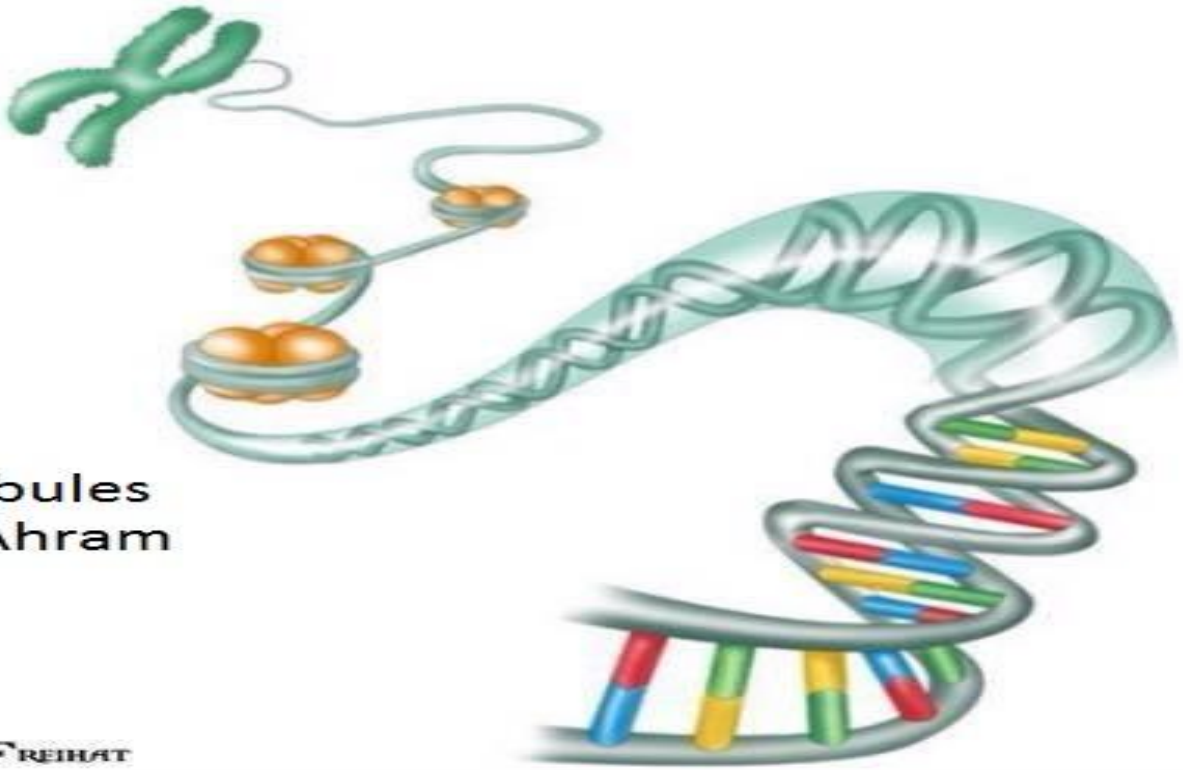
University of Jordan
Faculty of Medicine
Batch 2013-2019

# Genetics & Molecular Biology

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



Number # 7
**Title:** Microtubules
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# Lecture 7: the cytoskeleton and cell movement

## (Microtubules and intermediate filaments)

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**Faculty of Medicine**
**Second year, Second semester, 2014-2014**

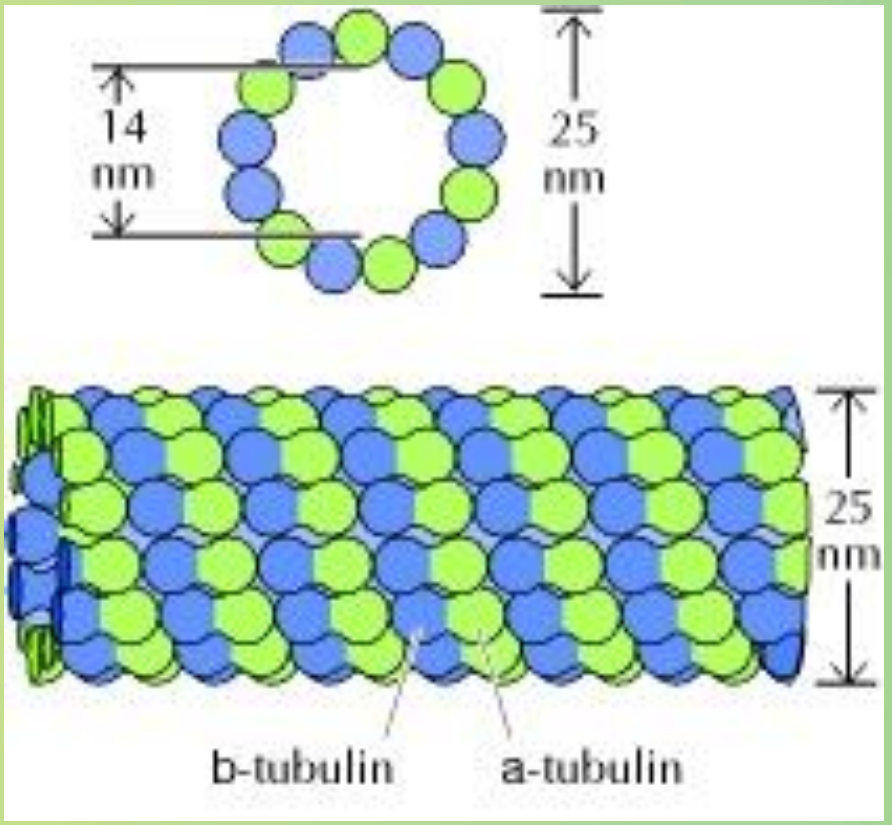
***Principles of Genetics and Molecular Biology***

# Overview

- Second predominant component of cytoskeleton
- They are rigid hollow rods
- They are dynamic structures that undergo continual assembly and disassembly within the cell.
- Functions:
  - Cell shape
  - Cell movements (some forms of cell locomotion)
  - Intracellular transport of organelles
  - Separation of chromosomes during mitosis

# Structure of microtubules

- Microtubules are composed of a single type of globular protein, called tubulin.
- Tubulin is a dimer consisting of two closely related polypeptides, α-tubulin and β-tubulin.

