بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | Lecture 7

The cytoskeleton

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Specialized regions

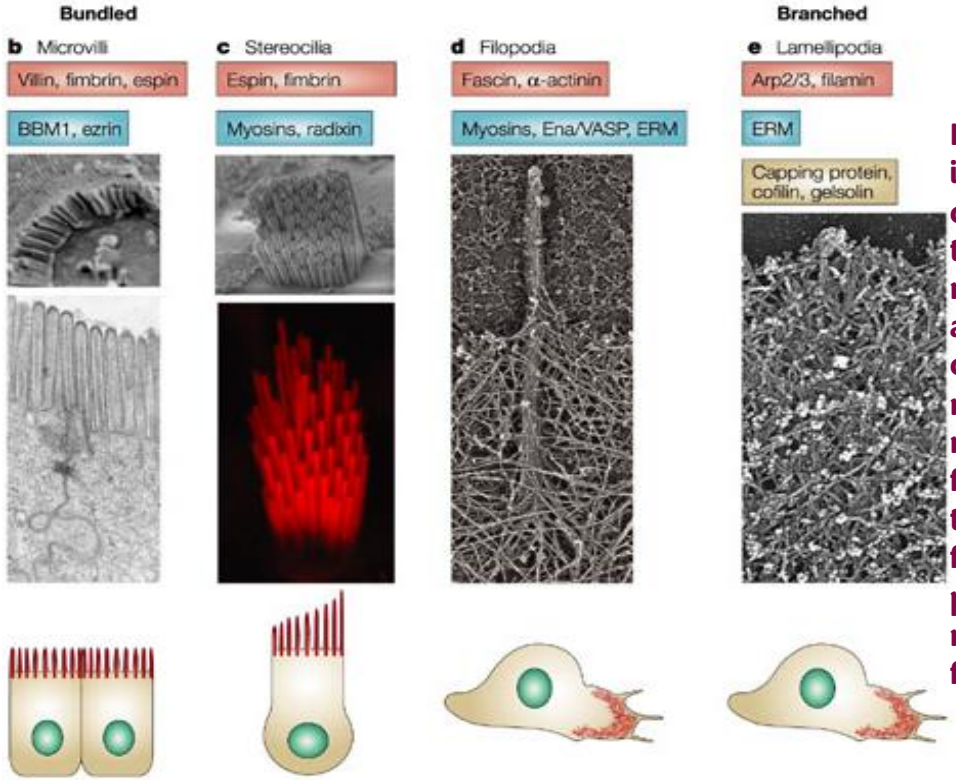
- The surfaces of most cells have a variety of protrusions or extensions that are involved in cell movement, phagocytosis, or specialized functions such as absorption of nutrients.
- Most of these cell surface extensions are based on actin filaments, which are organized into either relatively permanent or rapidly rearranging bundles or networks.

Microvilli finger like structure on the surface of intestines, its function to increase surface area to have maximum of absorption nutrients

Stereocilia, in the auditory cells to sense wavelength , frequency of sound

Filopodia , extention , a single finger underneath has a network

Lamelipodia it usually associated with Filopodia (lamelipodia underneath and as extension finger Filopodia)



It is found in motile cells while they are moving. The actin cytoskeleton is responsible for pushing the cell forward – pushing the membrane forward

Actin cytoskeleton can form differ structure inside cell and these structures are specialised region has specific functions which actin change the morphology state of cell determined by surrounding or environment .

