Lecture 8: The cytoskeleton and cell movement (Microtubules and intermediate filaments)

Dr. Diala Abu-Hassan, DDS, PhD

School of Medicine

Dr.abuhassand@gmail.com

Microtubules

Second principal component of cytoskeleton

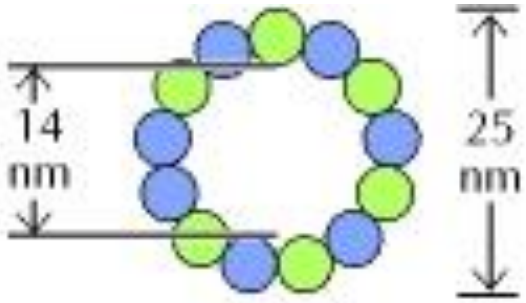
They are rigid hollow rods

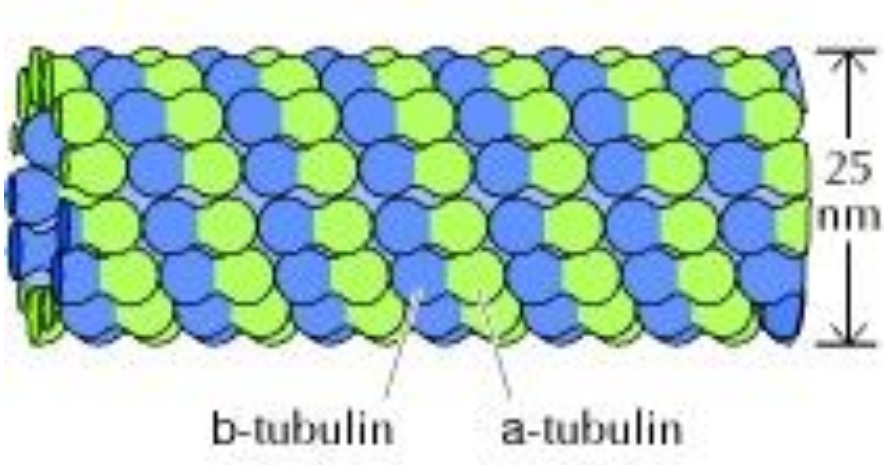
They are dynamic structures that undergo continual assembly and disassembly within the cell.

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 concentrated in the centrosome.**
  - **It initiates microtubule assembly.**

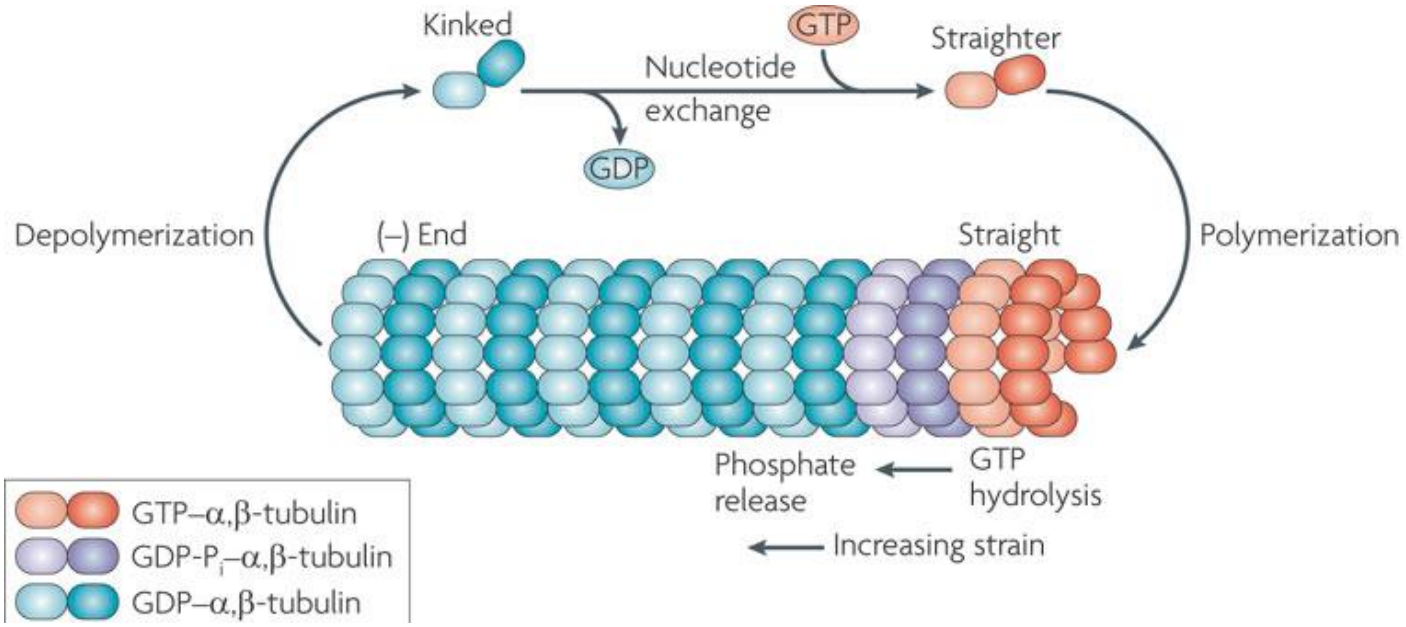
Polymerization of tubulin

Tubulin dimers polymerize to form protofilaments (head to tail arrays of tubulin dimers).

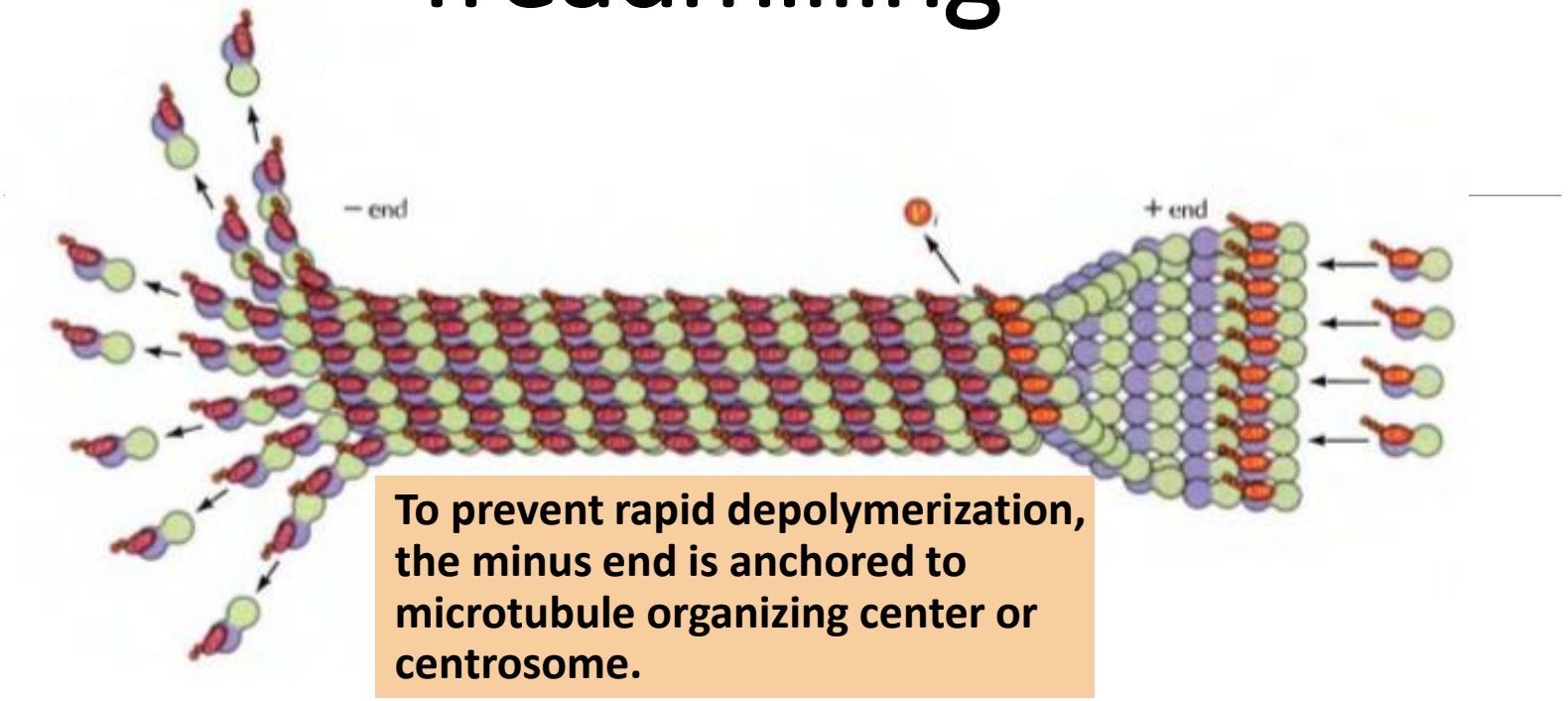
13 linear protofilaments assemble around a hollow core.