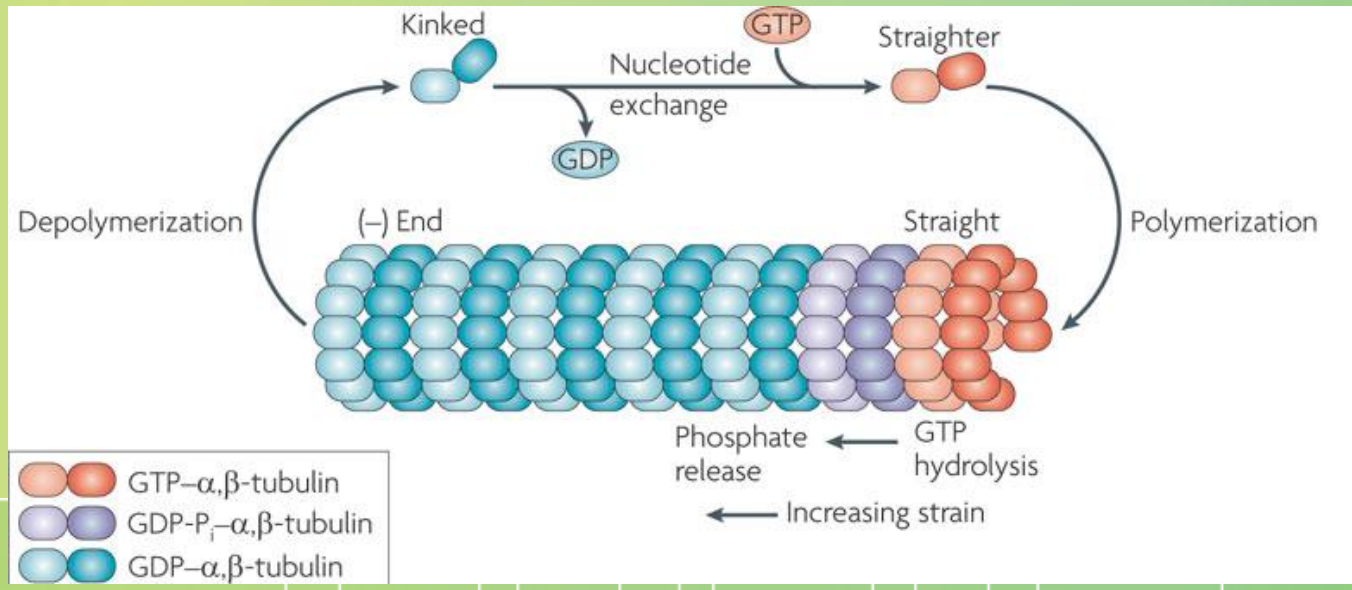



- γ-tubulin is specifically localized to the centrosome.
  - It initiates microtubule assembly.

# Polymerization of tubulin

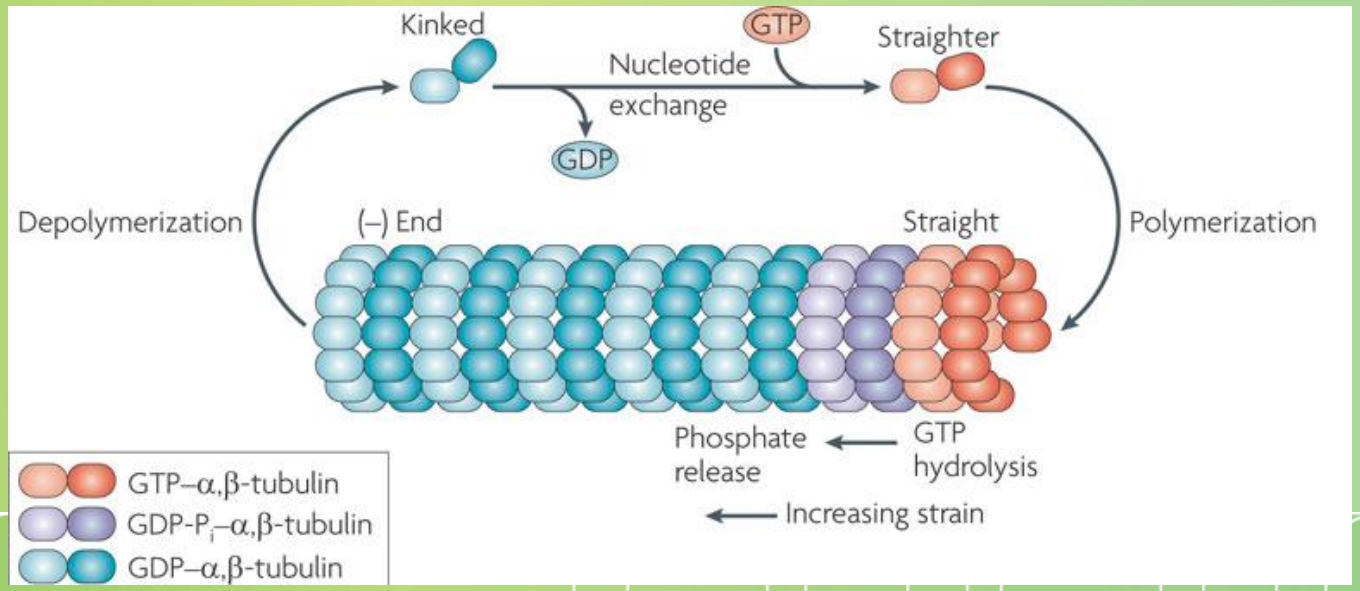
- Tubulin dimers polymerize to form protofilaments (a hollow core).
- Protofilaments are arranged in parallel and are composed of head-to-tail arrays of tubulin dimers.
  - Microtubules are polar structures : a fast-growing plus end and a slow-growing minus end.
  - Polarity determines the direction of movement along microtubules.