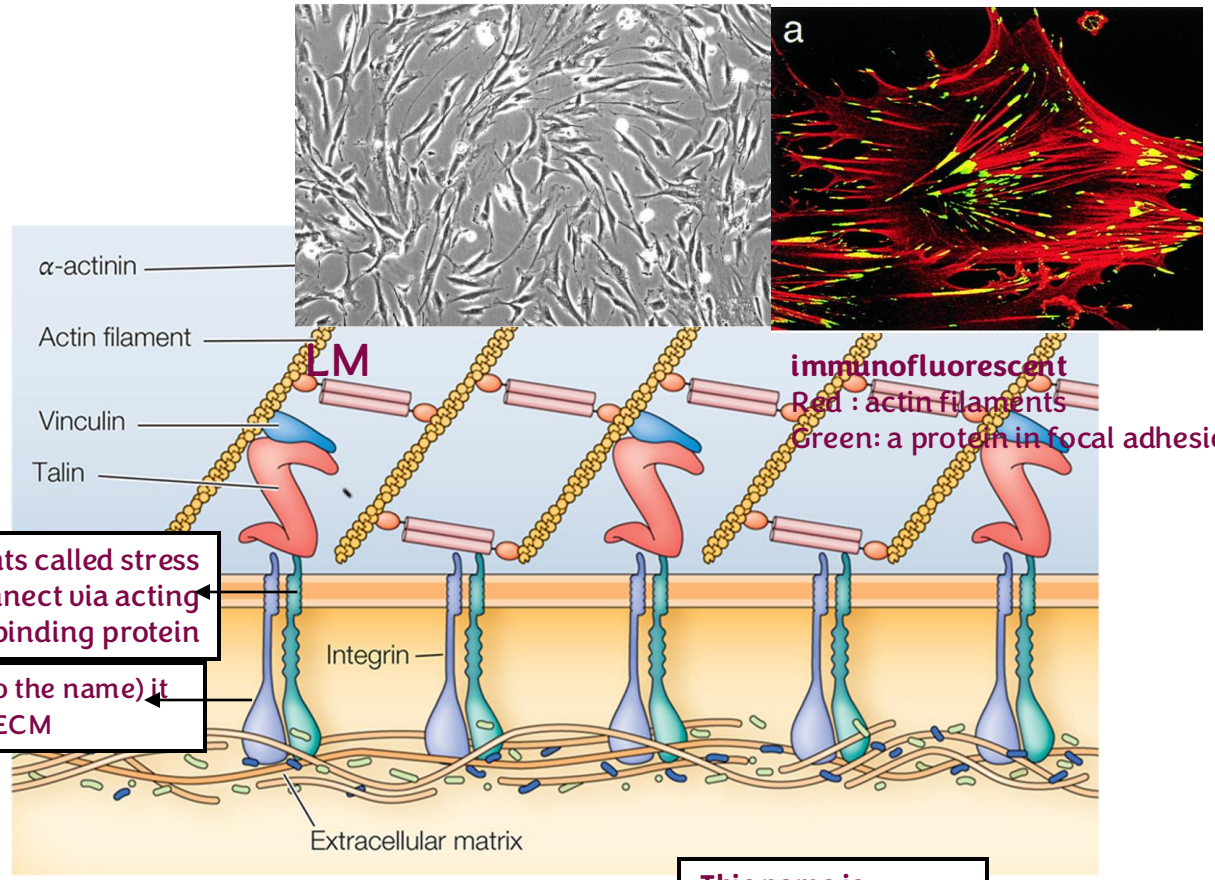
Actin organized as bundle

Specialized regions: focal adhesions

Show in the cell that grow in laboratory , when put the cell in plate and it adhere by focal adhesions with surface that called substratum

They are often seen in fibroblasts.

- Cultured fibroblasts secrete extracellular matrix proteins that cells bind to via **integrins**, and transmembrane proteins, at sites called **focal adhesions**, which themselves attach to bundles of actin filaments, called **stress fibers**.
- The actin bundles are stabilized by specialized actin-binding proteins.