- 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



To prevent rapid depolymerization, the minus end is anchored to microtubule organizing center or centrosome.

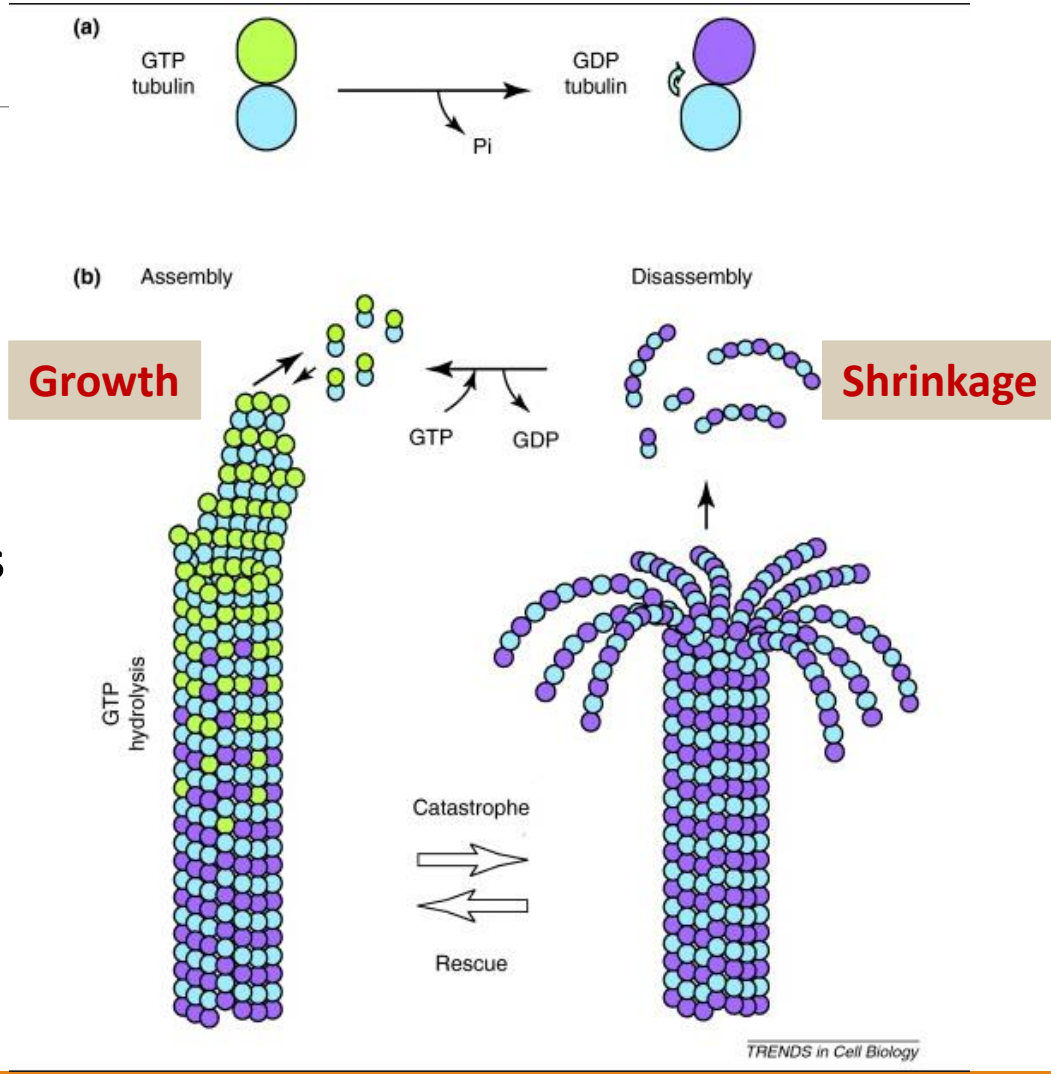
Treadmilling is rapid cycles of assembly and disassembly of microtubules

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 bonded 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)

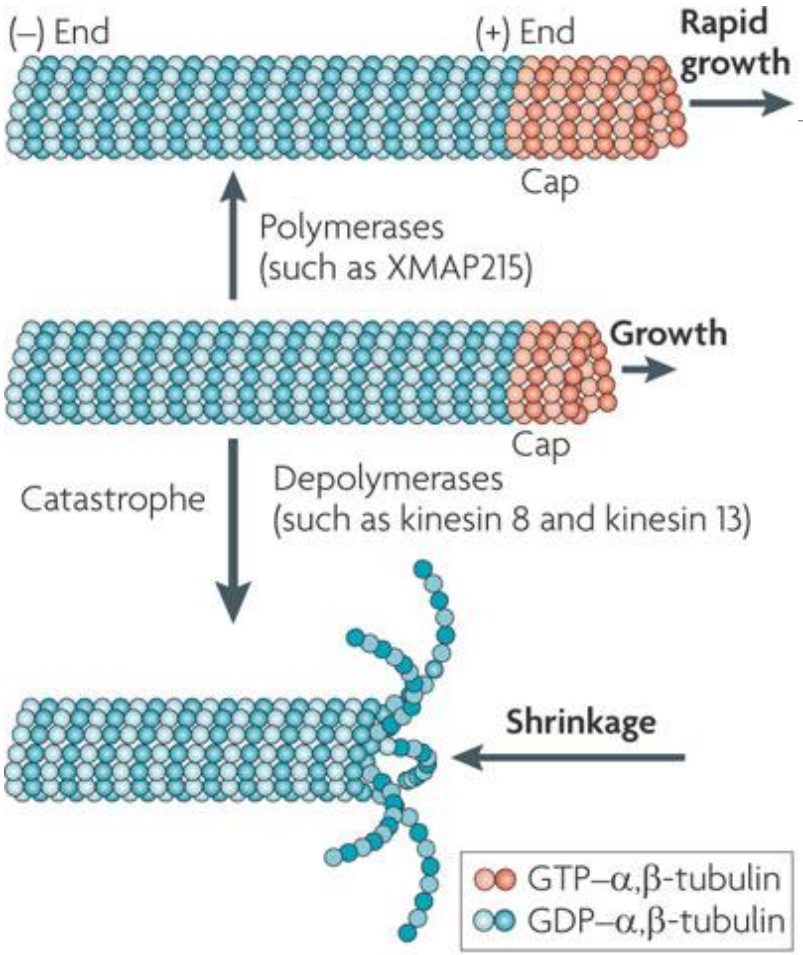
- **Alternating cycles of growth (rescue) and shrinkage (catastrophe).**
- **Growth or shrinkage is determined by the rate of tubulin addition relative to the rate of GTP hydrolysis.**
- **Catastrophe occurs when GTP is hydrolyzed at the plus end before new GTP-tubulin is added.**
- **Rescue occurs when GTP hydrolysis is slower than the addition of GTP-tubulin dimers.**



Application: Drugs that affect microtubule assembly

Experimental	Colchicine and colcemid bind tubulin, inhibit polymerization, and block mitosis.
Experimental and Cancer treatment	Vinblastine and vincristine bind specifically to tubulin and prevent their polymerization to form microtubules resulting in inhibition of rapidly dividing cells. Taxol stabilizes microtubule and blocks cell division.

Regulatory proteins



Nature Reviews | Molecular Cell Biology

Microtubule-associated proteins (MAPs) such as polymerases that accelerates growth at the plus end.

Depolymerases stimulate shrinkage by accelerating the dissociation of GTP-tubulin from the plus end.