**Both α- and β-tubulin bind GTP**



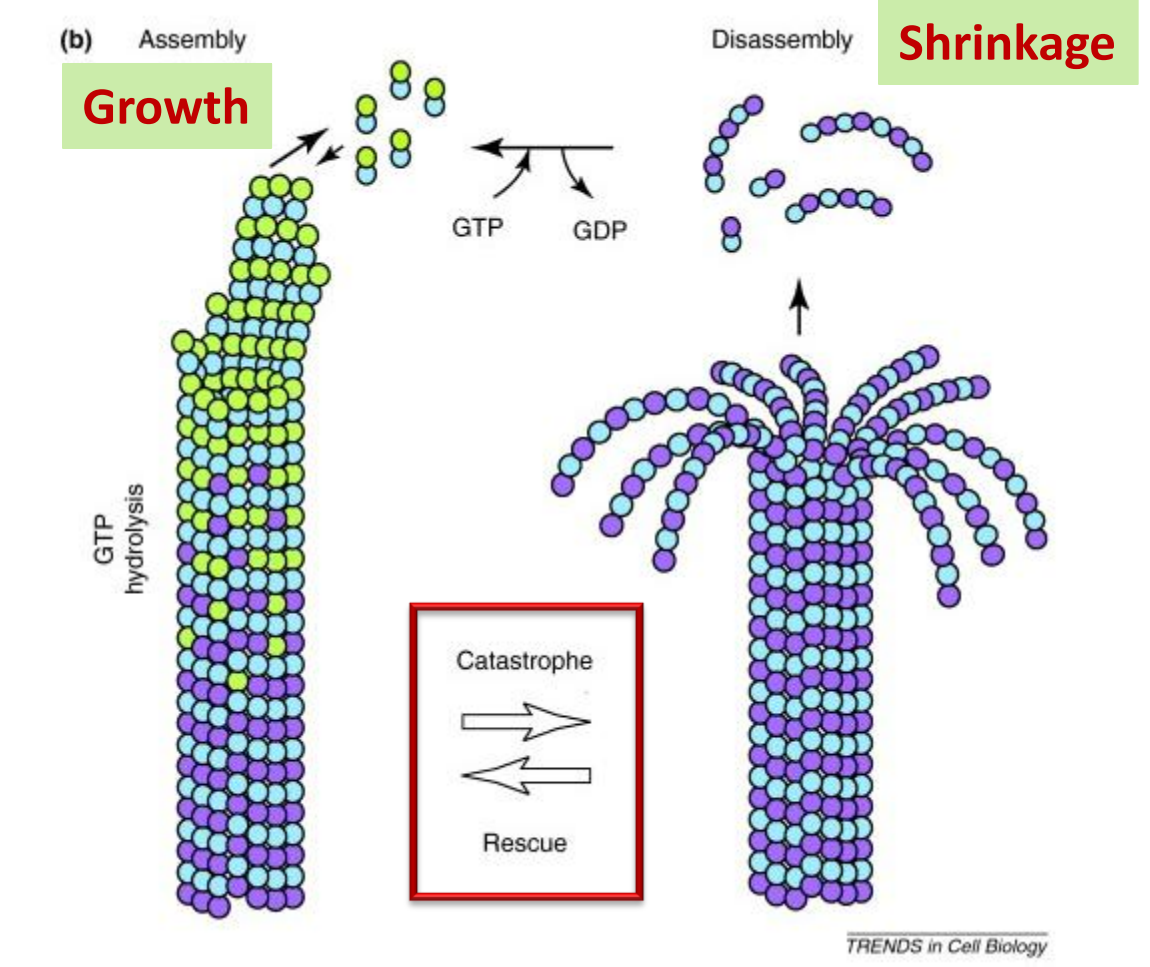
# Treadmilling

- Microtubules can undergo rapid cycles of assembly and disassembly (treadmilling) where tubulin molecules are continually lost from the minus end and replaced by the addition of tubulin molecules bound to GTP to the plus end.
- The GTP bound to β-tubulin is hydrolyzed to GDP during or shortly after polymerization weakening the binding affinity and favoring depolymerization.



# Dynamic instability
# (Rate of poymerization-depolymerization)

Catastrophe occurs when GTP is hydrolyzed at the plus end before new GTP-tubulin is added and there is a transition from growth to shrinkage.
On the other hand, transition from shortening to growth is called rescue.

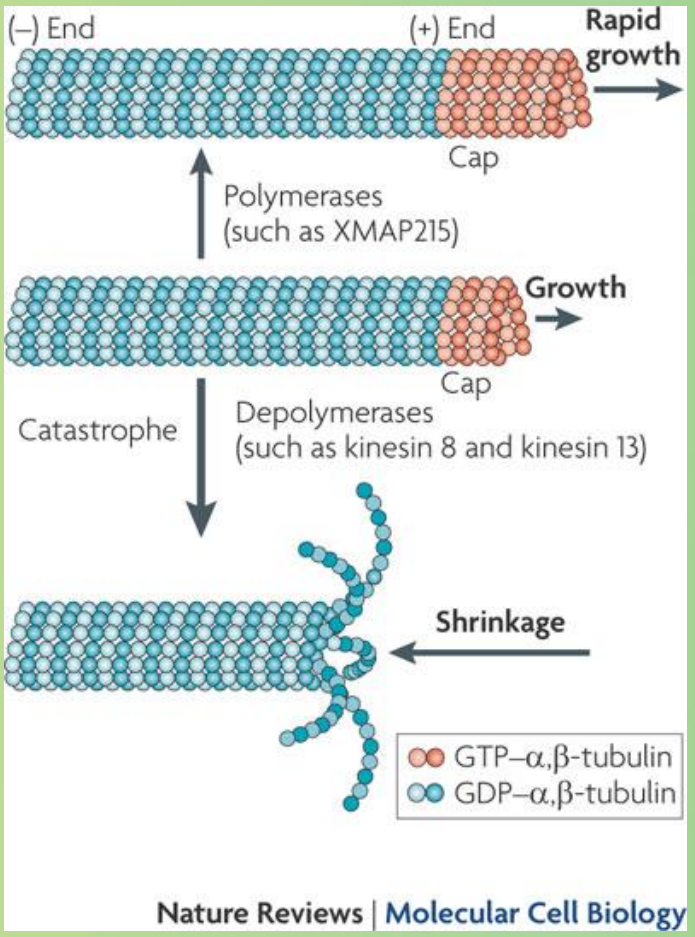


# Drugs

- Colchicine and colcemid bind tubulin, inhibit polymerization, and block mitosis.
- Vinblastine and vincristine bind specifically to tubulin and prevent their polymerization to form microtubules.
- Taxol stabilizes microtubule and blocks cell division.

# Regulatory proteins

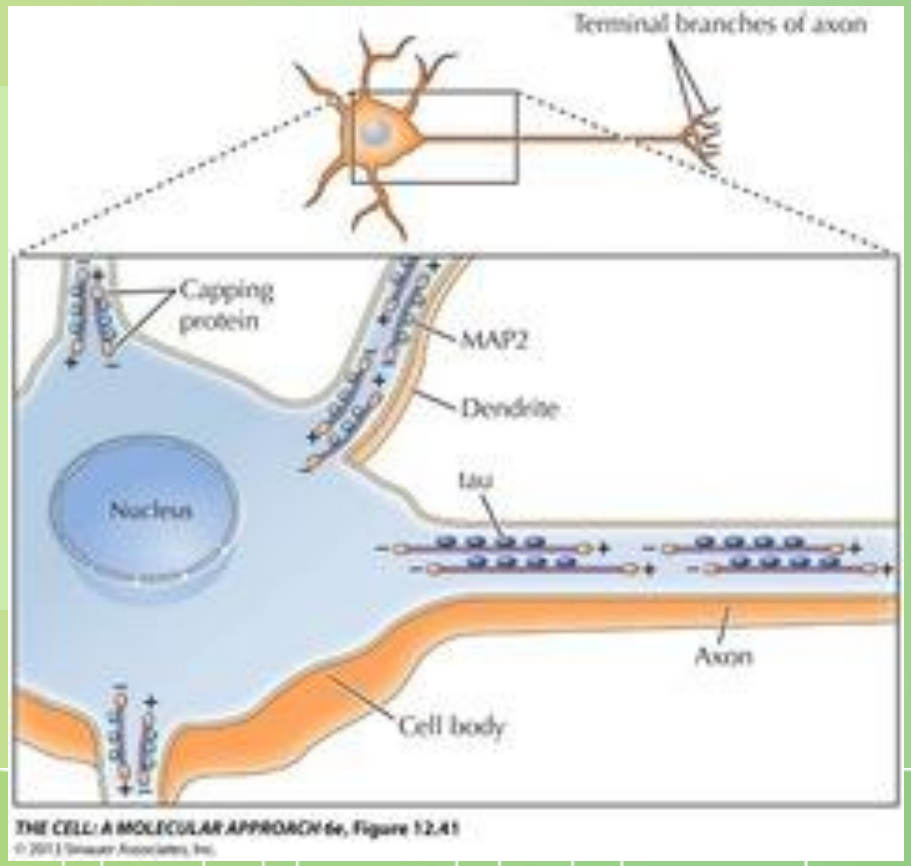
- Microtubule-associated proteins (MAPs) such as polymerase regulate growth and shrinkage at the plus end.
- depolymerases stimulate shrinkage by accelerating the dissociation of GTP-tubulin from the plus end.
- CLASP, a MAP, prevent disassembly (catastrophe) and promote restarting growth (rescue).