LM

immunofluorescent
Red : actin filaments
Green: a protein in focal adhesions

Focal adhesions: actin filaments linked with actin binding linked with receptors linked with ECM

Actin filaments called stress fibres connect via acting binding protein

this is actin bind protein (don't care to the name) it bind with receptors which liked with ECM

Any change outside the cell for example (integrin make interaction with liminal instead of collagen)it will send a signal to inside the cell to change the status of the cell or shape and so on.That what happens in cancer in differentiation of different cell inside our system

This name is important, these integrin are dimmer subunit (alpha,bata) linked filament with matrix

Specialized regions: focal adhesions

Cells need a surface to attach, called a substratum. In the lab, this is usually a plastic dish coated with proteins like fibronectin or collagen, while in the body, the natural extracellular matrix (ECM(acts as the substratum, made of proteins and sugars. Cells attach to both types of substratum at focal adhesions, where integrins connect the inside of the cell)actin filaments(to the outside proteins. Focal adhesions hold the cell in place, help it move, and send signals from the environment

Specialized structure:

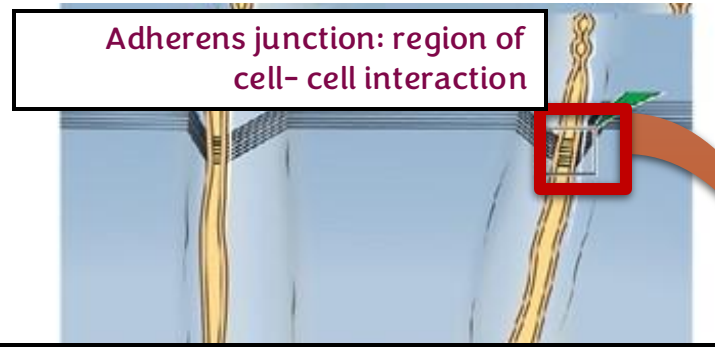
Adherins junctions

Adherens junctions are present in epithelial cells

- Cells interact with each other at **adherens junctions**, which are mediated by the transmembrane **cadherins**, which interact indirectly with actin filaments.
- The junctions form a continuous belt of actin filaments around each cell.
- Cadherins attach to actin filaments via **catenins** stabilizing the junctions.
- **Epithelial cells lose cadherins when they become cancerous becoming fibroblast-like.**

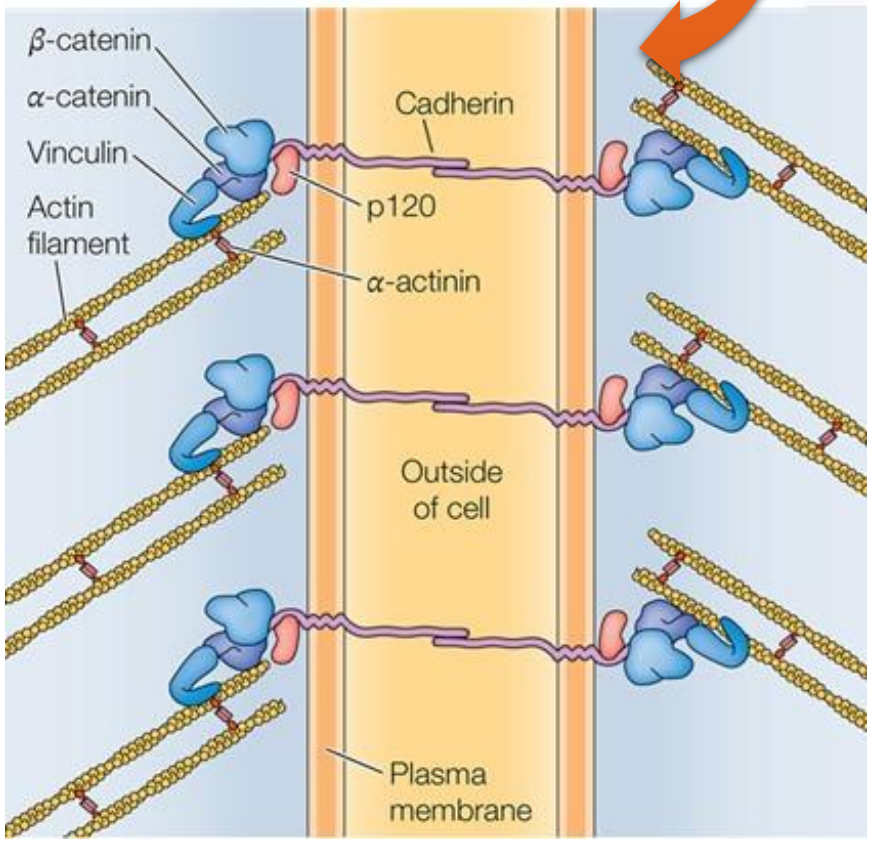
When loose cadherins mean loose cell – cell interaction they become elongated, motile , mesenchymal cells (fibroblast) these characteristics when normal cell become cancer cell.