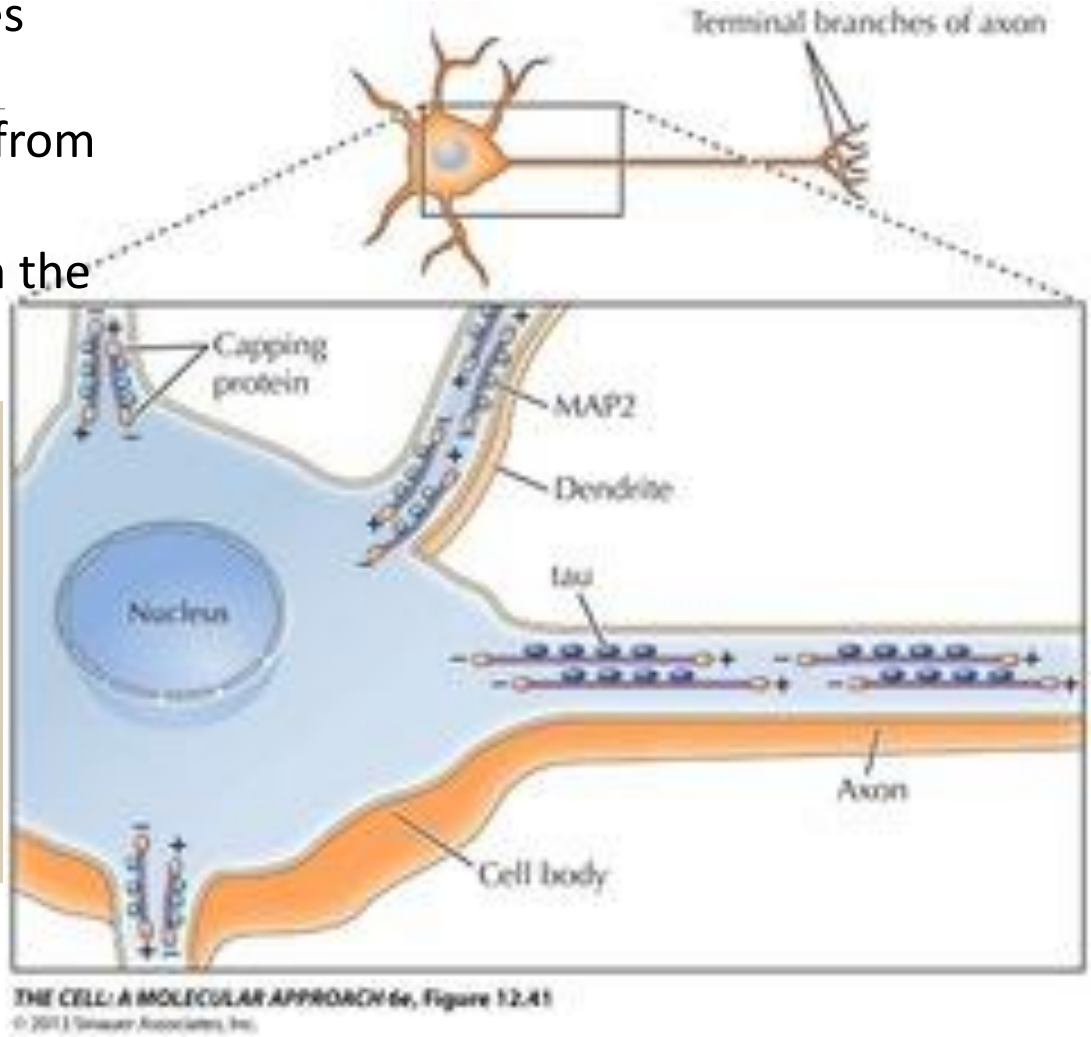
CLASP, a MAP, prevents disassembly (catastrophe) and promote restarting growth (rescue).

Organization of microtubules within neurons

Neurons have two types of processes extend from the cell body:

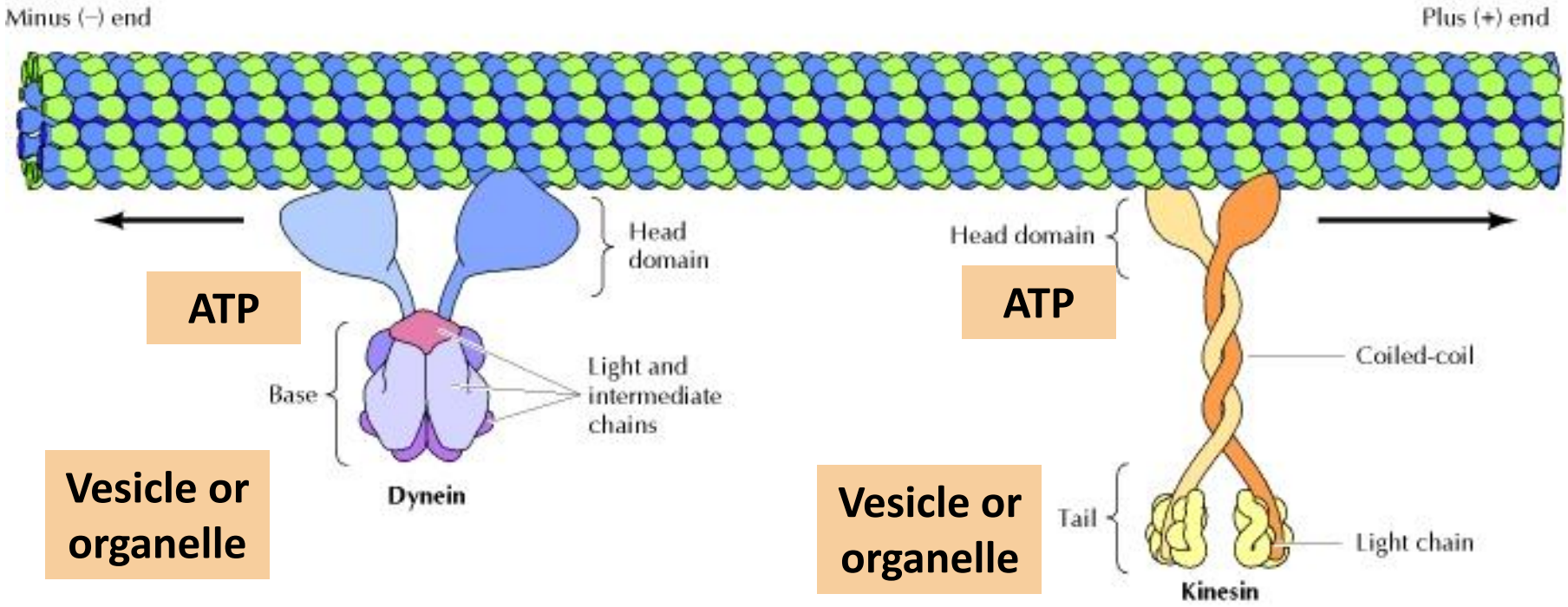
- **Dendrites**: short; receive stimuli from other nerve cells
- **Axon**: long; carries impulses from the cell body to other cells

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



THE CELL: A MOLECULAR APPROACH 6e, Figure 12.41

Vesicular transport



Microtubules-motor proteins such as kinesin and dynein move along microtubules in opposite directions

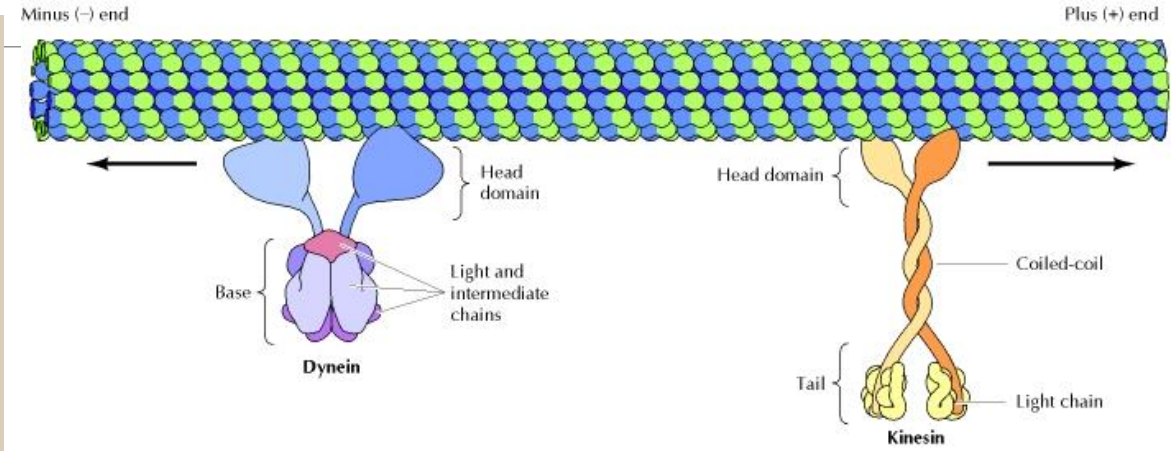
- kinesins move toward the plus end and dyneins move toward the minus end.