# Organization of microtubules within cells Example: neuron

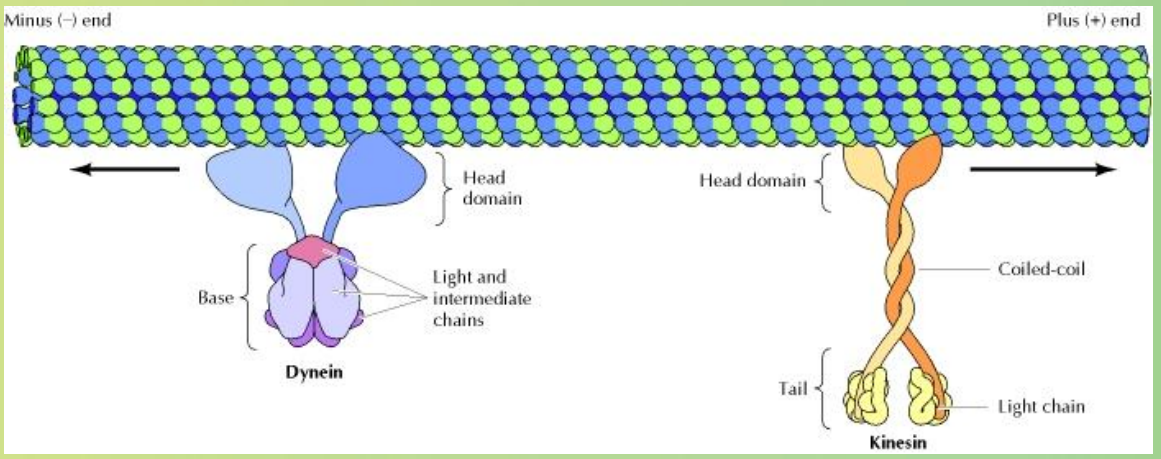
- **Neurons have two types of processes extend from the cell body:**
  - **Dendrites: short; receive stimuli from other nerve cells**
  - **Axon; long; carries impulses from the cell body to other cells**

- **In dendrites, microtubules are bound to MAP2 and are oriented in both directions.**
- **Microtubules in axons are bound to tau and are oriented with their plus ends pointing toward the tip of the axon.**



# Vesicular transport

- **Microtubules-motor proteins such as kinesin and dynein move along microtubules in opposite directions**
  - **kinesin move toward the plus end and dynein toward the minus end.**




**In neurons, kinesin assists in transporting vesicles and organelles toward the end of the axon. It gets its energy from the hydrolysis of ATP that is bound to the head domain that also binds to microtubule. The tail portion binds to cell components (e.g., membrane vesicles and organelles.**

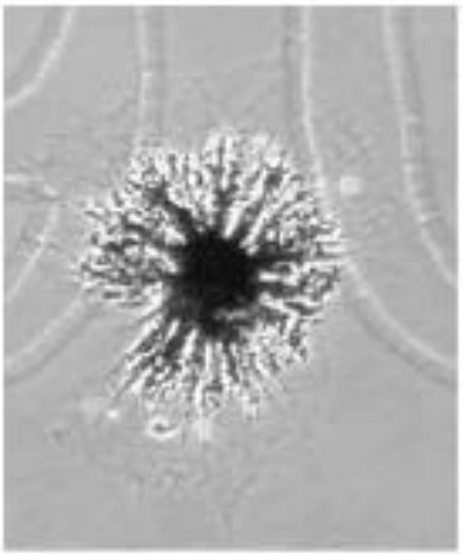
**The head domain of dynein forms the ATP-binding motor domains that are responsible for movement along microtubules. The basal portion of dynein is thought to bind to other subcellular structures, such as organelles and vesicles.**

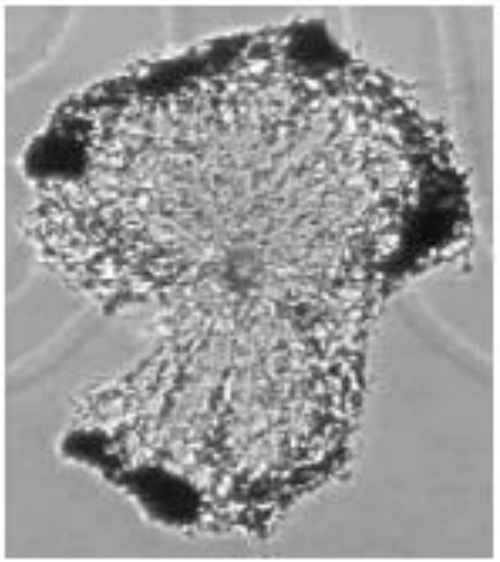
# Organelle organizations

- kinesin pulls the endoplasmic reticulum toward the cell periphery.
- Kinesin positions lysosomes away from the center of the cell
- Members of the kinesin family control the movements of mitochondria.
- Cytoplasmic dynein positions the Golgi apparatus in the center of the cell.
- Both kinesin and dynein transport selective mRNA molecules in cell.

# Stimulated movement

- Organelles will often have both types of motors on their surface, allowing cells to adjust their position.
- Melanocytes position the pigmented organelles, melanosomes, in response to the amount of light.
  - In the presence of ligh, kinesin moves melanosomes to the periphery of cells.
  - In the dark, dynein returns the melanosomes to the center of the cell.

