Lecture 6: the cytoskeleton (Microtubules and intermediate filaments)

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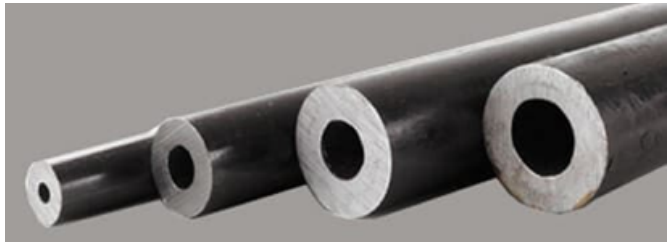
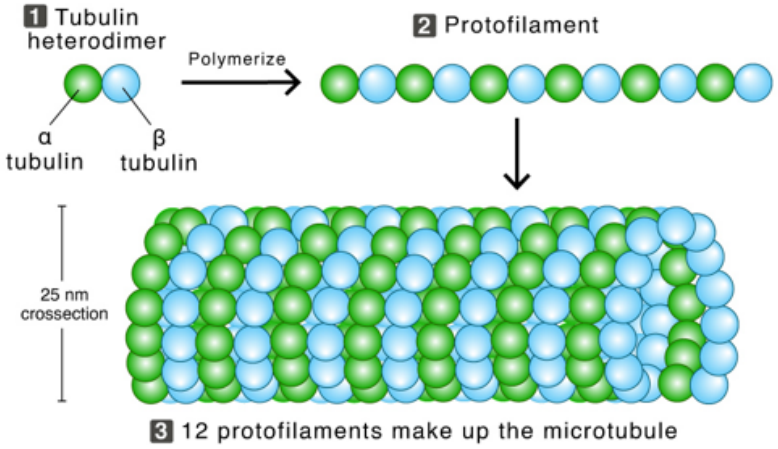
Overview

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



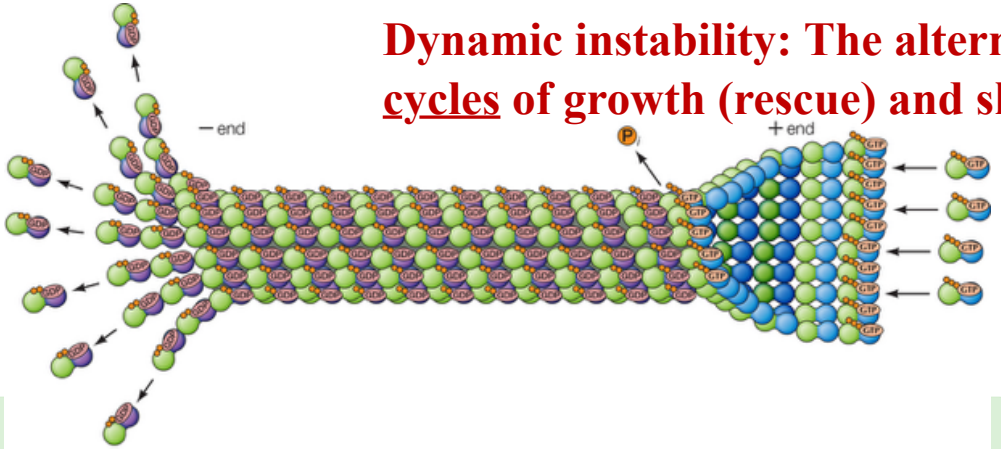
Structure of microtubules

- Microtubules are composed of a dimer of two globular proteins, α-tubulin and β-tubulin.
  - γ-tubulin is specifically localized to the centrosome and it initiates microtubule assembly for chromosomal separation.
- The tubulin dimers polymerize to form protofilaments (a hollow core) of arrays of the tubulin dimers.
- Both α- and β-tubulin bind GTP.



Treadmilling and dynamic instability

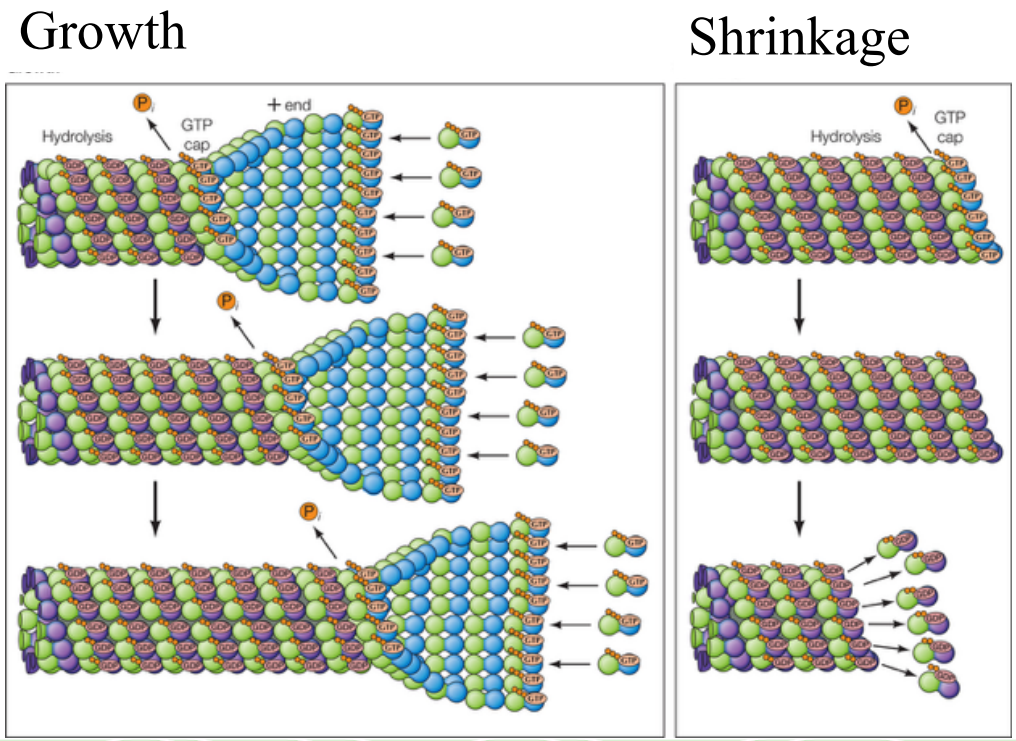
- Microtubules are polar structures with a fast-growing plus end and a slow-growing minus end.
  - Polarity determines the direction of movement along microtubules.
- Microtubules undergo assembly and disassembly (treadmilling) where tubulin molecules are lost from the minus end and replaced by the addition of tubulin molecules bound to GTP to the plus end.