Vesicular transport

https://www.youtube.com/watch?v=YAva4g3Pk6k

Kinesin

- **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.**



Dynein

- **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

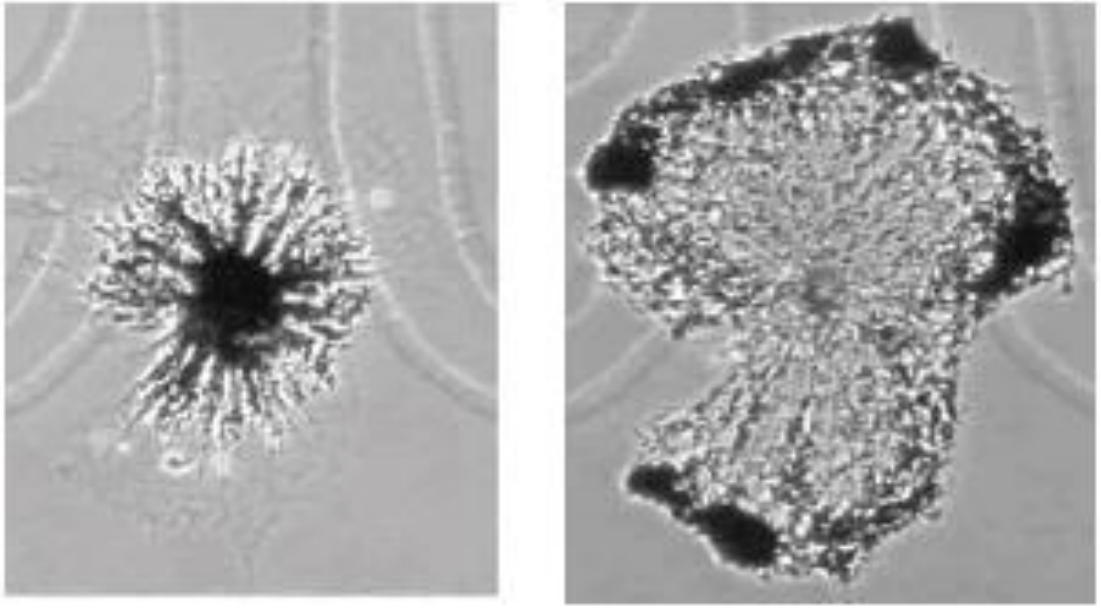
Members of the **kinesin** family control the movements of **mitochondria**.

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 often have both types of motors on their surface, allowing cells to adjust their position.



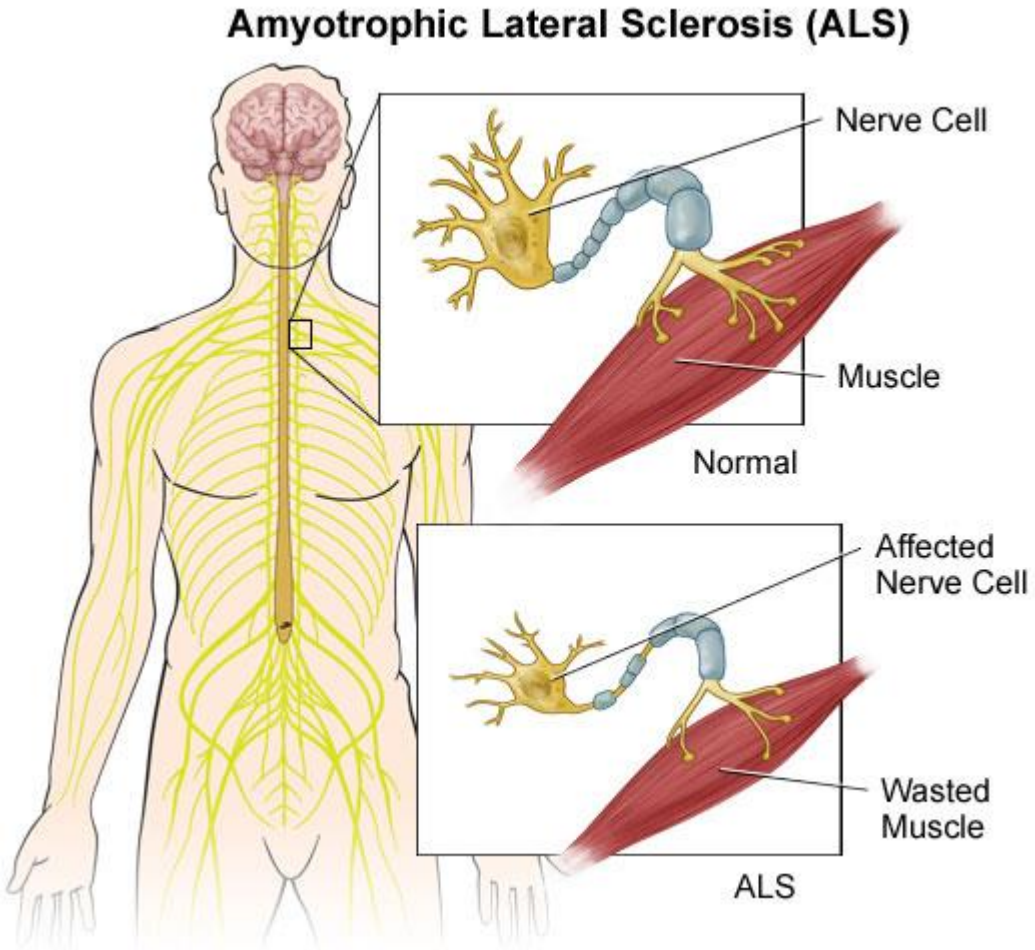
Melanocytes position the pigmented organelles, melanosomes, in response to the amount of light.

- In the presence of light, kinesin moves melanosomes to the periphery of cells.
- In the dark, dynein returns the melanosomes to the center of the cell.

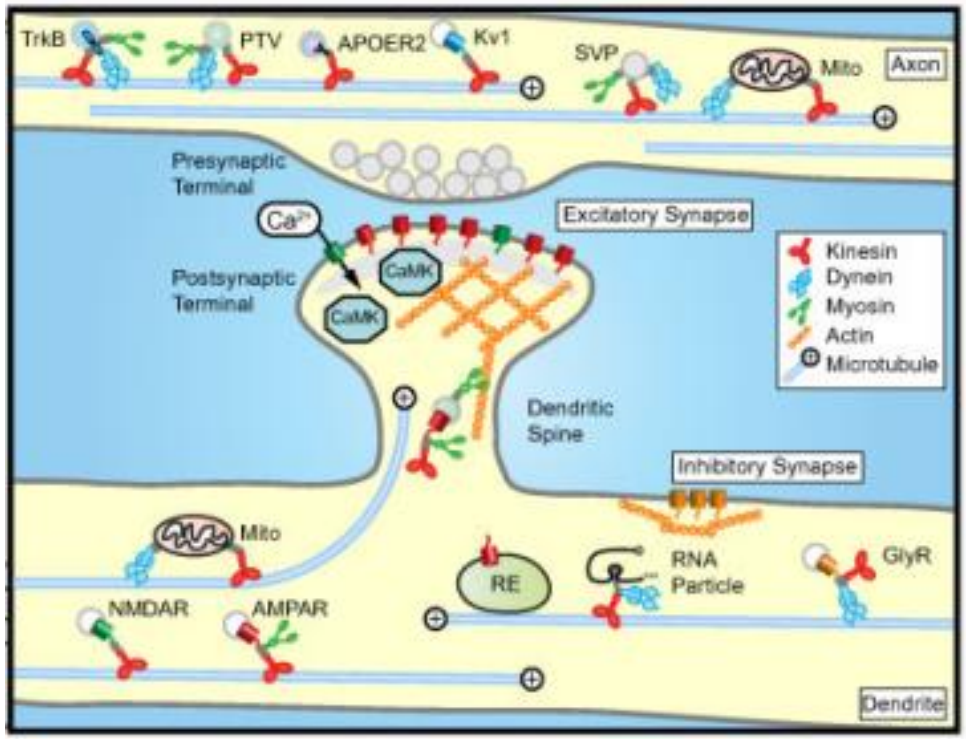
Application: Kinesins and diseases

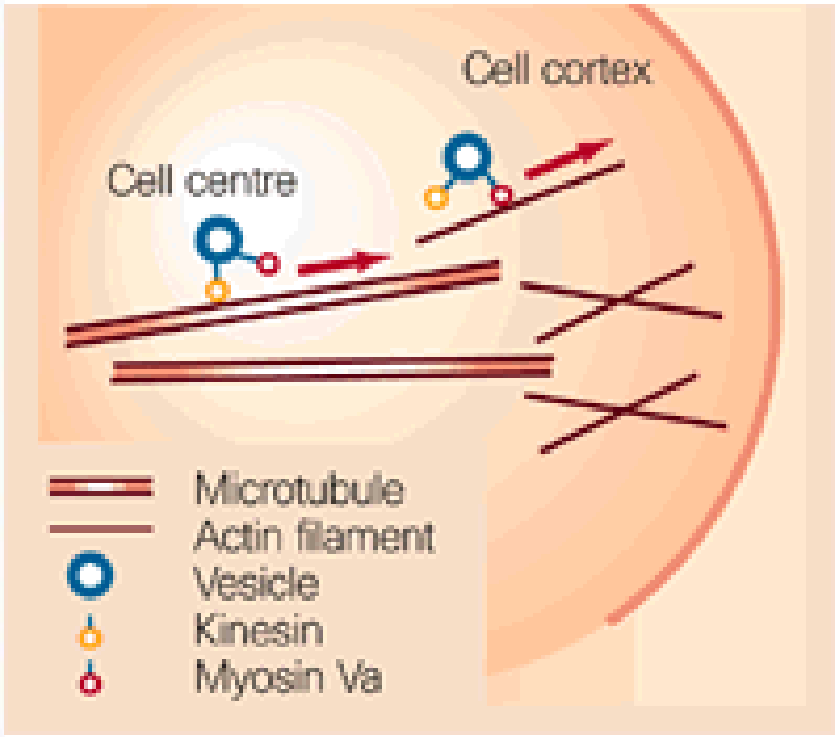
Mutations in certain kinesin proteins reduce the ability of neurons to move essential organelles from their cell bodies to their axons leading to neurodegeneration such as **amyotrophic lateral sclerosis (ALS).**