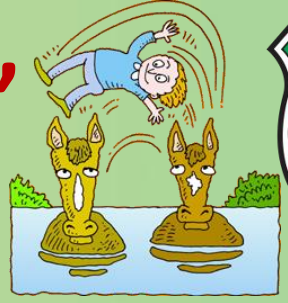




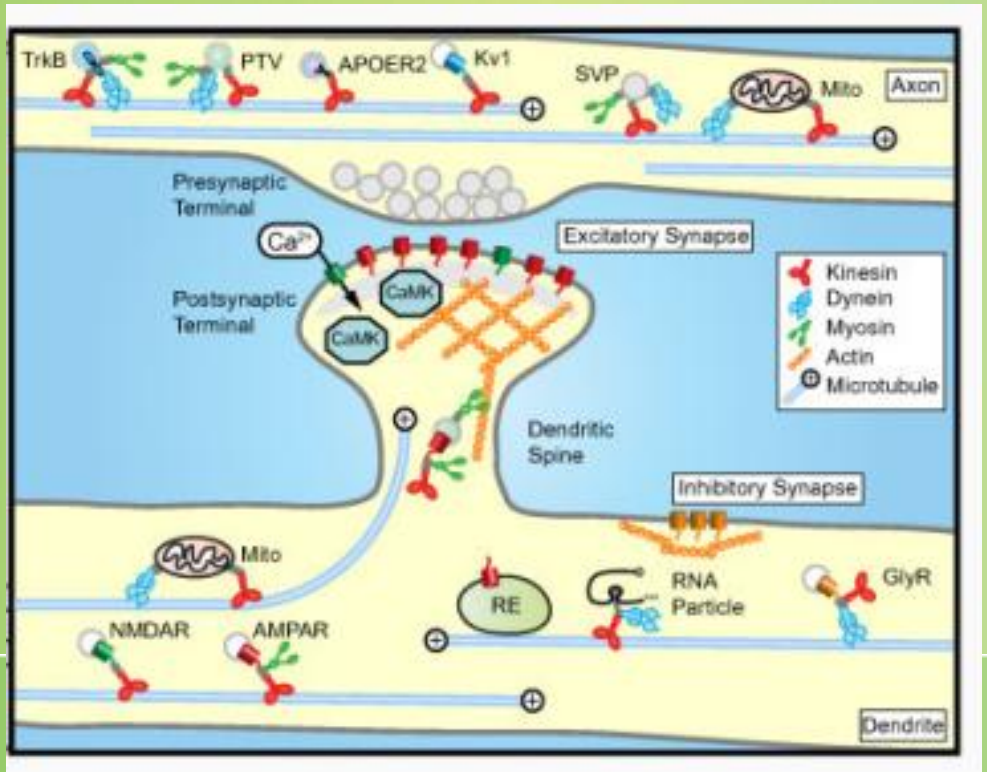
# Kinesins and diseases

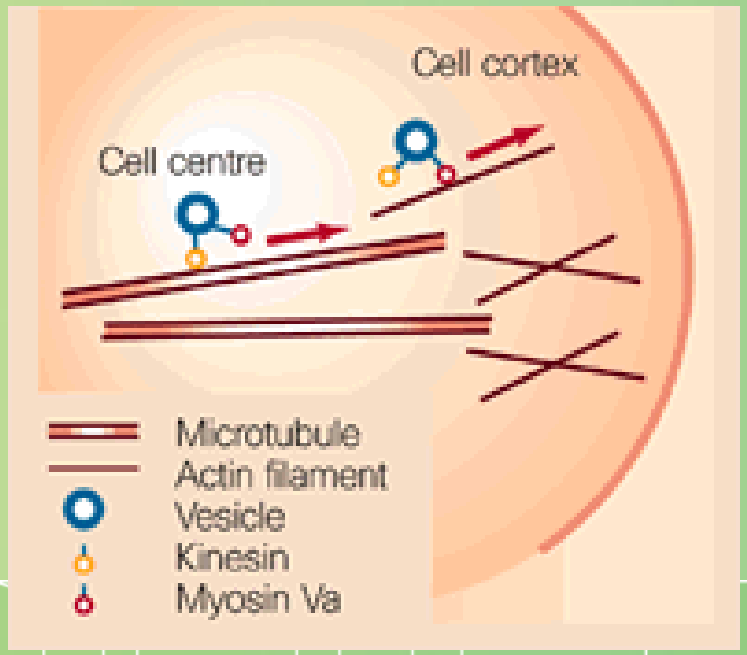
- Mutants in certain kinesin proteins reduce the ability of neurons move essential organelles from their cell bodies to their axons leading to neurodegeneration such as amyotrophic lateral sclerosis (ALS).
- Mutations in kinesins lead to peripheral neuropathies such as Charcot-Marie-Tooth.

# “Changing horses in midstream”



- Myosins of actins transport organelles over shorter distances compared to microtubules’s kinesins and dyneins.
- Kinesins and myosins transport organelles from the center of the cell towards the periphery, where myosins take over moving organelles near the plasma membrane.



# Intermediate filaments

# What are they?

- Intermediate filaments have a diameter that is intermediate between those of actin filaments and microtubule.
- They provide mechanical strength to cells and tissues.
- They are composed of a variety of proteins, which are classified into 5 groups based on similarities between their amino acid sequences.

# Types of IFs

- Types I and II are expressed in epithelial cells with each type of cell synthesizing at least one type I (acidic) and one type II (neutral/basic) keratin.
  - Hard keratins are used for production of structures such as hair, nails, and horns.
  - Soft keratins are abundant in the cytoplasm of epithelial cells.
- Type III:
  - Vimentin, which is found in fibroblasts, smooth muscle cells, and white blood cells.
  - Desmin is specifically expressed in muscle cells.
- Type IV: neurofilament (NF) found in the axons of motor neurons.
- Type V: nuclear lamins, omponents of the nuclear envelope.

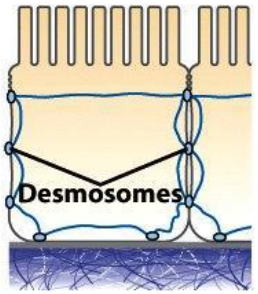
# Types of IFs

| Type | Protein | Site of expression |
|---|---|---|
| I | Acidic keratins | Epithelial cells |
| II | Neutral or basic keratins | Epithelial cells |
| III | Vimentin | Fibroblasts, white blood cells, and other cell types |
| | Desmin | Muscle cells |
| | Glial fibrillary acidic protein | Glial cells |
| | Peripherin | Peripheral neurons |
| IV | Neurofilament proteins | |
| | NF-L | Neurons |
| | NF-M | Neurons |
| | NF-H | Neurons |
| | α-Internexin | Neurons |
| | Nestin | Stem cells of central nervous system |
| V | Nuclear lamins | Nuclear lamina of all cell types |

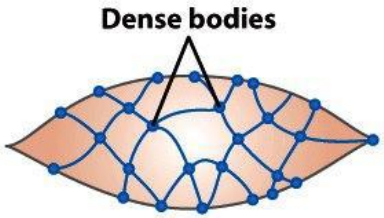
# Types of IFs

**TABLE 18-1 The Major Classes of Intermediate Filaments in Mammals**

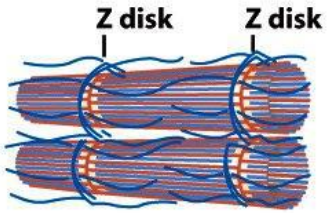
| CLASS | PROTEIN | DISTRIBUTION | PROPOSED FUNCTION |
|---|---|---|---|
| I | Acidic keratins | Epithelial cells | Tissue strength and integrity |
| II | Basic keratins | Epithelial cells | |
| III | Desmin, GFAP, vimentin | Muscle, glial cells, mesenchymal cells | Sarcomere organization, integrity |
| IV | Neurofilaments (NFL, NFM, and NFH) | Neurons | Axon organization |
| V | Lamins | Nucleus | Nuclear structure and organization |



