University of Jordan
Faculty of Medicine

GENETICS & Molecular Biology

Number: 24

Done By: Abdullah S. Sulaiman

Corrected By: Neveen Azzam

Subject: **Microtubules and intermediate filaments**

Doctor: Diala Abu-Hassan

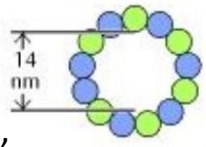
Price:

Date:

Last time we discussed actin filament (microfilaments). Today we are going to discuss microtubules as a part of cytoskeleton as well as the intermediate filaments (IFs).

☞ Let's see some **differences between actin filaments and *microtubules*** :

- Larger in term of size with 25nm diameter.
- Different functions as they are used for organelles and vesicular transport (intracellular movements), and movement + separation of chromosomes during mitosis.
- Use GTP/GDP instead of ATP/ADP
- Made of three different types of monomers "tubulins" (α-, β- and γ-), but the mainly two are α-tubulin and β-tubulin. If you take a cross-section to the microtubules you notice 13 monomers of tubulin assemble around a hollow core.

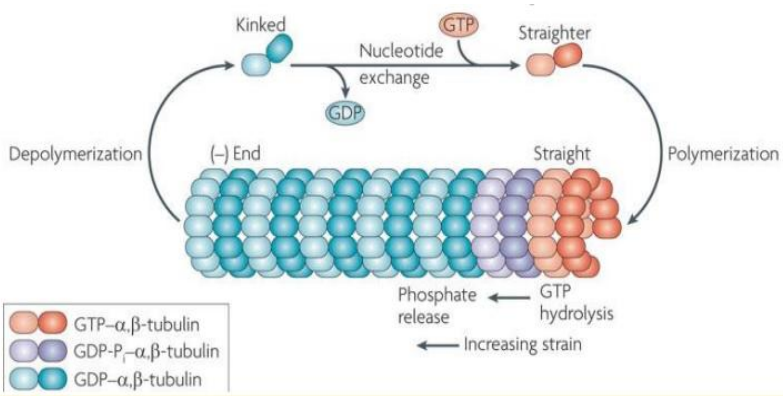


✦ **γ-tubulin** is concentrated in the centrosome and it initiates microtubule assembly.

ꕥ **How do these monomers assemble to form the microtubule?**
It is formed from basic unit structure called protofilament which is a line of tubulin protein dimers (alpha>beta>alpha>beta….), then 13 linear protofilaments are arranged close to each other to make a **hollow tube** of microtubule.

ꕥ How does the assembly occur?
If the molecule (α, β-tubulin) is bounded to **GTP** then the conformation is straighter and suitable to fit in the (+) end of the microtubule. Where in the (-) end there is more depolymerization due to the presence of more kinked tubulin-GDP molecules that can't fit well.

Both α- and β-tubulin bind GTP



Microtubules are polar structures

Have + end where more assembly/ polymerization happen

And – end where more disassembly happen

- The feature of rapid cycles of assembly and disassembly is called **Treadmilling**.
- The same process in actin is also called treadmilling.
- Sometimes , the assembly and disassembly are not equal in both ends and we don't have treadmilling as the length is not stable (**Dynamic instability**) :

Growth or shrinkage is determined by .. the rate of tubulin addition relative to the rate of GTP hydrolysis

- More GTP hydrolysis > shrinkage and catastrophe.
- More tubulin-GTP addition > growth and rescue.

Microtubules can be anchored from its (-) end to some structures as centrosomes during division.

Application: <u>Drugs that affect microtubule assembly :</u>

✓ **Experimental:** are used in experiments for science.
Colchicine and ***Colcemid*** bind tubulin, ***inhibit polymerization*** and block mitosis.

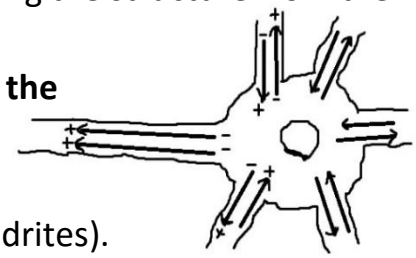
✓ **Cancer treatment** (anti-cancerous agents) as well as for experiments:
Vinblastine and ***vincristine*** bind specifically to tubulin and ***prevent their polymerization*** to form microtubules resulting in inhibition of rapidly dividing cells. ***Taxol*** ***stabilizes microtubule*** (prevent the shortening) and ***blocks cell division***.