Mutations in kinesins lead to peripheral neuropathies such as **Charcot-Marie-Tooth disease.**



“Changing horses in midstream”





Myosins transport organelles over shorter distances compared to 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 (IFs)

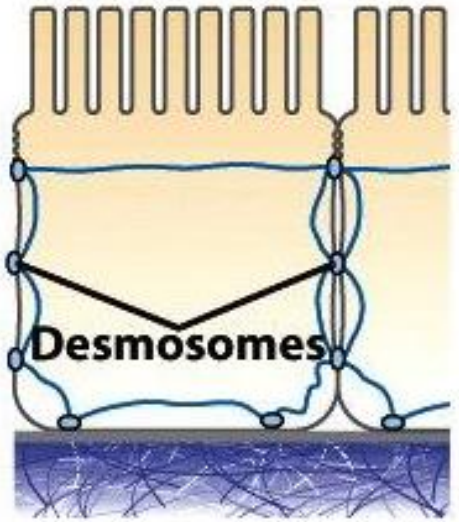
- ✓ Their diameter is intermediate between those of actin filaments and microtubule.
- ✓ They provide mechanical strength to cells and tissues.
- ✓ They provide a scaffold for localization of cellular processes
- ✓ Not involved directly in cell movement.
- ✓ They are composed of a variety of proteins, which are classified into 5 groups based on similarities between their amino acid sequences.

Types of IFs Proteins

Types I and II are expressed in epithelial cells

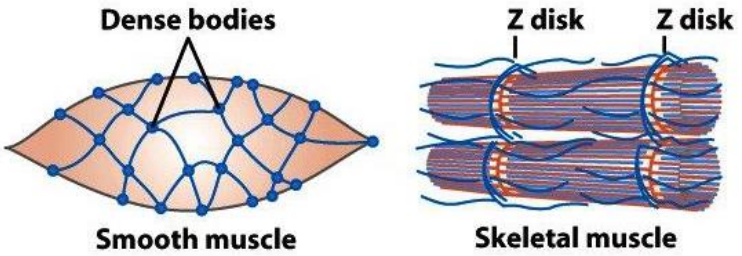
Each cell type synthesizes at least one type I (acidic) and one type II (neutral/basic) keratin.

- **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 is found in fibroblasts, smooth muscle cells, and white blood cells.
- Desmin is specifically expressed in muscle cells.

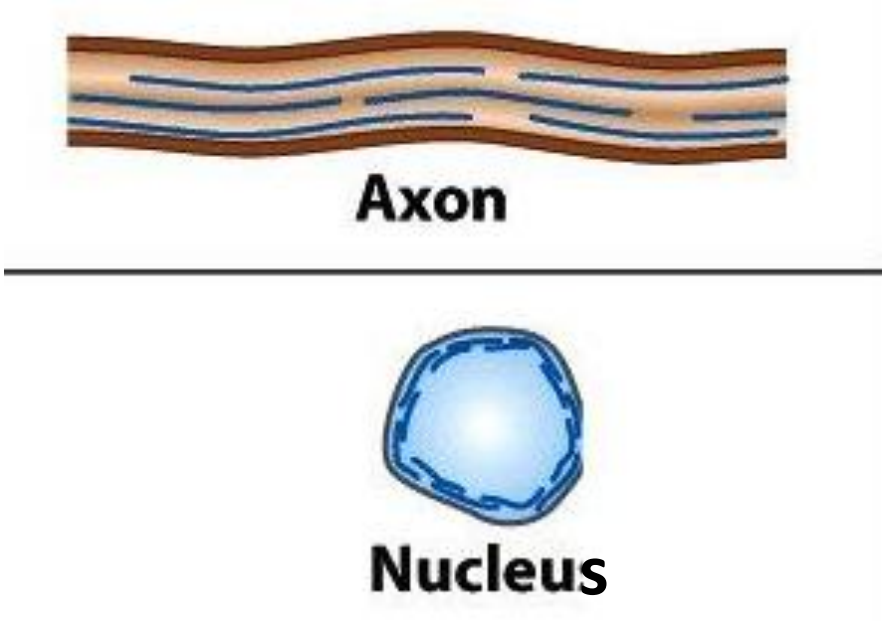


Types of IFs Proteins

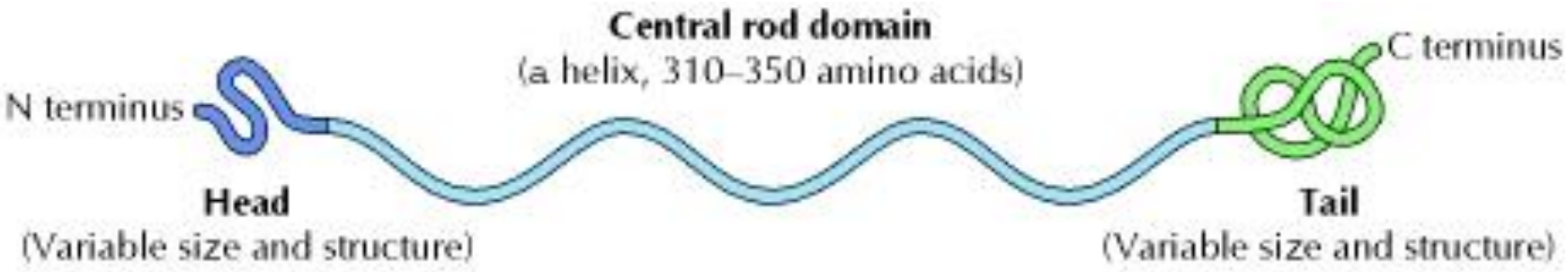
Type IV: neurofilament (NF) found in mature neurons and the axons of motor neurons.

Nestin in stem cells

Type V: nuclear lamins, components of the nuclear envelope.