Epithelial cell



Smooth muscle



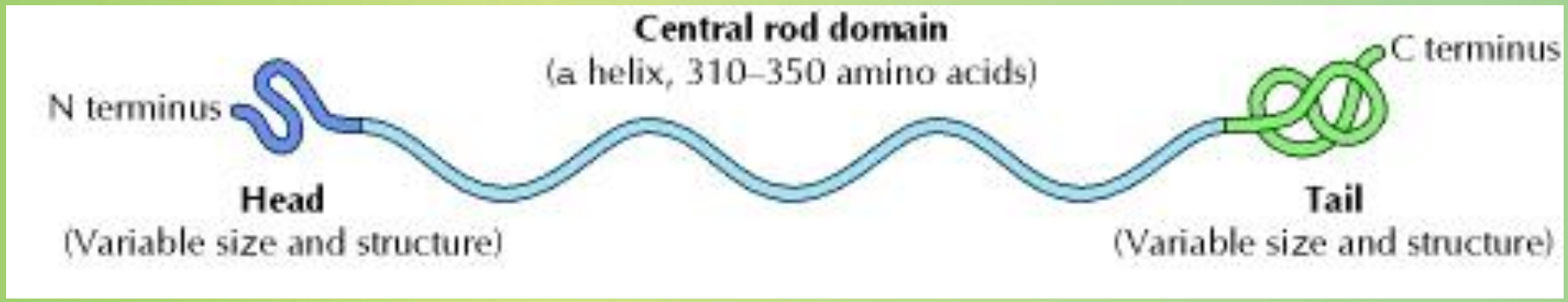
Skeletal muscle


Axon

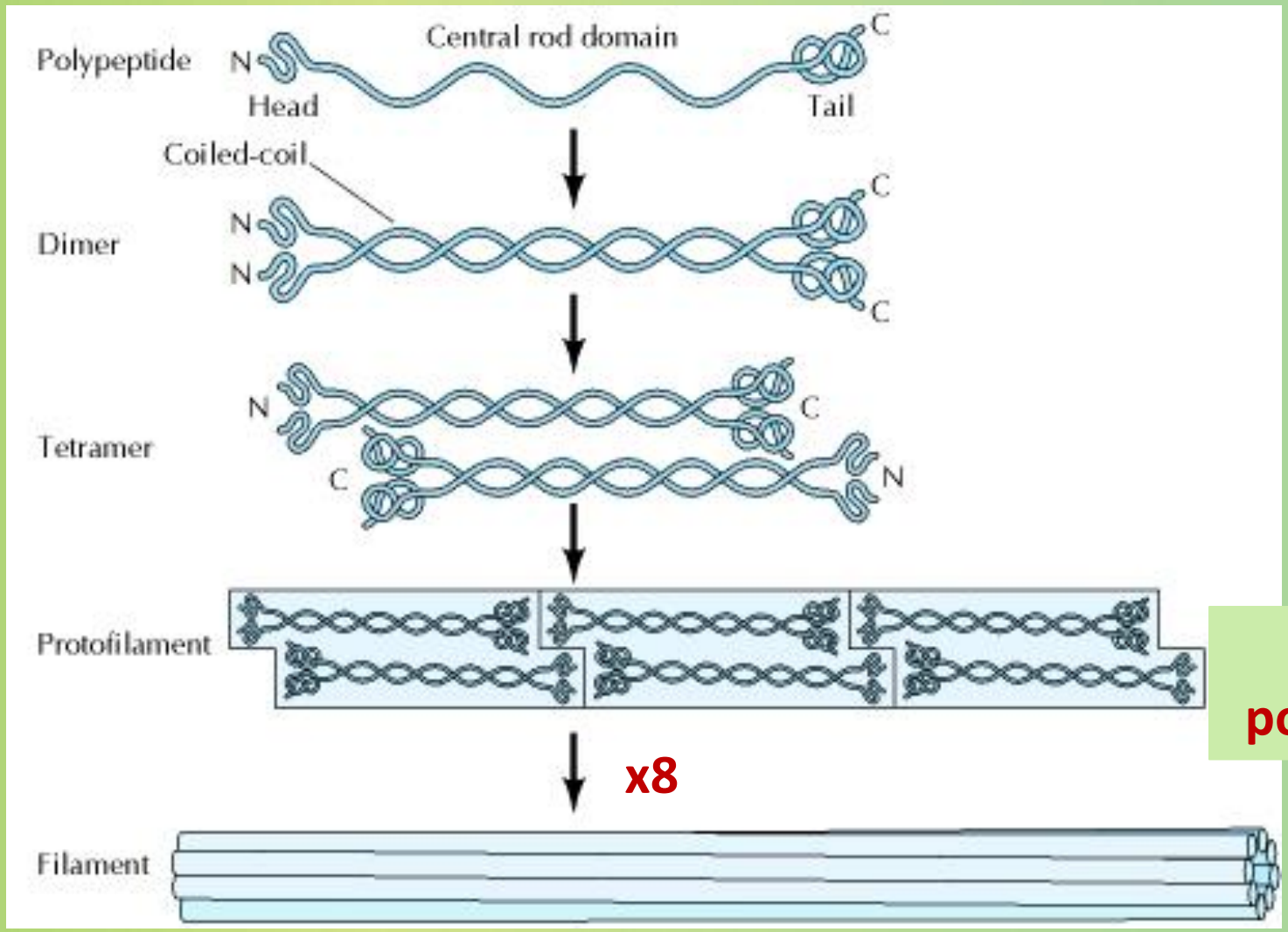

Nucleu

# Structure of IFs

- A central α-helical rod domain for filament assembly
- Flanking amino- and carboxy-terminal domains that vary among the different intermediate filament proteins in size, sequence, and secondary structure that determine the specific functions of the different intermediate filament proteins.



# Assembly of IFs



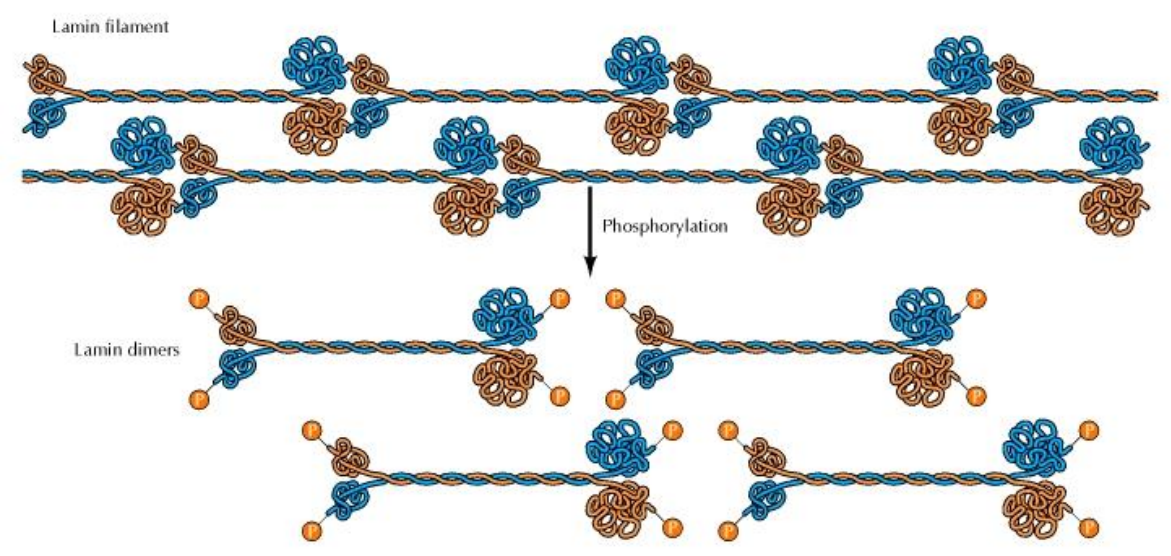

No polarity

# Interaction of IF types

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

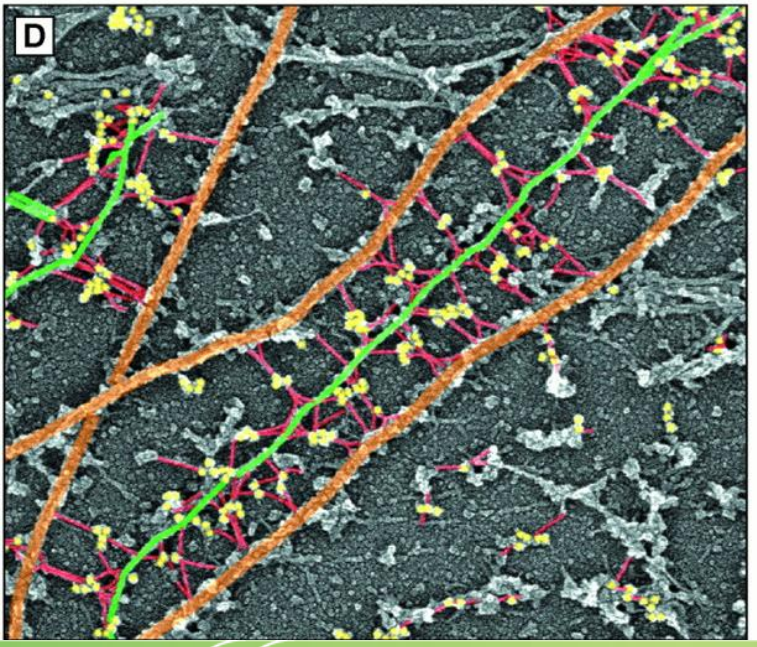
# Relative to actins and microtubules

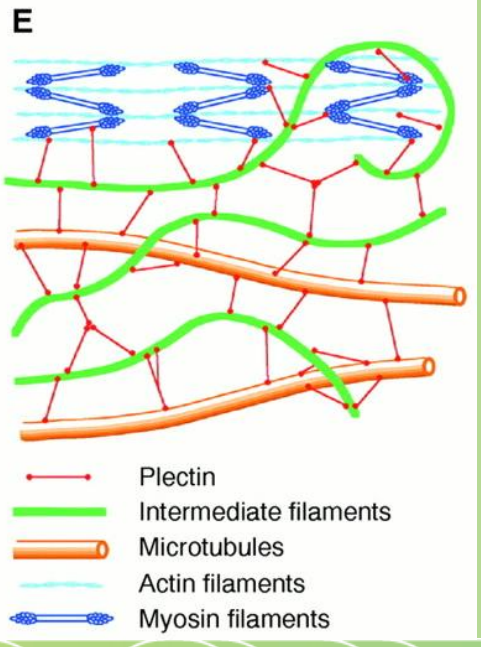
- More stable
- More dynamic within cells
- Not regulated by GTP, but regulated by phosphorylation
  - When nuclear lamins and vimentins are phosphorylated, they are disassembled.