https://sketchfab.com/3d-models/intercellular-junctions-c49bf33b4efe4d32ae0bdfab5d344b4d

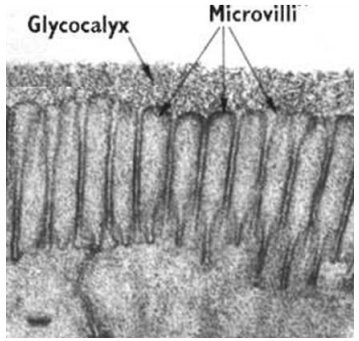


Adherens junction: region of cell- cell interaction

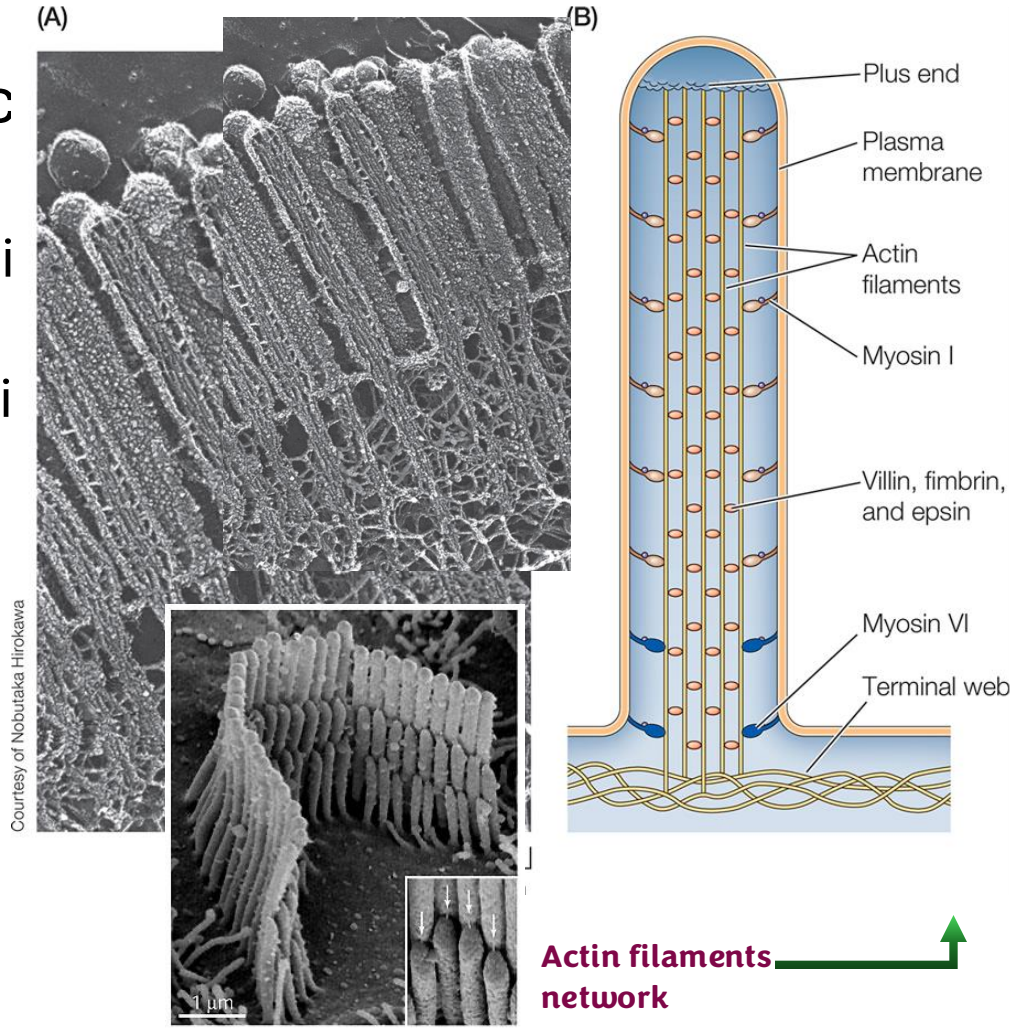
This receptor **Cadherin** is linked indirectly with actin cytoskeleton via actine binding proteins one of them called **catenin.**