❖ As there are regulatory proteins for actin, the same thing applies here. We have **Microtubule-associated proteins (MAPs)** :

- Some of them are **polymerases** > accelerate polymerization. (growth)
- Others are **depolymerases** such as kinesin 8 and 13. (shrinkage)
- **CLASP**, prevent the disassembly at one side and promote the assembly at the other side (affects both ends).

How are these structures (microtubules) involved in cellular functions?

I. Firstly let's talk about neurons :
The *dendrites* receive stimuli from the surrounding environment and transmit the signals to the cell body, and the *axon* transmits the signal away from the cell body of neuronal cell. Therefore, due to the polarity of the microtubules there is a direction of movement along the structure from the (-) end to the (+) end.
So, we can conclude a **bidirectional movement in the dendrites** (on for transmitting the signals towards the cell body and one for vesicular transport of dendrites' proteins from the body toward the dendrites).



On the other hand, we would have a **one direction of movement in the axon** towards the synapse. Here there should be more assembly to support the axon along its way.

II. The movement of vesicles along these microtubules depends on different motor molecules; each has two heads for walking. Examples of these :

a. Dynein: moves from (+) end to (-) end, so the vesicular transport doesn't depend only on the movement (polarity) of the microtubules but also on the motor proteins.
b. Kinesin: moves from (-) end to (+) end.

❖ The common features between kinesin and dynein are :
- Two head domains that can bind ATP - this process need energy
- Connecting region (coiled-coil in kinesin while it's smaller in dynein)
- Tail or base where can bind other structures.

ꕥ What makes them move?
ATP binding to the heads will affect the conformation which will differ from the conformation result by ADP binding.
One conformation will enhance microtubule binding and the other will enhance the detachment[No need to know is it ATP or ADP the one which causes attachment]

ꕥ How do these two molecules contribute to **organelles movement**?

Mostly the nucleus is located at the centre of the cell, microtubules start next to the nucleus "(-) end", radiating out towards the periphery/ plasma membrane "(+) end".

✓ **Kinesin**: pulls the endoplasmic reticulum (to extend it) toward the cell periphery, positions lysosomes away from the center of the cell (as the anchoring around the nucleus {(-) end} and radiating towards the periphery {(+) end}), and controls the movements of mitochondria.
✓ Cytoplasmic **dynein**: positions the Golgi apparatus in the center of the cell (in close proximity to the ER).
✓ Both **kinesin** and **dynein** transport selective mRNA molecules in cell, depending on the direction of movement.

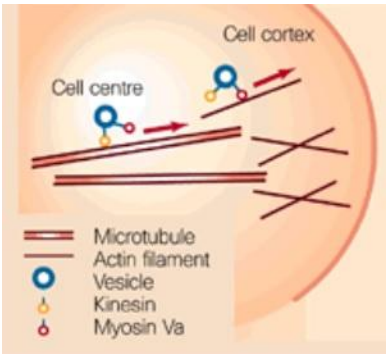
Application :

✦ **Stimulated movement**: Why do we get darker when we are exposed to light?
Melanocytes position the pigmented organelles, melanosomes (where melanin is synthesized), in response to the amount of light:
- When light is present it *stimulates* kinesin to move melanosomes to the periphery of cells, so they can be released into keratinocytes.
- In the dark, dynein returns the melanosomes to the center of the cell.

✦ **Kinesins and diseases :**

- ✓ **Mutations in certain kinesin proteins** reduce the ability of neurons to move essential organelles from their cell bodies to their axons **leading to neurodegeneration** such **as** amyotrophic lateral sclerosis **(ALS).**
- ✓ **Mutations in kinesins** lead to ***peripheral*** **neuropathies** such as **Charcot-Marie-Tooth disease.**
  CNS is affected in ALS while it's the PNS in case of Charcot-Marie-Tooth disease.

✦ **Changing horses in midstream :**



Kinesins will move and carry organelles toward the periphery then **myosins** will take over and move the organelles near the plasma membrane.
(Kinesins walk on microtubules, where myosins walk on actin filaments).
A probable explanation is the present of the **cortical cytoskeleton** which is made of actin filaments just underneath the plasma membrane, and to reach our final destination we need myosin to walk on the actin.

☞ Now let's discuss the ***intermediate filaments*** (IFs) :

- Their diameter is intermediate between those of actin filaments and microtubule.
- They are composed of a variety of proteins, which are classified into 5 groups based on similarities between their amino acid sequences.
  (So many types of monomers depending on the cell type and the function of these filaments).
- They provide mechanical strength to cells and tissues (connect actin filaments to microtubules)
- Not involved directly in cell movement.
- They provide a scaffold for localization of cellular processes.