# Intracellular Organization of IFs

- Both keratin and vimentin filaments attach to the nuclear envelope to position and anchor the nucleus within the cell.
- Ifs can associate not only with the plasma membrane but also with the actin filaments and microtubules.

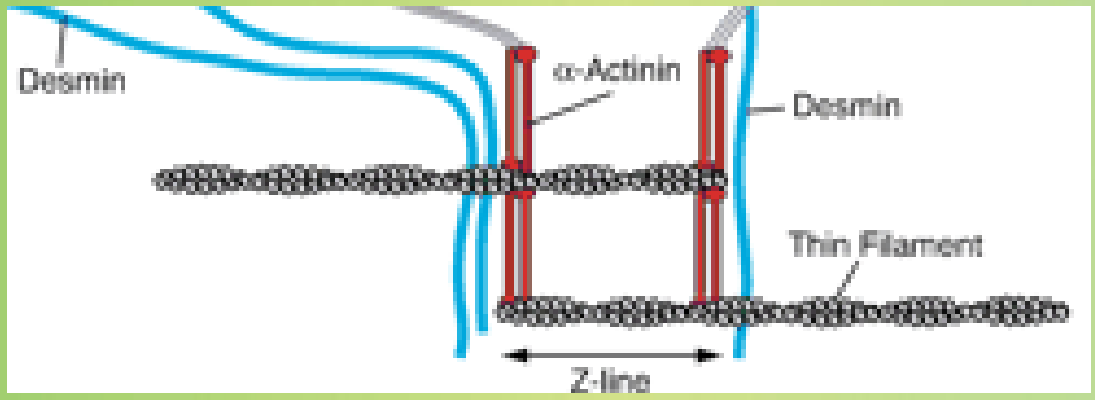


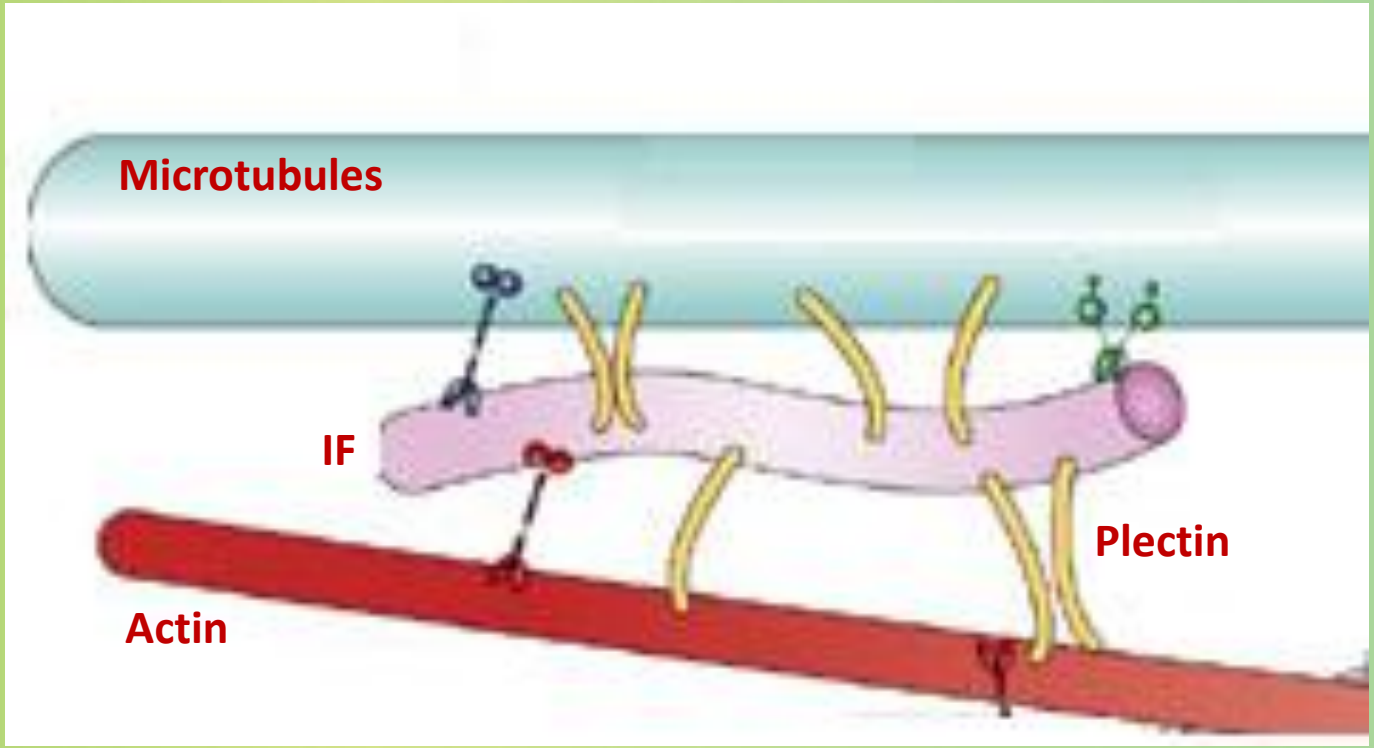

IFs provide a scaffold that integrates the components of the cytoskeleton and organizes the internal structure of the cell.

# Examples

- Desmin connects the actin filaments in muscle cells to one another and to the plasma membrane, thereby linking the actions of individual contractile elements.
- Neurofilaments in mature neurons are anchored to actin filaments and microtubules by neuronal members of the plakin family.
  - Neurofilaments provide mechanical support and stabilize the cytoskeleton in the long, thin axons of nerve cells.