Specialized structures:
Microvilli



- Finger-like extensions of the plasma membrane that are found on the surfac of cells involved in absorption such as:
  - The apical surface (brush border) of intesti epithelial cells.
  - Stereocilia: a specialized form of microvilli the surface of auditory hair cells.
- The microvilli are stabilized by the anchorage of the actin bundles to the spectrin-rich actin cortex.



Underneath there actin filaments network flexible called cortex mean (out the cell) which stabilise the finger

Specialized regions:

mean dynamic, keep changing

Transient surface protrusions for cell movement, phagocytosis, nerve cell extensions

This process requires dynamic changes of the actin cytoskeleton, which first extends and then surrounds the bacteria

Fake arm

- **Pseudopodia**: Extensions of actin filaments cross-linked into a network for **phagocytosis**.

present in phagocytic cells like macrophages ,their arms surround bacteria cells making phagocytosis (cell eating)

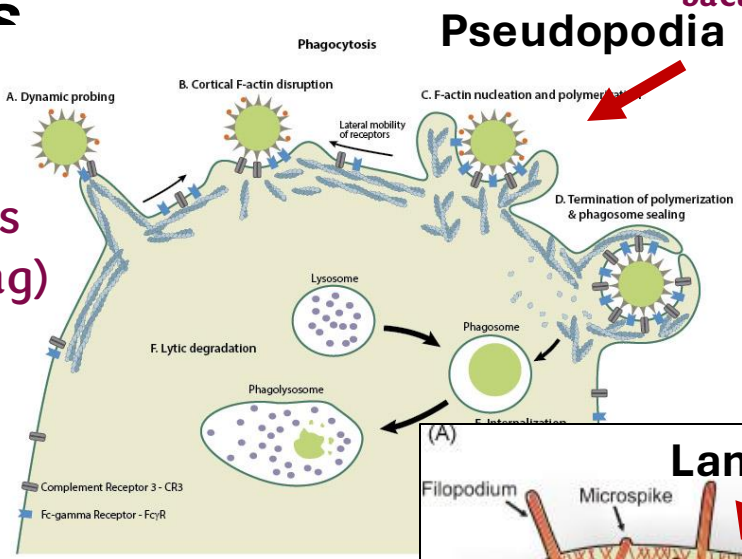
- **Lamellipodia**: Broad, sheetlike extensions made of a network of actin filaments at the leading edge of **moving** fibroblasts.