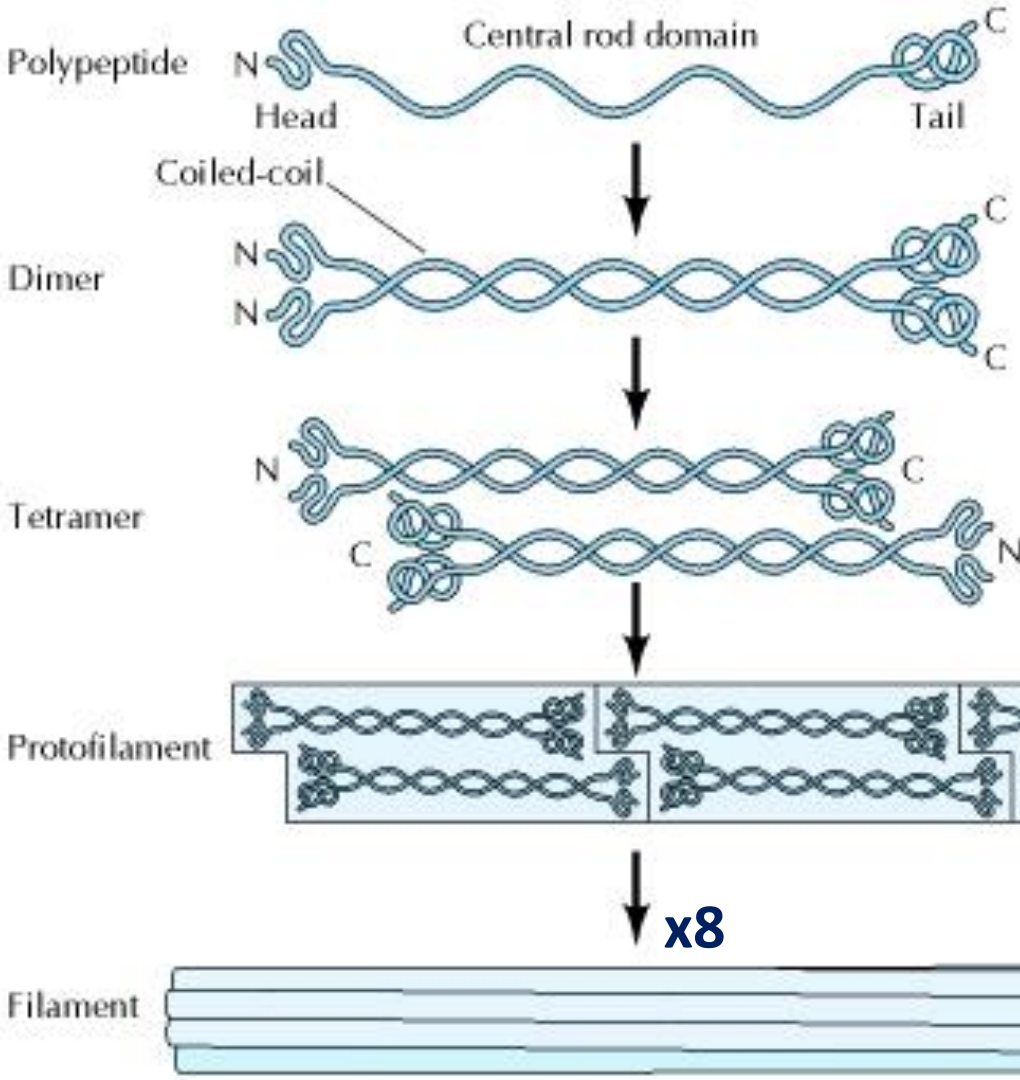
Structure of IFs



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



Antiparallel

End to end assembly

No polarity

A more stable structure that actin filaments or microtubules

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.
- ✓Phosphorylation affects the assembly and disassembly of IFs.

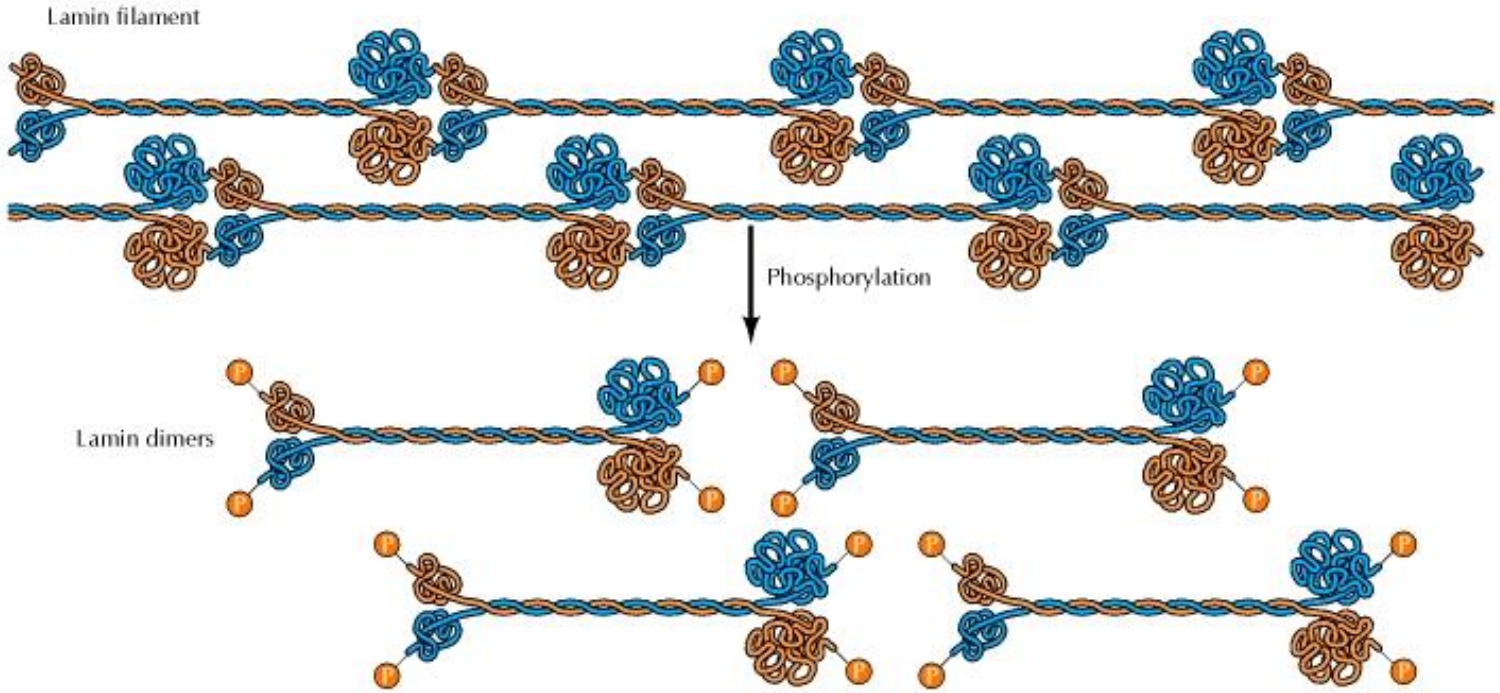
IFs versus actins and microtubules

More stable

More dynamic within cells

Not regulated by GTP, but regulated by phosphorylation

- When nuclear lamins and vimentins are phosphorylated, they disassemble.

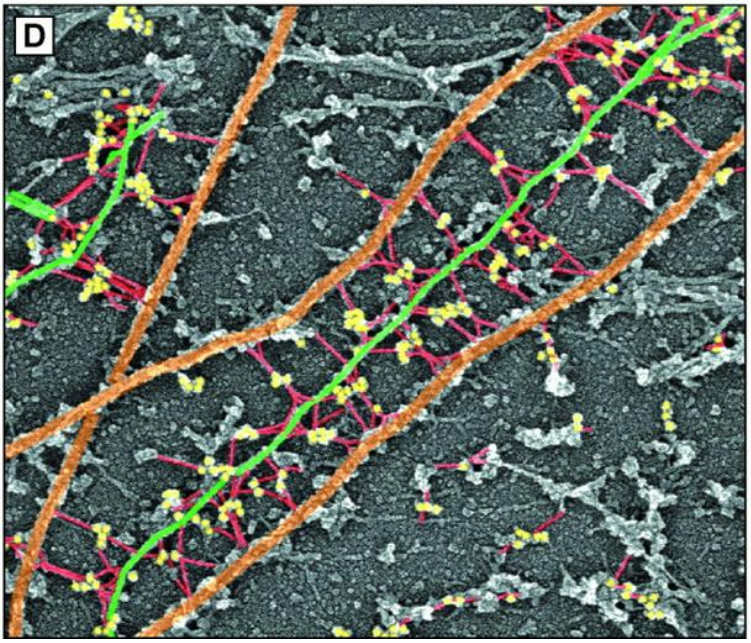


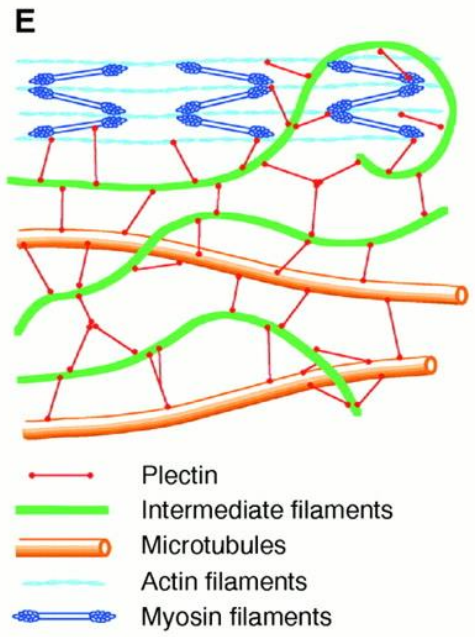
Intracellular Organization of IFs

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

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.