Dynamic instability: The alternation of microtubules between cycles of growth (rescue) and shrinkage (catastrophe).

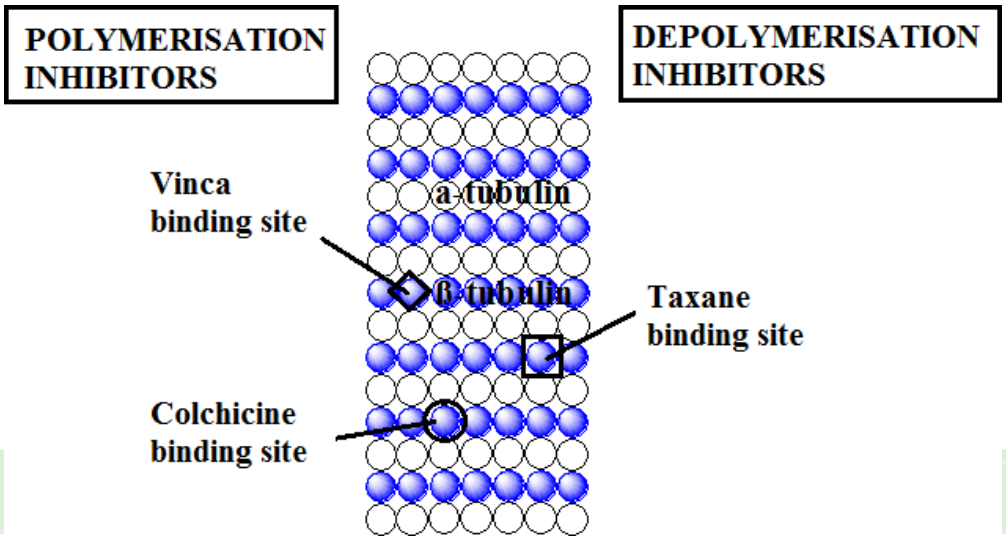
The reason behind dynamic instability

- Dynamic instability results from GTP hydrolysis of β-tubulin during polymerization, which reduces its binding affinity for neighboring molecules.
- Growth of microtubules continues as long as new GTP-bound tubulin molecules are added more rapidly than GTP hydrolysis.
- Faster GTP hydrolysis than the addition of new subunits leads to the disassembly and shrinkage of microtubules.



Drugs

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



Regulatory proteins

- Microtubule-associated proteins (MAPs) regulate the dynamic behavior of microtubules by

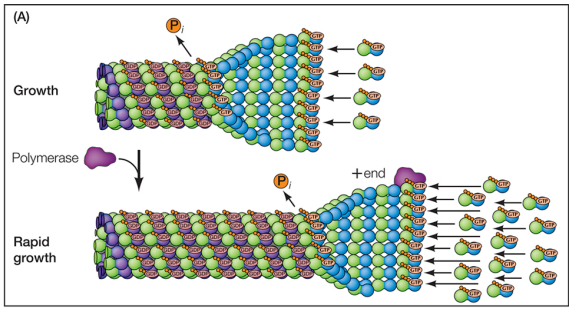
1. Regulating:

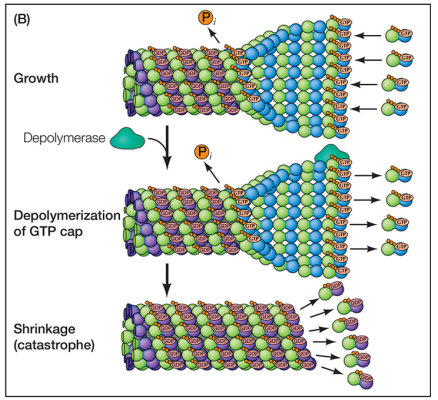
A. growth or polymerization (by polymerases) or

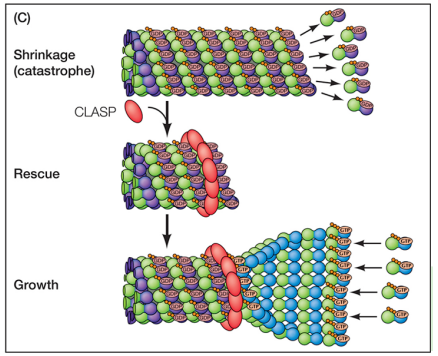
B. shrinkage or depolymerization (by depolymerases) at the plus ends of microtubules,

2. Suppressing microtubule catastrophe and promoting rescue:

- CLASP proteins rescue microtubules from catastrophe.