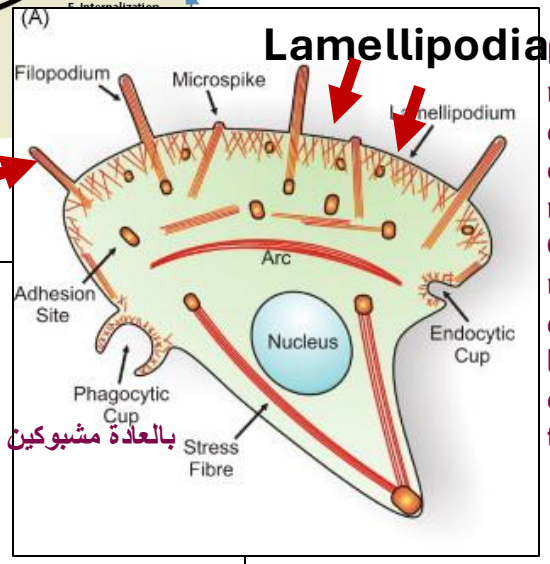
It's present in moving cells , to push cell forward

- **Filopodia**: Very thin projections of the plasma membrane, supported by actin bundles, that extend from lamellipodia for **sensory purposes**.

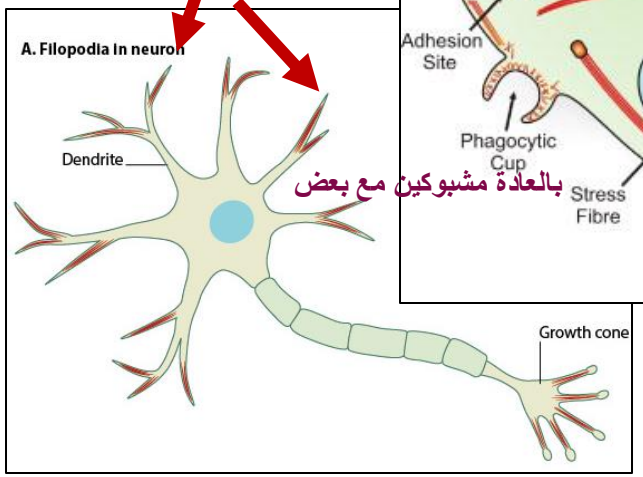
present in neurons

- The formation and retraction of these structures during cell movement is based on the regulated assembly and disassembly of actin filaments.





Filopodia



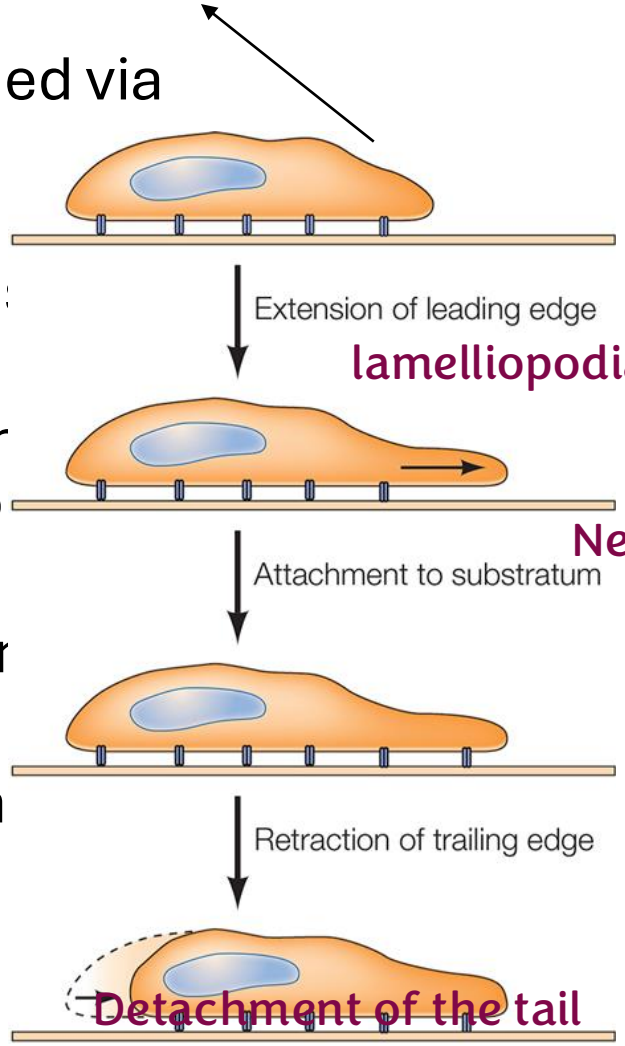
بالعادة مشبوكين مع بعض

Filopodia sense nutrients and determine the direction of cell movement. Once the nutrients are detected, the lamellipodia extend along the filopodia