❖ **Types of IF proteins :**

1. **Types I and II**: are expressed in epithelial cells and called *keratin*.
   Type I is acidic keratin and type II is (neutral/basic) keratin.
   Keratin provides strength and stability and is divided into:
   - **Hard** keratins are used for production of structures such as hair, nails, and horns.

- **Soft** keratins are abundant in the cytoplasm of epithelial cells.

These types of proteins are found as a part of connections between cells, called desmosomes and hemidesmosomes.

2. **Type III**: different types of protein component :
   Vimentin is found in fibroblasts, smooth muscle cells, and white blood cells.
   Desmin is specifically expressed in muscle cells (related to Z-lines).

3. **Type IV**:
   Neurofilament (NF) found in mature neurons and the axons of motor neurons.
   Nestin in stem cells (important in development of cells).
4. **Type V**: *nuclear lamins*, components of the nuclear envelope.

☞ Let's talk about the **structure and the assembly of different types of IFs** :
IFs are made of different proteins depending on the cell type and function, but they ***share a central α-helical rod domain for filament assembly***, while different sequences, sizes and secondary structures at the N-terminus (head) and at the C-terminus (tail).

Assembly: (see the figure in the slides)

i. Two polypeptides dimerize in the same direction (N to N and C to C).
ii. Then two dimers can associate together to form a tetramer in anti-parallel direction (so no polarity here).
iii. These tetramers would assemble end to end to form a protofilament.
iv. 8 of the protofilaments would assemble to form an intermediate filament.
As there is no polarity the structure is more stable.

Note: IFs are modified by phosphorylation, which can regulate their assembly and disassembly within the cell, **phosphorylation of the filaments themselves leads to disassembly**. It is Not GTP/GDP dependent as others.

☞ **IFs can interact with each other or with other components of cytoskeleton as the following :**

- ✓ **Keratin** filaments are always assembled from ***heterodimers*** containing one type I (acidic) and one type II polypeptide (neutral/basic).
- ✓ The type III proteins can assemble into filaments containing only a single polypeptide {***homodimer***} (e.g., vimentin) ***or*** consisting of two different type III proteins {***heterodimer***} (e.g., vimentin plus desmin).
- ✓ The type III proteins do not form copolymers with the keratins (type II).
- ✓ α-internexin, a type IV protein, can assemble into filaments by itself, but the NFs copolymerize to form ***heteropolymers.***

❖ **Intracellular Organization of IFs:**

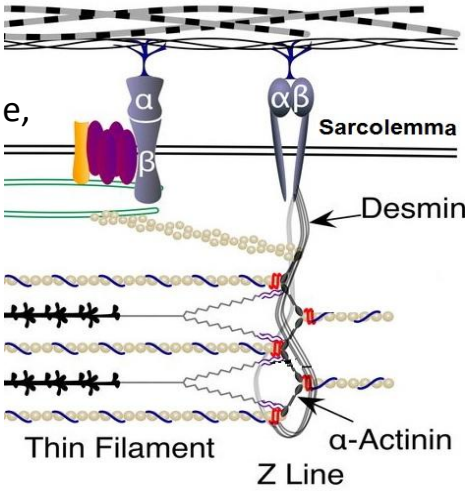
IFs form an elaborate **network** in the cytoplasm extending from a ring surrounding the nucleus to plasma membrane.

IFs can associate with the plasma membrane, the actin filaments and microtubules.

☞ **Let's see how they work in muscles : (Desmin IFs in muscles)**

Desmin connects the actin filaments in muscle cells to one another and to the plasma membrane, thereby linking the actions of individual contractile elements. While **alpha-actinin** only connects between actin filaments.

Desmin mutations cause muscle defects such as early onset cardiomyopathy.



☞ The major type of IFs in neurons is **neurofilament(NF, type IV):**

Neurofilaments in mature neurons (especially motor neurons) are anchored to actin filaments and microtubules by neuronal members of the **plakin** family (intermediate proteins).

Neurofilaments provide mechanical support and stabilize the cytoskeleton in the long, thin axons of nerve cells (especially motor neurons).

Notes : **(for more see** http://sites.sinauer.com/cooper6e/flashcards12.html **)**

γ-tubulin ring complex :A protein complex that nucleates the formation of microtubules.

Desmosome: A region ***of contact between epithelial cells*** at which keratin filaments are anchored to the plasma membrane.

Hemidesmosome : A region of ***contact between cells and the extracellular matrix*** at which keratin filaments are attached to integrin.

Plakin :A member of a family of proteins that link intermediate filaments to other cellular structures.

Taxol :A drug that binds to and stabilizes microtubules.

Sorry for any mistakes ☺

Special thanks to Hanin Saleh