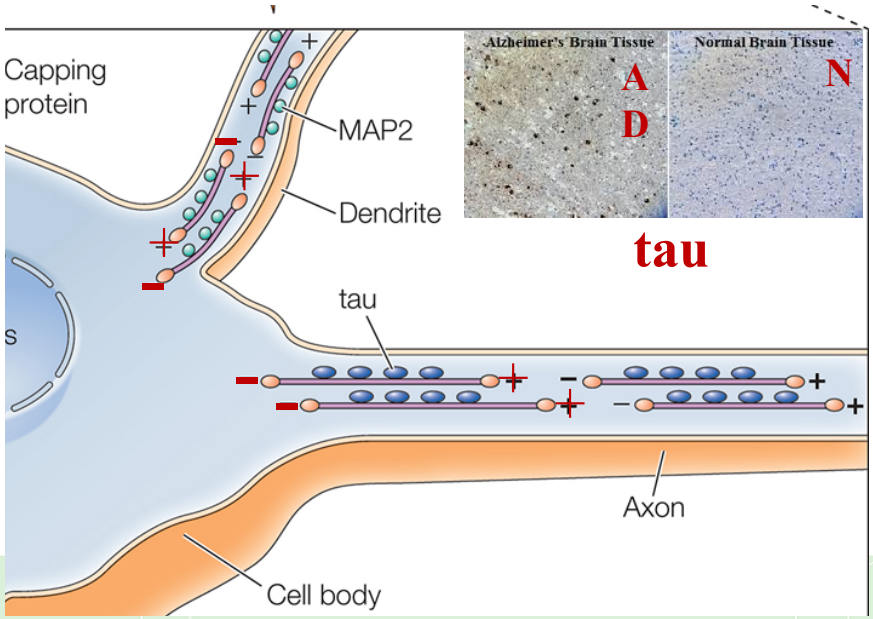






Organization of microtubules within cells
Example: neuron

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



The plus and minus ends of microtubules in nerve cells terminate in the cytoplasm.

In dendrites, microtubules are oriented in both directions.

In axons, microtubules are oriented with their plus ends pointing toward the tip of the axon.

Axons contain tau protein, which is the main component of lesions found in the brains of Alzheimer's patients.

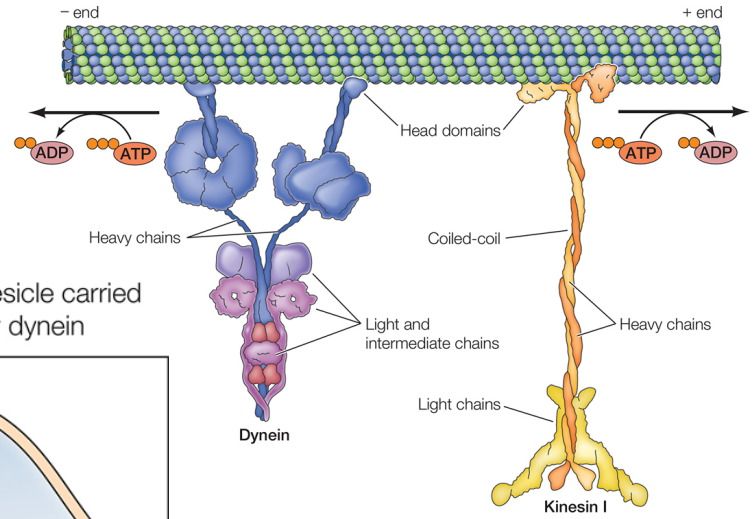
Microtubules-motor proteins
e.g., kinesin and dynein

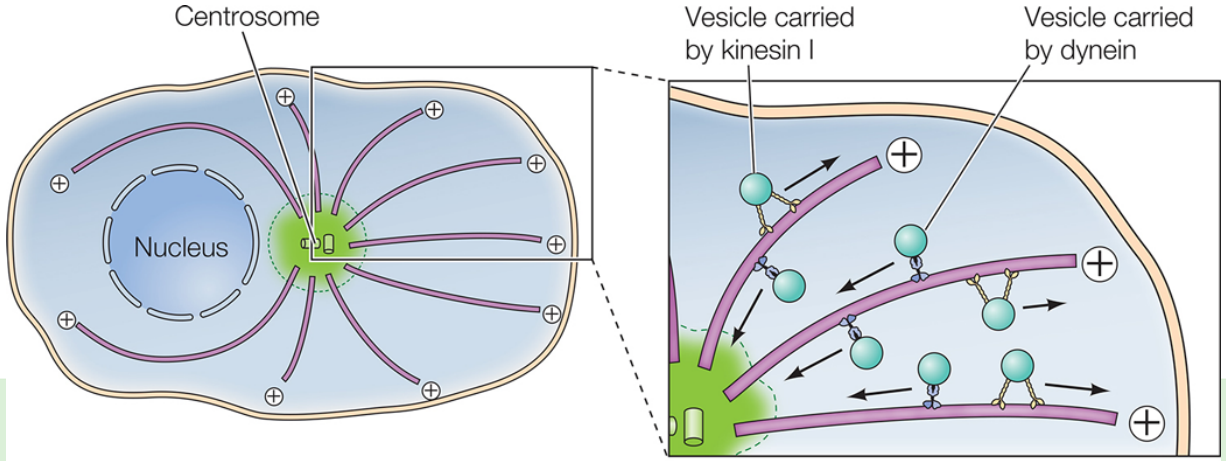
- Microtubules-motor proteins use ATP to move along microtubules in opposite directions.

Disclaimer: Exceptions exist

- Kinesin moves toward the plus end.
- Dynein moves toward the minus end.

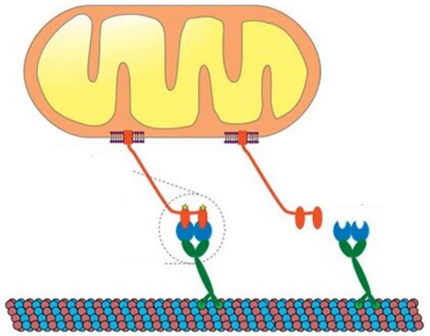
Part of the molecules bind microtubules and the other part binds to the vesicle or organelle.