Cell migration

- Cell-substratum attachment is normally maintained via stress fibers and focal adhesions.
- Movement is initiated via sensing an attractant (wounds, chemokines, etc.) and polarity is establi
- Actin-bundling proteins and focal adhesion protei transported to the leading edge in connection with integrins forming protrusions (lamellipodia, filopo psuedopodia).
- Actin filaments are extended via polymerization ar branching.
- At trailing end, focal adhesions are broken down a cells dissociate.

These are focal adhesions , between cells and substratum (cellular matrix)



lamelliopodia and Filopodia

New

Detachment of the tail

Rho family proteins

- The formation of cell surface protrusions in response to extracellular stimuli is regulated by small GTP-binding proteins of the Rho family.
  - Rho: formation of stress fibers and contraction
  - Rac: formation of lamellipodia
  - Cdc42: formation of filopodia (cell direction)

in cell membrane as network

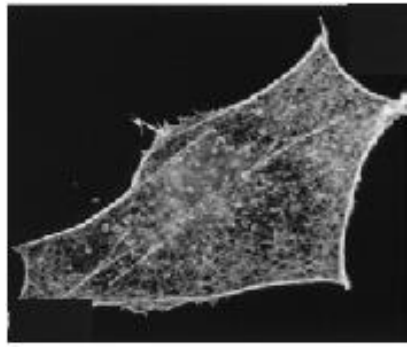
make sensing where cell should move

We have two type of G- protein :

1. **Large trimeric G protein**(alpha, bata , gamma) bind with GPCR

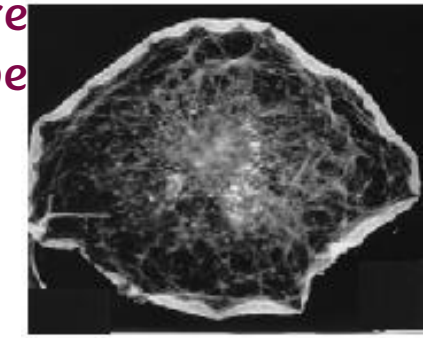
2. **Small monomer G-protein called Rho family** (Rho, RAC , Cdc42: it's name come from number of its molecular weight 42kda and it present in the yeast

Other small monomeric G- protein we talked about: Rab(for vesicular targeting) , Ras (for signal transduction)

