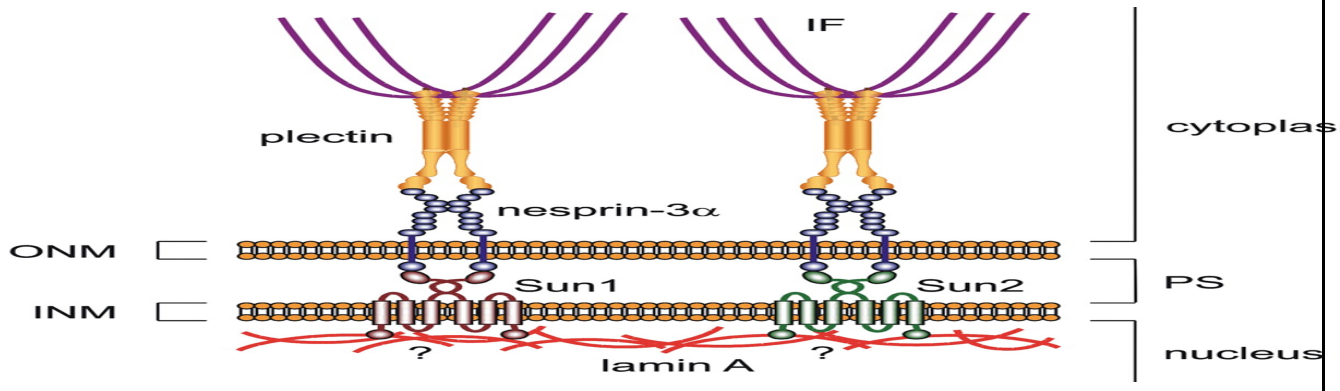
a. Desmin intermediate filament in muscles: we have type 3 of intermediate filaments , alpha actinian in red, desmin make a connection between the actin filament and the other components.

   - If there is a mutation in desmisn molecule there will be a problem in the smooth muscles, especially in the heart, and that will lead to cardiomyopathy.

b. In type 4 (neurofilament) will anchor the microtubules and the actin filaments

   The proteins that make the connection with the other components of cytoskeleton is the (blacken family) like plectin.(bridges microtubule to actin filaments and stabilizes

them and increases the mechanical stability of the cell).

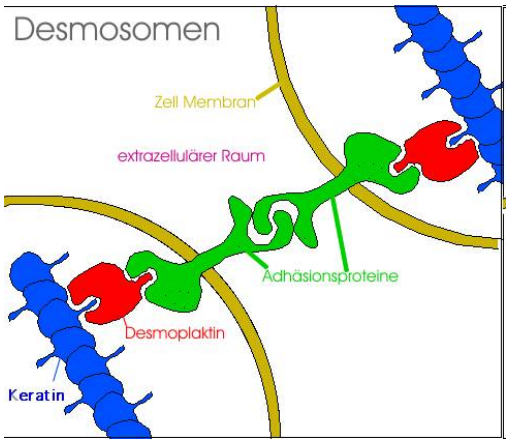


5. **How these intermediate filaments contribute to cellular junctions like (desmosome)**

Two membrane proteins from different cells interact with each other, and interact from the inside to a connector molecule that is connected to the cytoskeleton.

Integral membrane protein binds to a connector protein (that differs according to the kind of intermediate filament that binds to it)

In this example we talk about type 1,2 ,so the intermediate filament is composed of keratin, so the connector protein will be desmoplakin that will bind to a membrane protein and make the structure of the desmosome.



******Hemidesmosome:

Connects the cell to the ECM.

Membrane protein (integrin).

Connector protein (plectin: that binds to intermediate filaments type 4).

The plectin will interact with integrin and then with other protiens until it's connected to the components of the ECM.

6. Application on intermediate filament diseases：

Experiment done in mice where they disrupted the structure of intermediate filament type 3: vimentin and there was no effect on growth or movement.

So they concluded that disruption of intermediate filaments is not lethal.

The experiments were done using Transgenic mice which expressed mutated keratin gene and that resulted in severe skin abnormalities (blisters due to epidermal cell lysis following mild mechanical trauma).

a. Human epidermolysis bulossa: mutation in keratin gene that interfere with the normal assembly of keratin filaments causing skin blisters after minor trauma (wearing tight shoes will reactivate them).
b. ALS ：related to mutation in intermediate filament type 4 leading to progressive loss of motor neurons which in turn lead to muscle atrophy, paralysis, and eventual death.