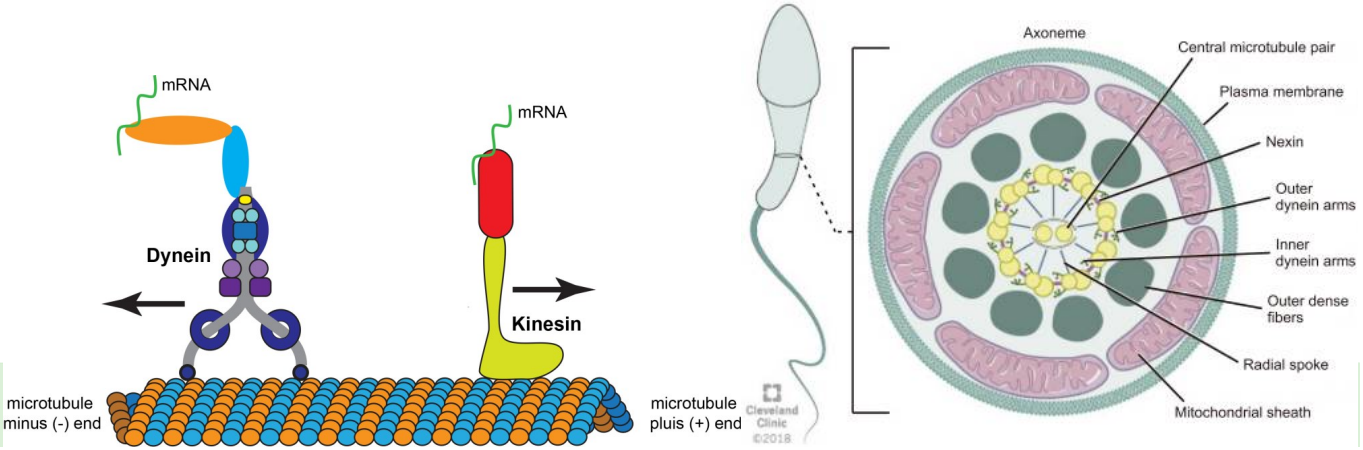




Other functions of microtubules

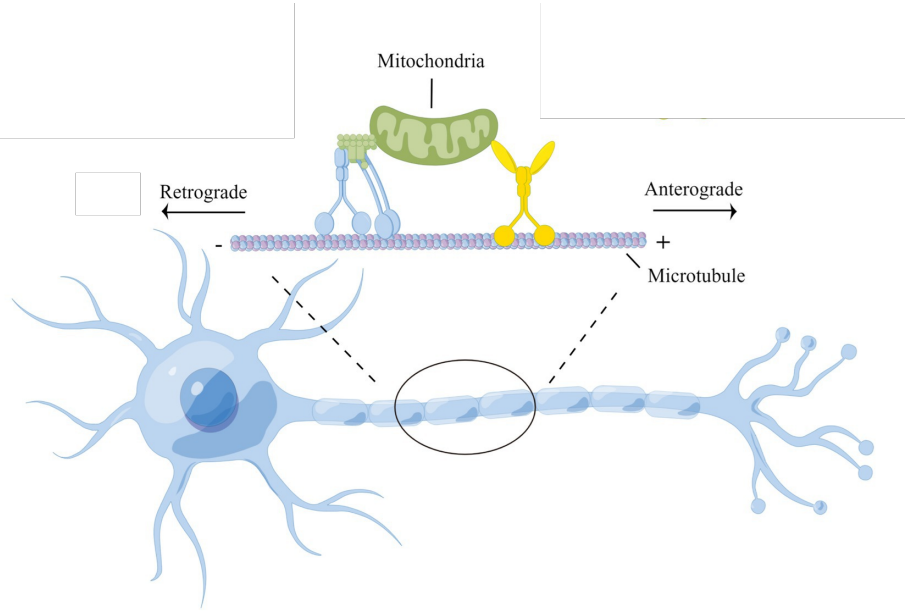
- Microtubules and their associated motor proteins position membrane-enclosed organelles (such as the ER, Golgi apparatus, lysosomes, peroxisomes, and mitochondria) within the cell.
- Microtubules are responsible for sperm motility.
  - Infertility!
- Kinesin and dynein transport selective mRNA molecules in cells.





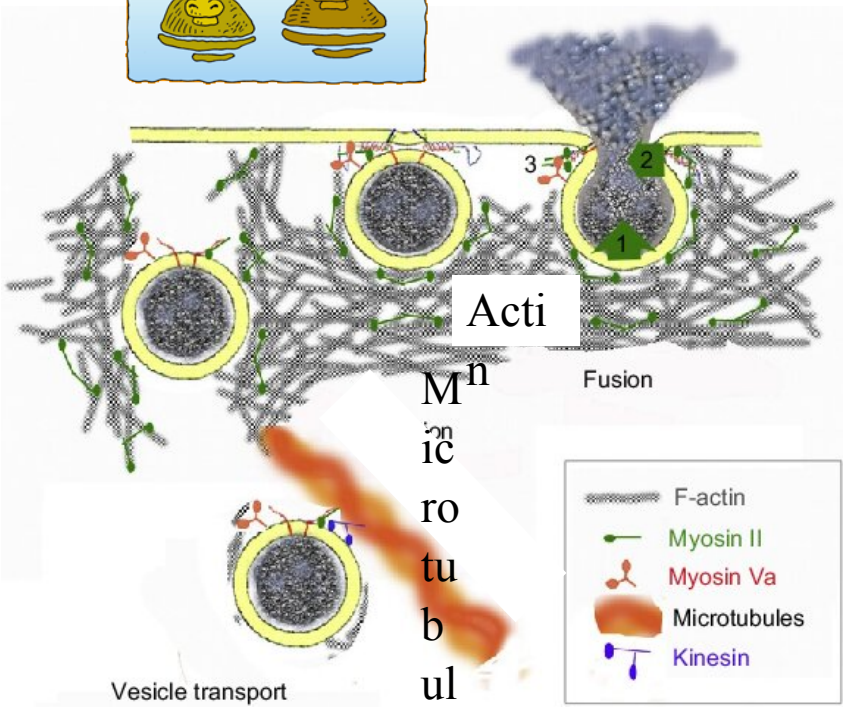
Neuronal axonal transport and diseases

Mutants of dynein and kinesin proteins reduce the ability of neurons to move organelles and proteins in neurons leading to neurodegeneration such as in amyotrophic lateral sclerosis (ALS; loss of muscle control), Alzheimer's disease (dementia) and Charcot-Marie-Tooth disease.