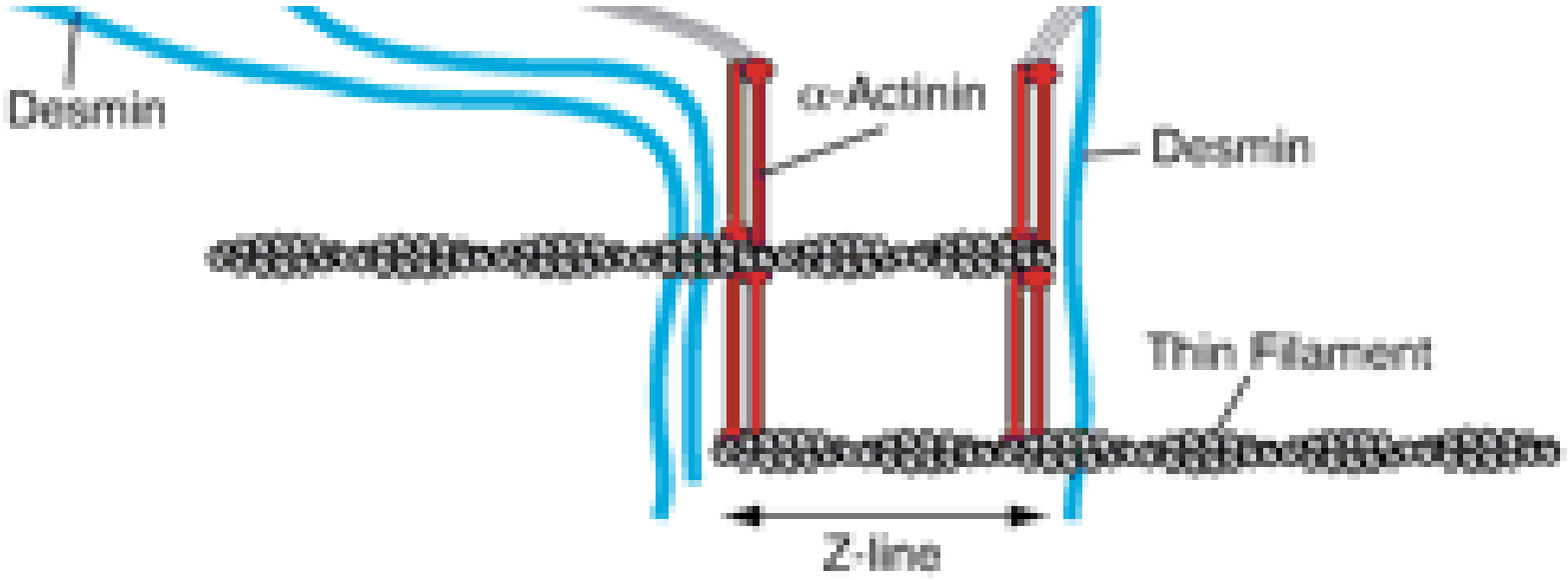




IFs provide a scaffold that integrates the components of the cytoskeleton and organizes the internal structure of the cell.

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.



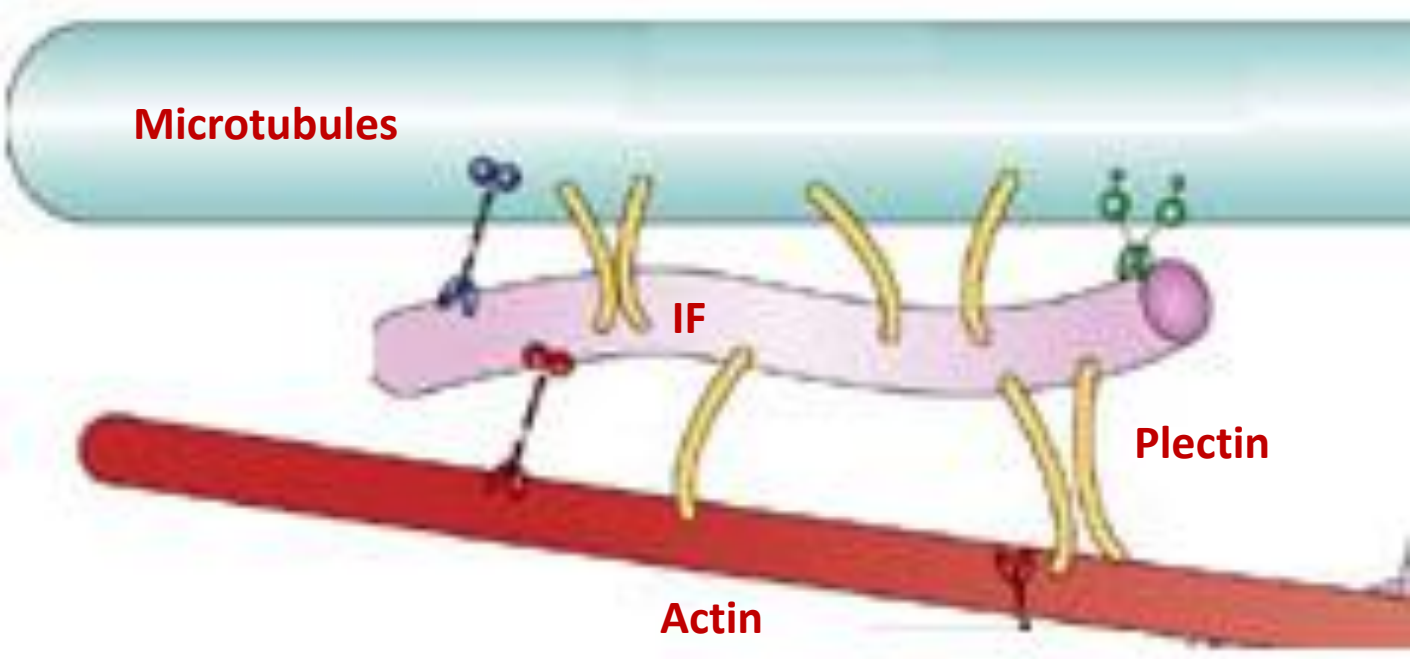
Desmin mutations cause muscle defects such as early onset cardiomyopathy

Neurofilaments in neurons

- 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 connects IFs to other cytoskeletal elements



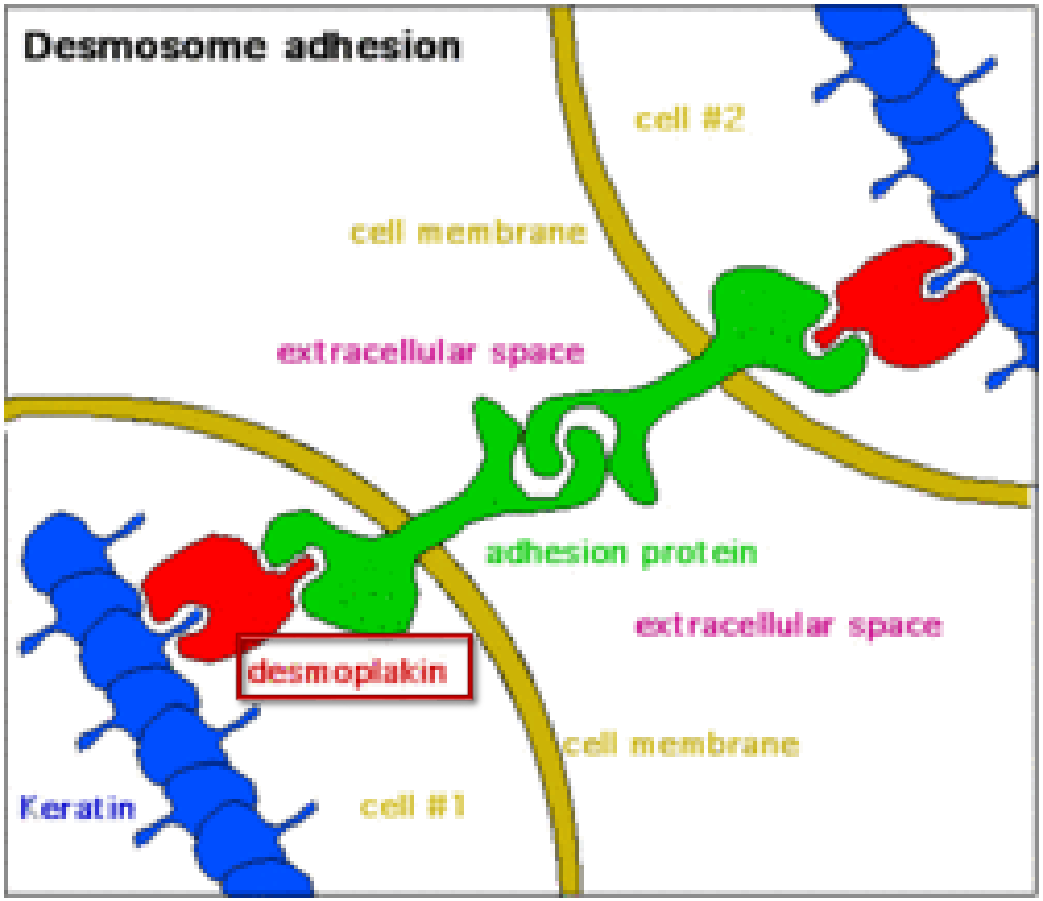
Plectin bridges <u>microtubules</u> to actin filaments and stabilizing them and increasing the mechanical stability of the cell.