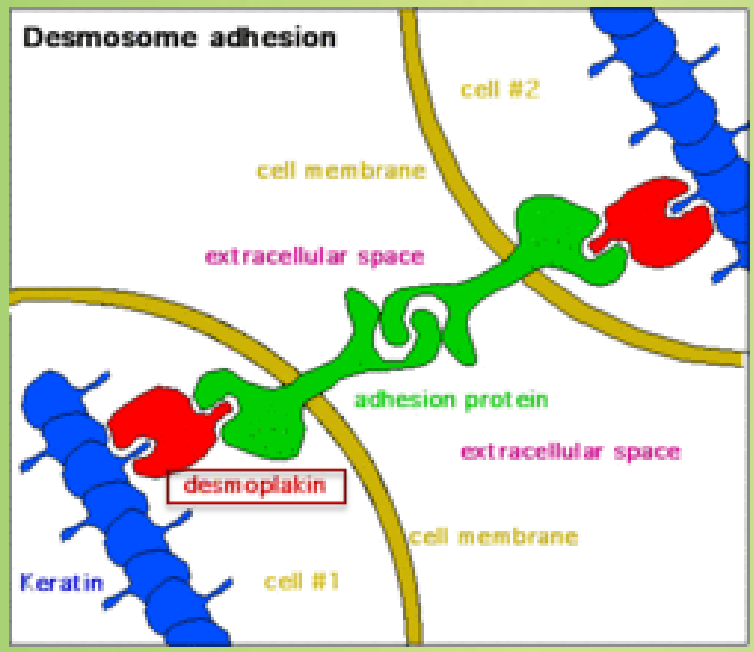
# Plectin (example)



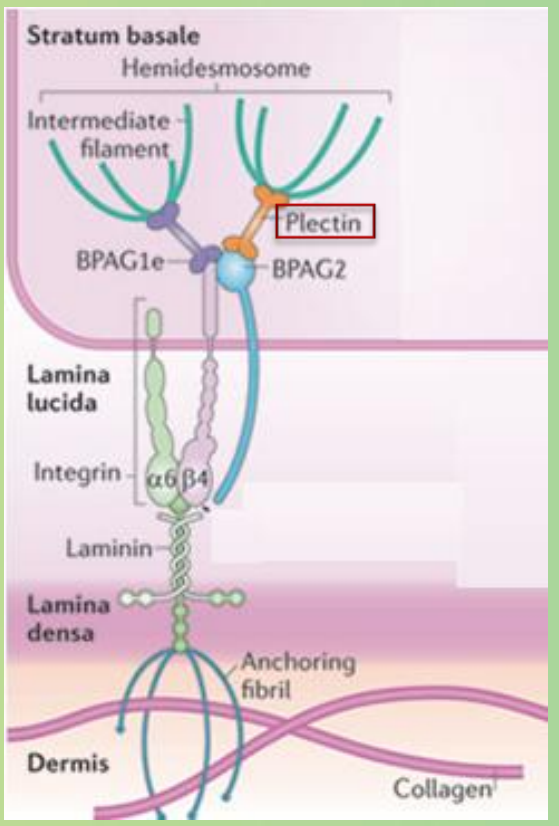

**Plectin bridges intermediate filaments to actin filaments and stabilizing them and increasing the mechanical stability of the cell.**

# The keratin filaments of epithelial cells are tightly anchored to the plasma membrane at two areas of specialized cell contacts, desmosomes and hemidesmosomes

## Desmosomes



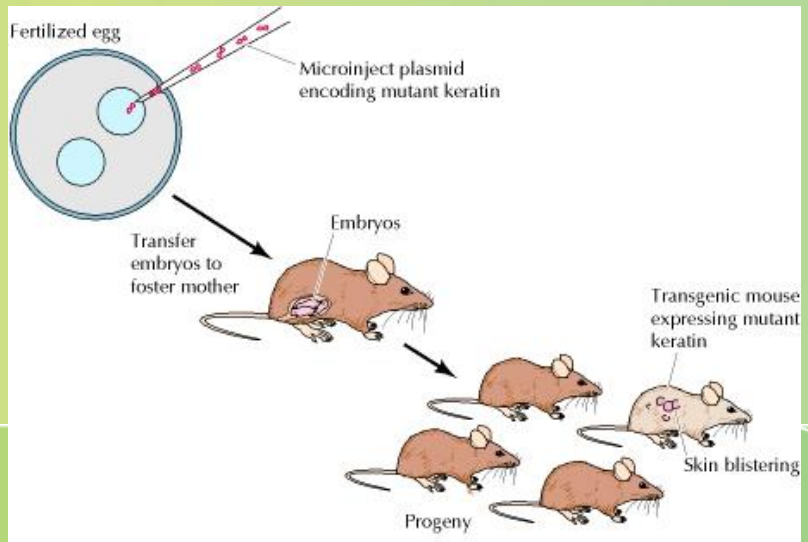

## Hhemidesmosomes




Keratin filaments anchored to both sides of desmosomes serve as a mechanical link, thereby providing mechanical stability to the entire tissue.

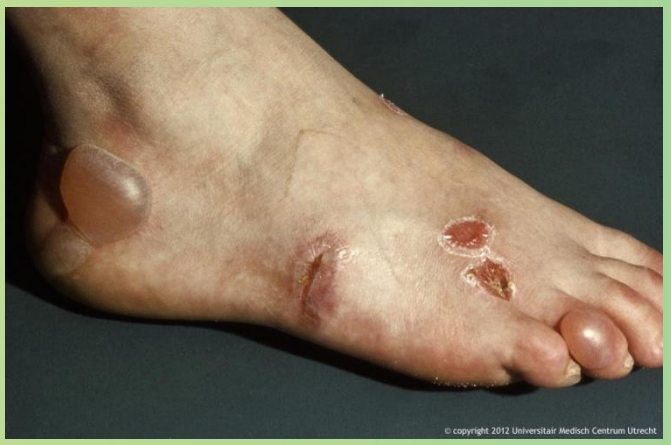
# IFs and diseases

- **Previously, disruption of vimentin in fibroblast cells did not affect cell growth or movement.**
  - **Hypothesis: IFs are most needed to strengthen the cytoskeleton of cells in the tissues of multicellular organisms.**
- **Support; transgenic mice expressing mutated kerains resulted in mice with severe skin abnormalities (blisters due to epidermal cell lysis following mild mechanical trauma).**

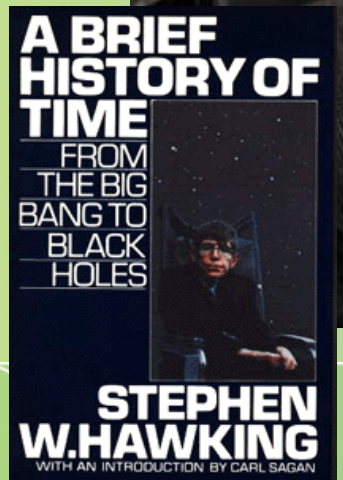


# Human diseases

- **Human epidermolysis bullosa simplex is caused by keratin gene mutations that interfere with the normal assembly of keratin filaments.**




- **Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease is characterized by the accumulation and abnormal assembly of neurofilaments.**




The greatest enemy of knowledge is not ignorance, it is the illusion of knowledge.

–Stephen Hawking

## Table 7.2 The Structure and Function of the Cytoskeleton

| Property | Microtubules | Microfilaments (Actin Filaments) | Intermediate Filaments |
|---|---|---|---|
| Structure | Hollow tubes; wall consists of 13 columns of tubulin molecules | Two intertwined strands of actin | Fibrous proteins supercoiled into thicker cables |
| Diameter | 25 nm with 15-nm lumen | 7 nm | 8–12 nm |
| Protein subunits | Tubulin, consisting of $\alpha$-tubulin and $\beta$-tubulin | Actin | One of several different proteins of the keratin family, depending on cell type |
| Main functions | Maintenance of cell shape (compression-resisting "girders")<br>Cell motility (as in cilia or flagella)<br>Chromosome movements in cell division<br>Organelle movements | Maintenance of cell shape (tension-bearing elements)<br>Changes in cell shape<br>Muscle contraction<br>Cytoplasmic streaming<br>Cell motility (as in pseudopodia)<br>Cell division (cleavage furrow formation) | Maintenance of cell shape (tension-bearing elements)<br>Anchorage of nucleus and certain other organelles<br>Formation of nuclear lamina |