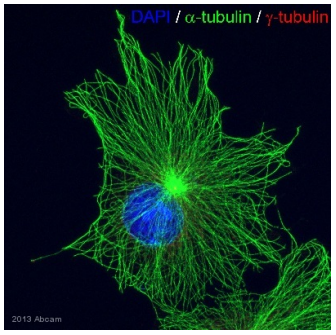
“Changing horses in midstream”

- Myosins of actin filaments 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.

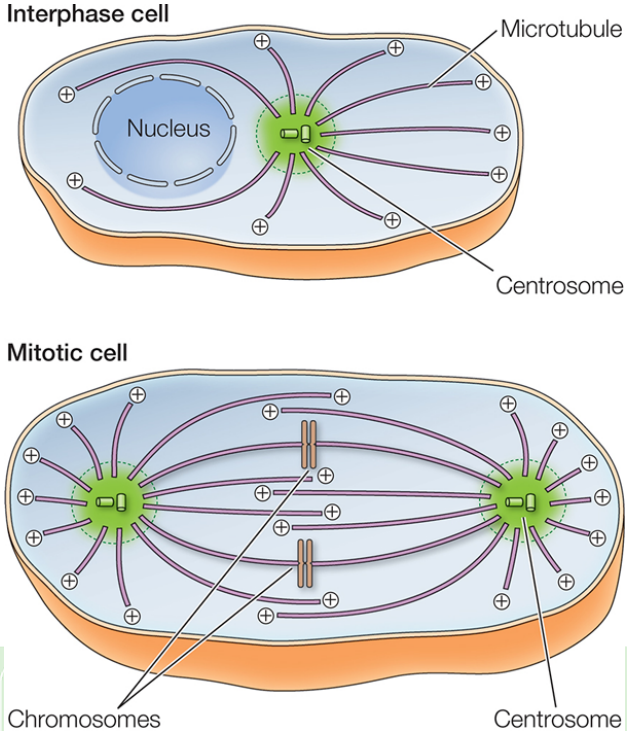


Centrosome

A microtubule-organizing center



- The centrosome serves as the initiation site for the assembly of microtubules, which then grow outward toward the periphery of the cell with their minus ends anchored in the centrosome.
  - In interphase cells, the centrosome is located near the nucleus and microtubules extend outward to the cell periphery.
  - During mitosis, duplicated centrosomes separate, and microtubules reorganize to form the mitotic spindle.



Intermediate filaments

What are they?

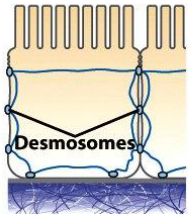
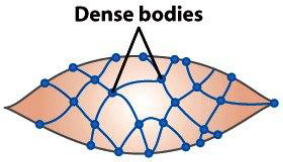
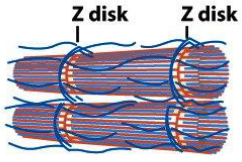
- Intermediate filaments have a diameter that is intermediate between actin filaments and microtubules.
- Functions:
  - They provide mechanical strength to cells and tissues.
  - They provide a scaffold that integrates the components of the cytoskeleton
  - They organize the internal structure of the cell.

Types of IFs

- They are composed of a variety of proteins, which are classified into 5 groups based on similarities between their amino acid sequences.
  - Types I and II are expressed in epithelial cells which synthesize at least one type I (acidic) and one type II (neutral/basic) **keratin**.
    - Hard keratins are found in hair and nails.
    - Soft keratins exist in the cytoplasm of epithelial cells.
- Type III:
    - Vimentin exist in fibroblasts, smooth muscle cells, and white blood cells.
    - Desmin is specifically expressed in muscle cells.
- Type IV: neurofilaments (NF) found in the axons of motor neurons.
- Type V: nuclear lamins, components of the nuclear envelope.

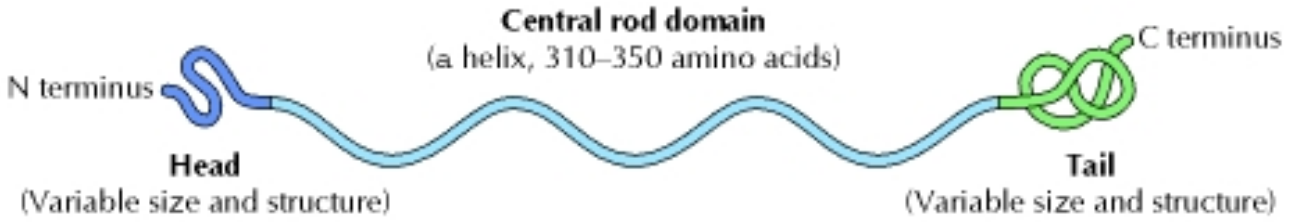
Types of IFs

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	 Epithelial cell
II	Basic keratins	Epithelial cells		
III	Desmin, GFAP, vimentin	Muscle, glial cells, mesenchymal cells	Sarcomere organization, integrity	 Smooth muscle  Skeletal muscle
IV	Neurofilaments (NFL, NFM, and NFH)	Neurons	Axon organization	 Axon
V	Lamins	Nucleus	Nuclear structure and organization	 Nucleu