Cellular Junctions: Desmosomes

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

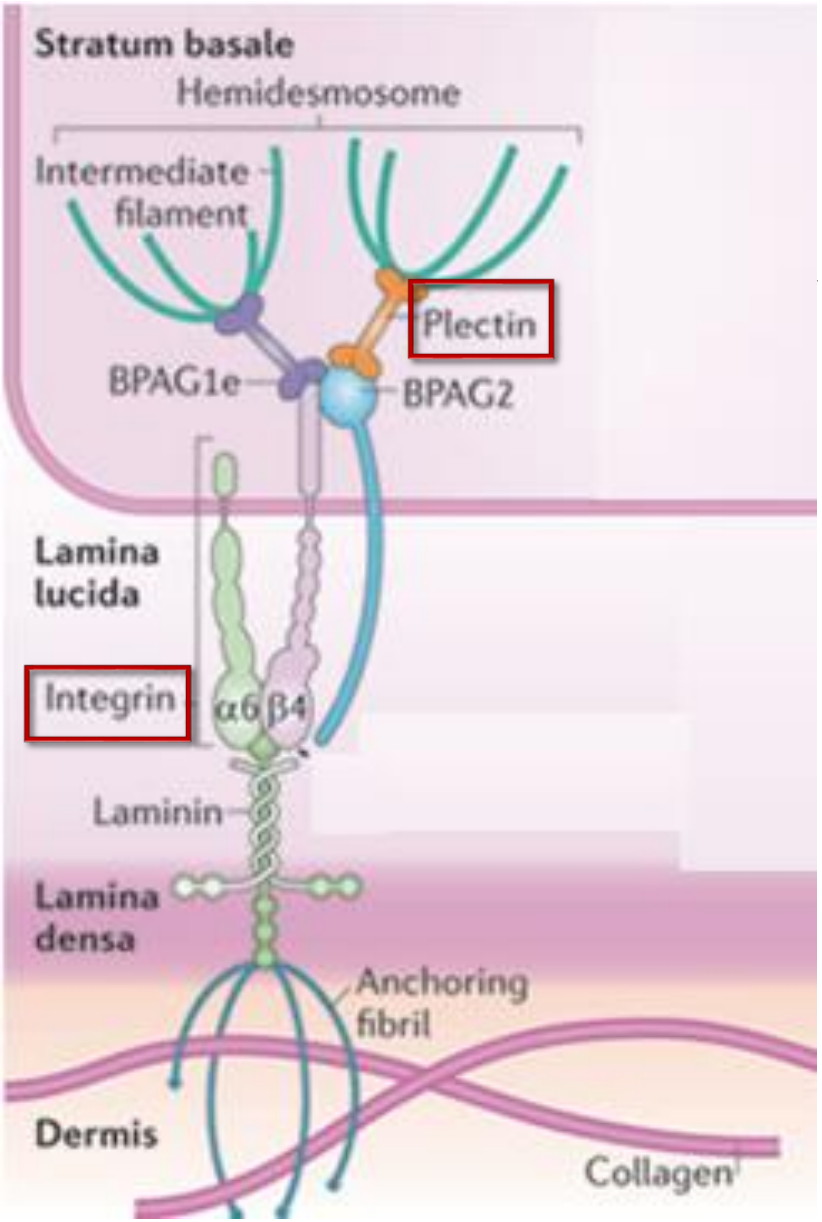
Desmosomes anchor IFs to regions of cell-cell contacts

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



Hemidesmosomes

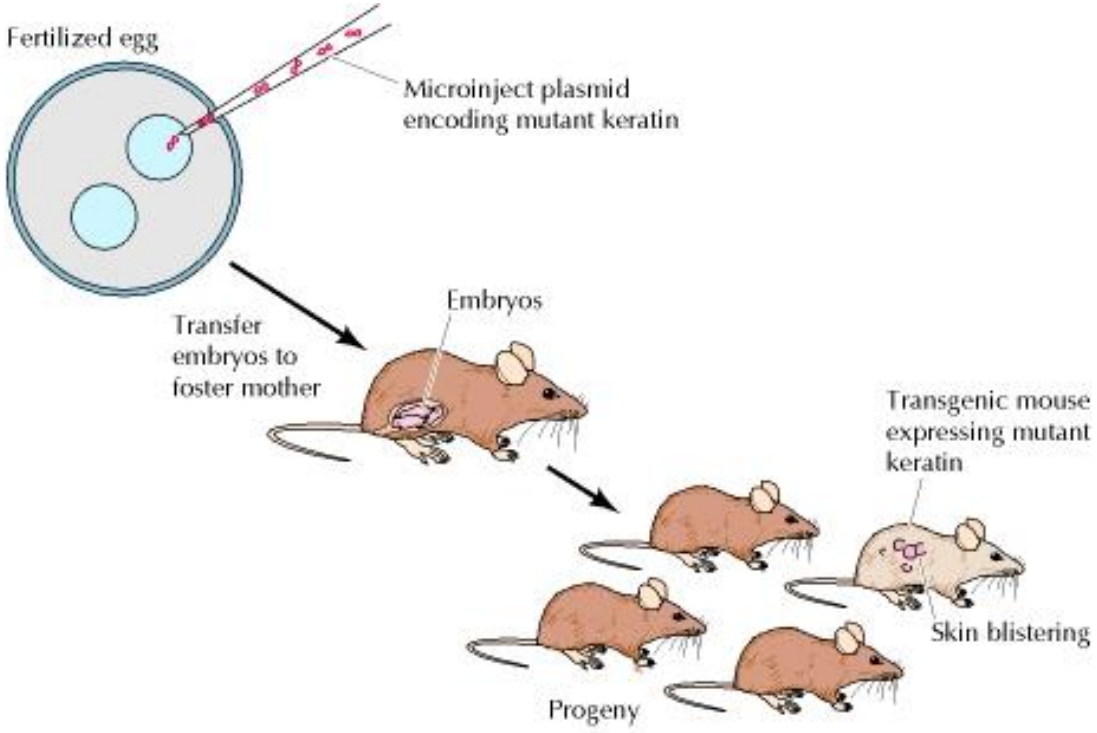
Hemidesmosomes anchor IFs to regions of cell-substratum contacts



Application: IFs and diseases

Previously, disruption of vimentin in fibroblast cells did not affect cell growth or movement.

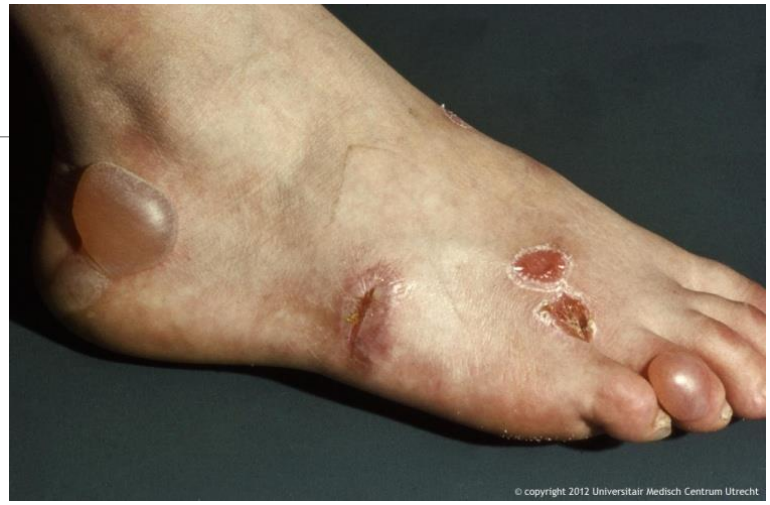
- Hypothesis: IFs are most needed to strengthen the cytoskeleton of cells in the tissues of multicellular organisms.



Transgenic mice expressing mutated keratins resulted in mice with severe skin abnormalities (blisters due to epidermal cell lysis following mild mechanical trauma).

IFs and Human diseases

Human epidermolysis bullosa simplex is caused by keratin gene mutations that interfere with the normal assembly of keratin filaments causing skin blisters after minor trauma.



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

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 α-tubulin and β-tubulin	Actin	One of several different proteins of the keratin family, depending on cell type
Main functions	Maintenance of cell shape (compression-resisting "girders") Cell motility (as in cilia or flagella) Chromosome movements in cell division Organelle movements	Maintenance of cell shape (tension-bearing elements) Changes in cell shape Muscle contraction Cytoplasmic streaming Cell motility (as in pseudopodia) Cell division (cleavage furrow formation)	Maintenance of cell shape (tension-bearing elements) Anchorage of nucleus and certain other organelles Formation of nuclear lamina