Structure of IFs

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



Assembly of IFs

No polarity

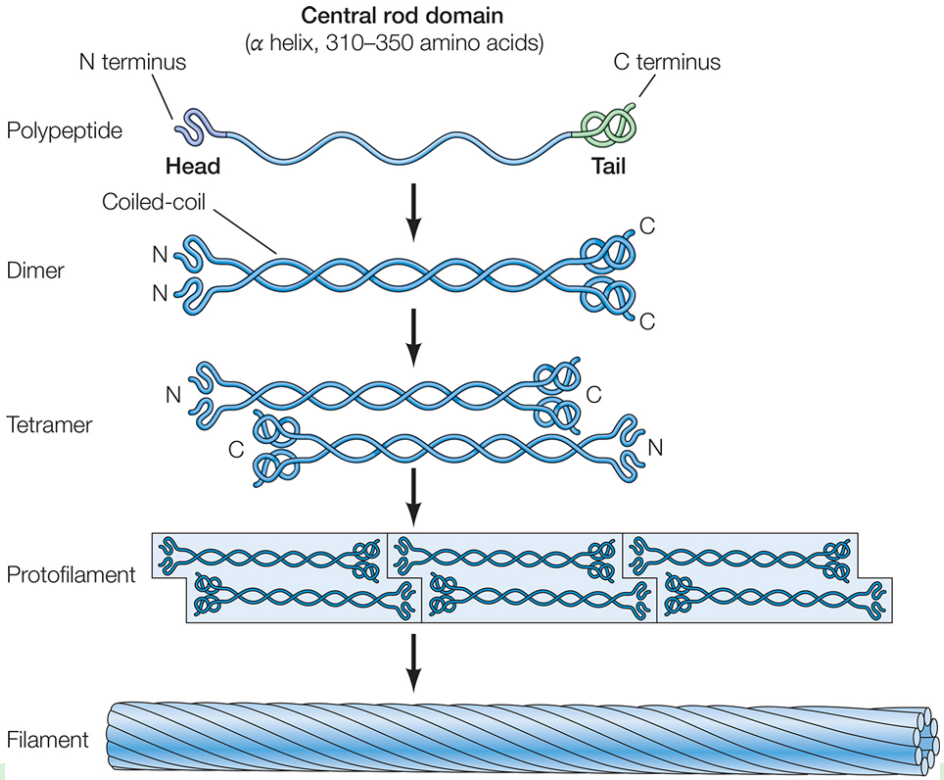
Intermediate filament proteins contain central α-helical rod domains and N-terminal head and C-terminal tail domains.

The central rod domains of two polypeptides wind around each other in a coiled-coil structure to form dimers.

Dimers then associate in a staggered antiparallel fashion to form tetramers.

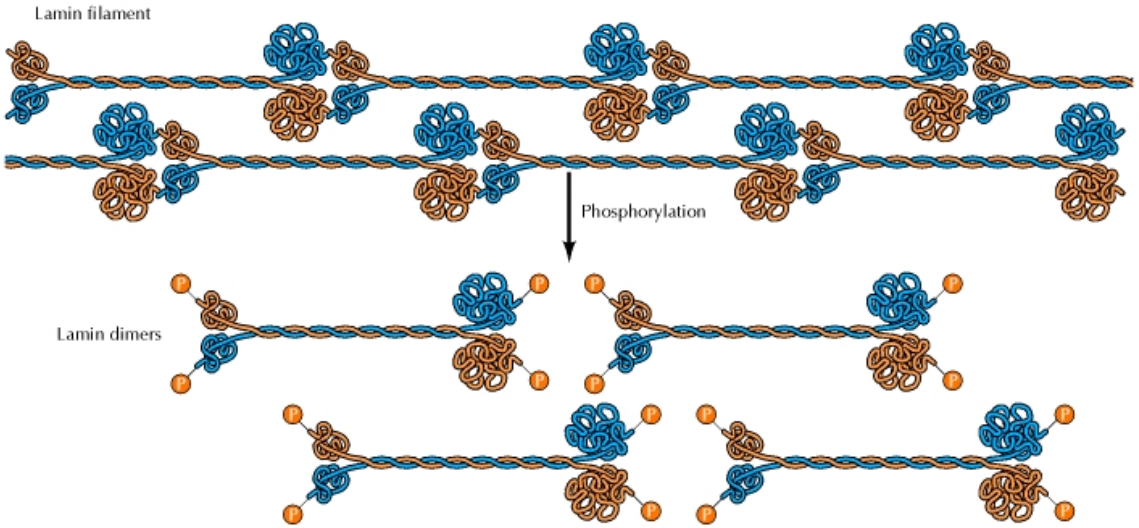
Tetramers associate end to end to form protofilaments and laterally to form filaments.

Each filament contains approximately eight protofilaments wound around each other in a ropelike structure.



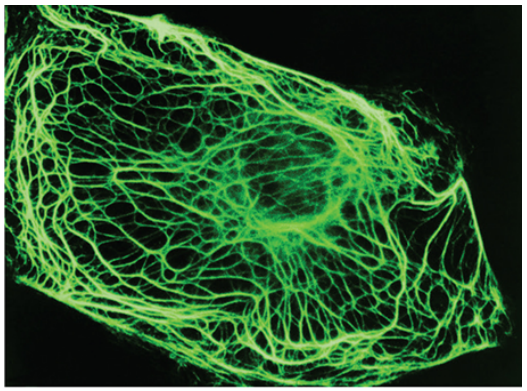
Relative to actins and microtubules

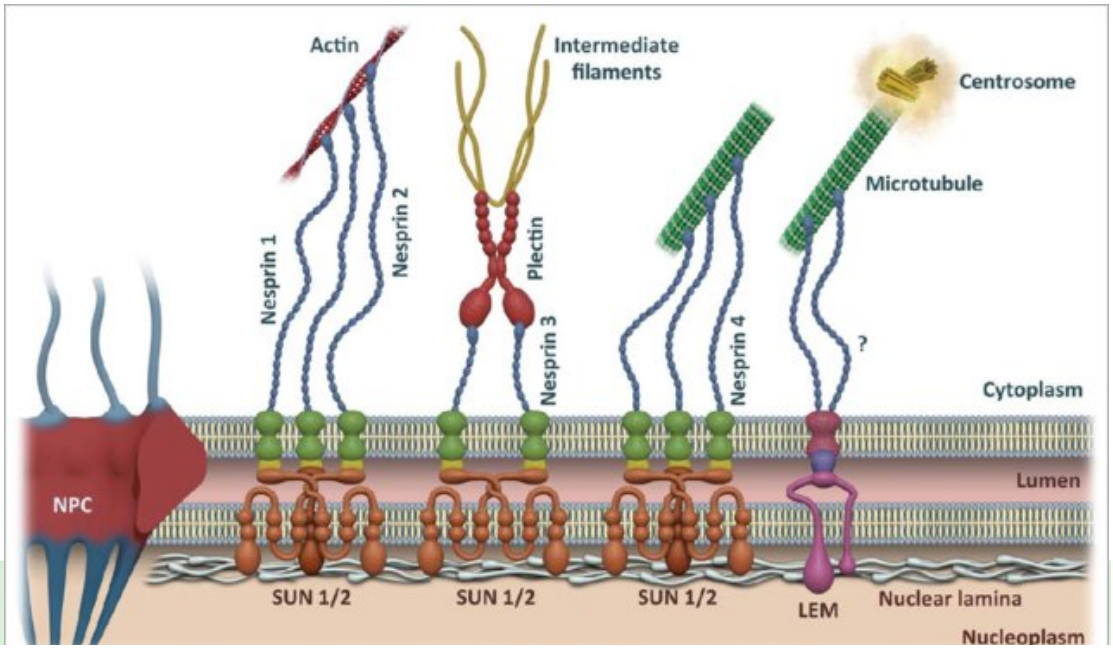
- More stable
- Not dynamic
- Not regulated by GTP, but regulated by phosphorylation
  - When nuclear lamins and vimentins are phosphorylated, they are disassembled.



Intracellular Organization of Ifs
The nucleus

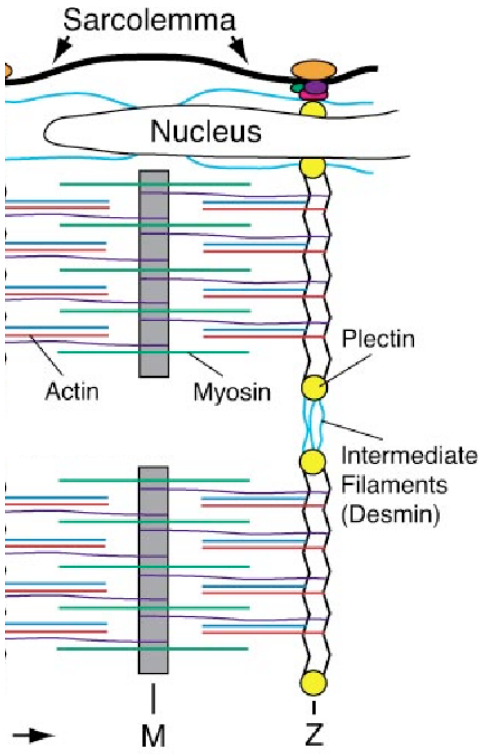
- Both keratin and vimentin filaments attach to the nuclear envelope to position and anchor the nucleus within the cell.





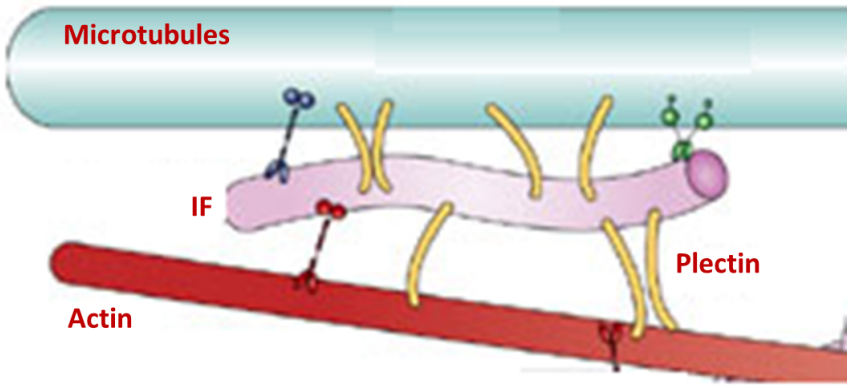
Intracellular Organization of Ifs
Muscle cells

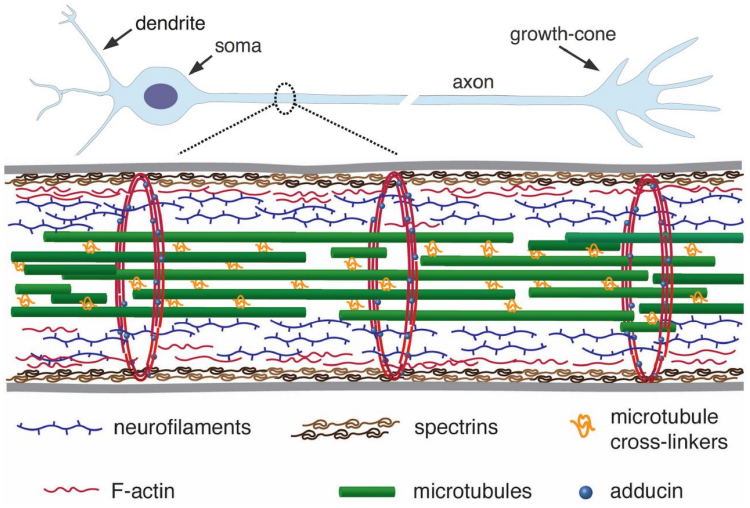
- The IF desmin connects the actin filaments in muscle cells to one another and to the plasma membrane, thereby linking the actions of individual contractile elements.



Intracellular Organization of Ifs
Neurons

- Neurofilaments in axons of mature neurons bridge actin filaments and microtubules stabilizing them and increasing the mechanical stability of the cell.

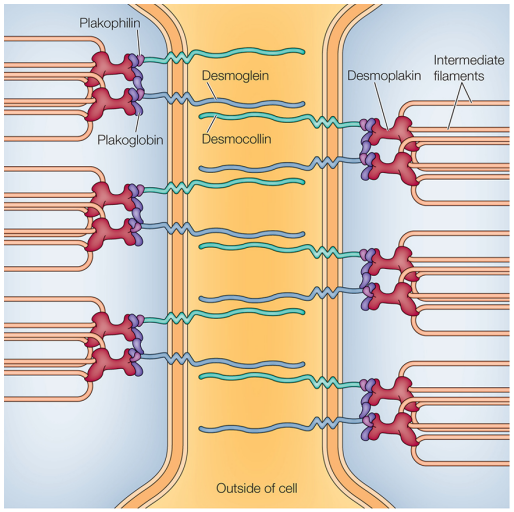




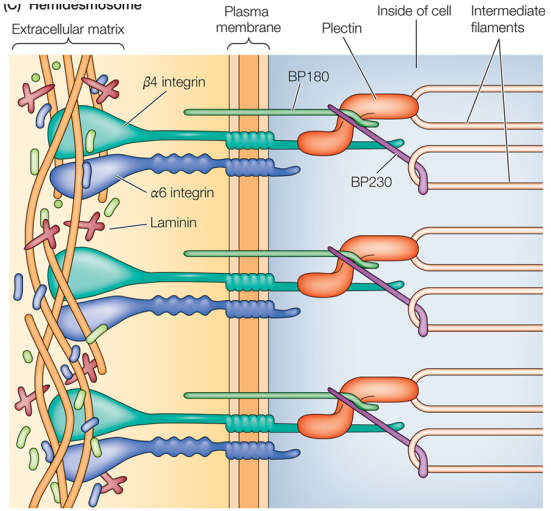
Intracellular Organization of IFs
Epithelial cells

- The intermediate filaments provide mechanical stability to the tissue at desmosomes and hemidesmosomes.
- **Desmosomes**: Junctions between cells connected to each other via cadherin-like transmembrane proteins and intermediate-binding proteins (<u>similar to adherens junctions</u>).
- **Hemidesmosomes**: Junctions linking intermediate filaments to the extracellular matrix via integrins (transmembrane receptors) and intermediate filament-binding proteins (like focal adhesions).

<u>Desmosomes</u>
<u>Cell-cell contacts</u>



<u>Hemidesmosomes</u>
<u>Cell-substratum contacts</u>



IFs and diseases

- It appears that the primary role of intermediate filaments is to strengthen the cytoskeleton of cells in the tissues of multicellular organisms.

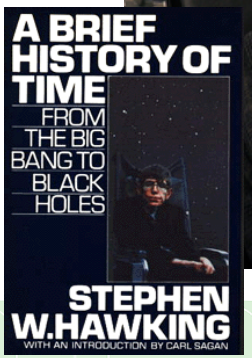
Transgenic mice expressing mutated keratins suffer from severe skin abnormalities (blisters due to epidermal cell lysis following mild mechanical trauma).

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



Exp Neurol. 2022 September ; 355: 114143. doi:10.1016/j.expneurol.2022.114143.

Multiple roles for the cytoskeleton in ALS

Xinbei Liu[a], **Jessica L. Henty-Ridilla**[a,b,*]

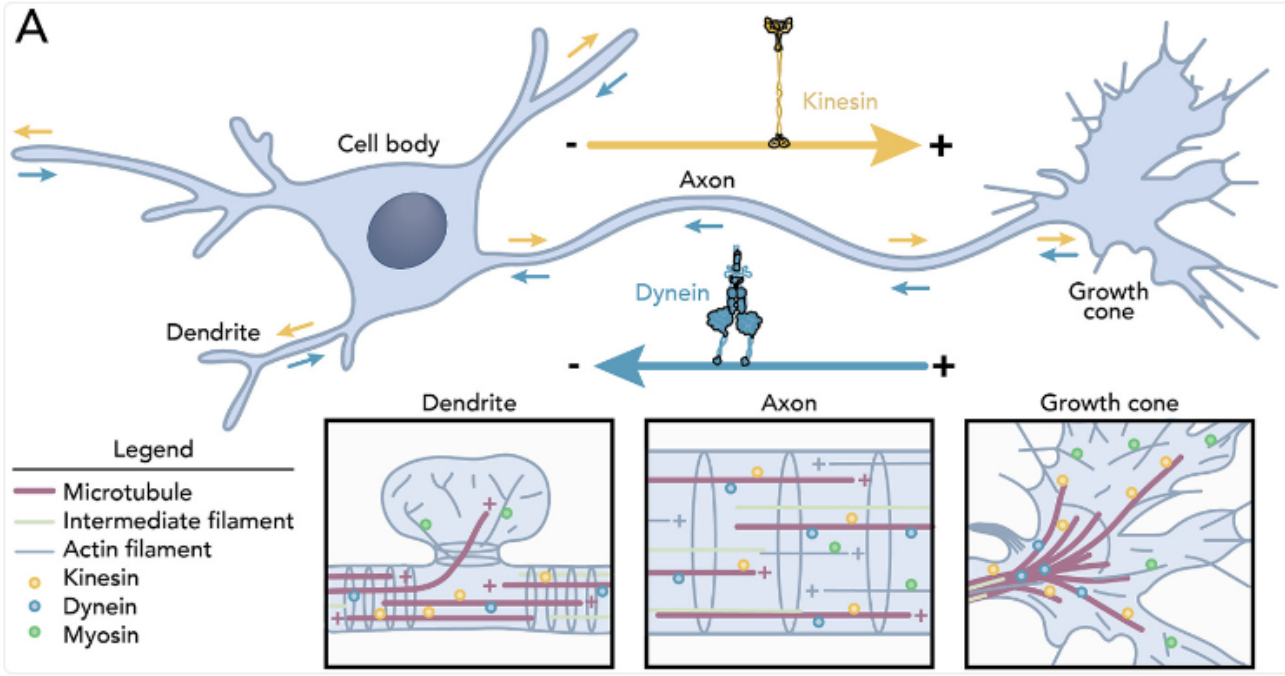


Table 7.2 The Structure and Function of the Cytoskeleton

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