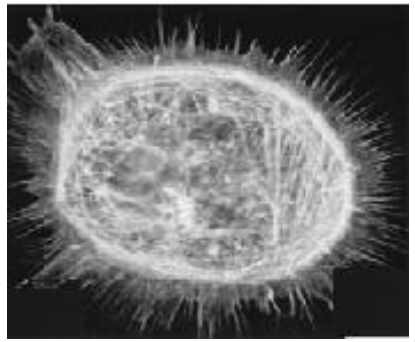


Normal cell

Rho



Rac

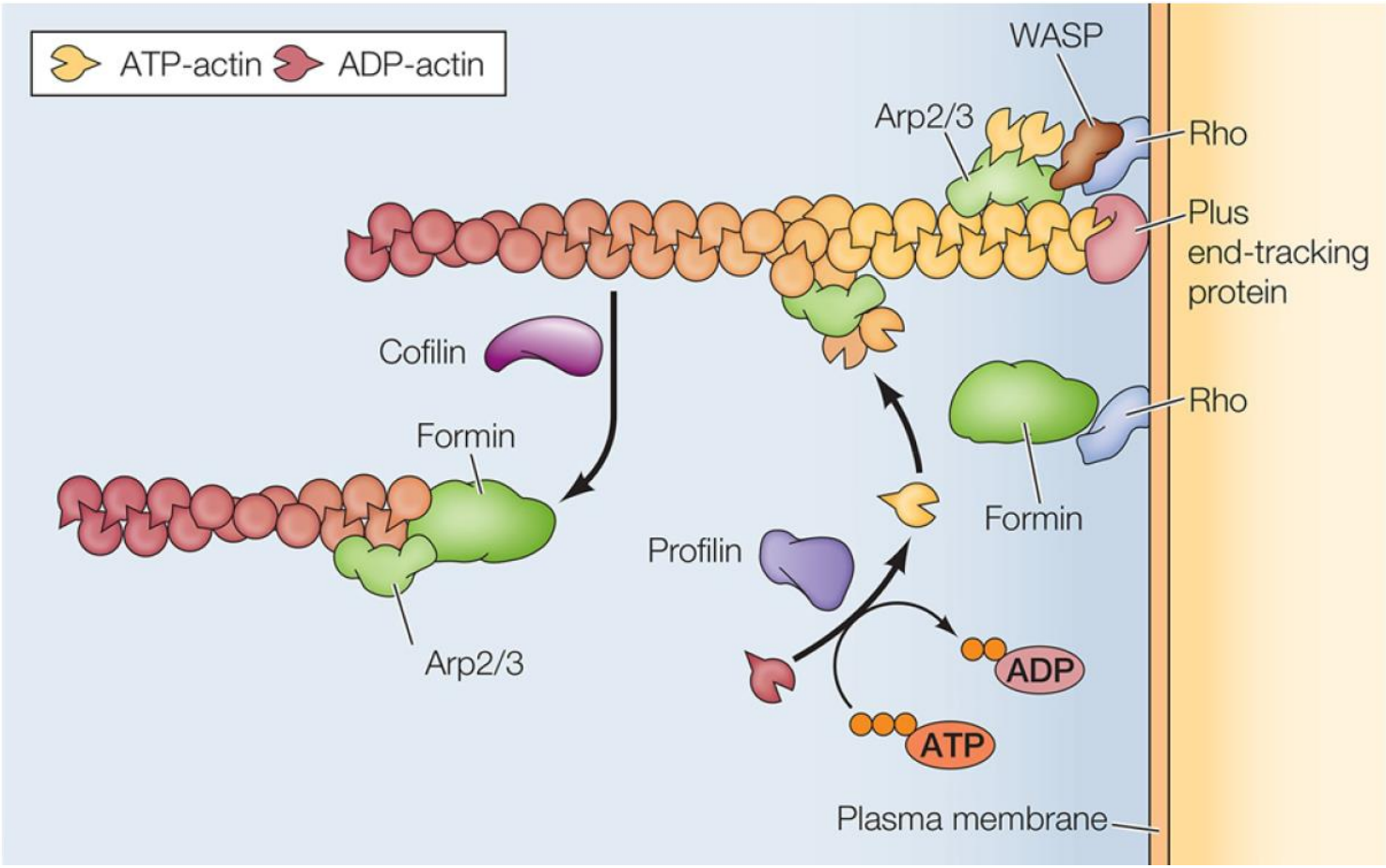


Cdc42

Actions of the Rho family

They work on the actin binding proteins , make a polymerisation /depolymerisation / Growth of the actin micro filaments/ shrinkage of the actin micro filament/branching.

- Profilin
- Formin
- Arp2/3
- Cofilin



Lecture 6: the cytoskeleton (Microtubules and intermediate filaments)

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Second year, Second semester, 2024-2025

Overview

- Microtubules are rigid hollow rods.
- They are dynamic structures that undergo continual assembly and disassembly within the cell. keep changing ,growing and shrinking
- Functions:
  - Cell shape

    Microtubules Maintain (support) the cell shape ,
    the actin filaments which **determine** the cell shape
  - Intracellular transport of organelles

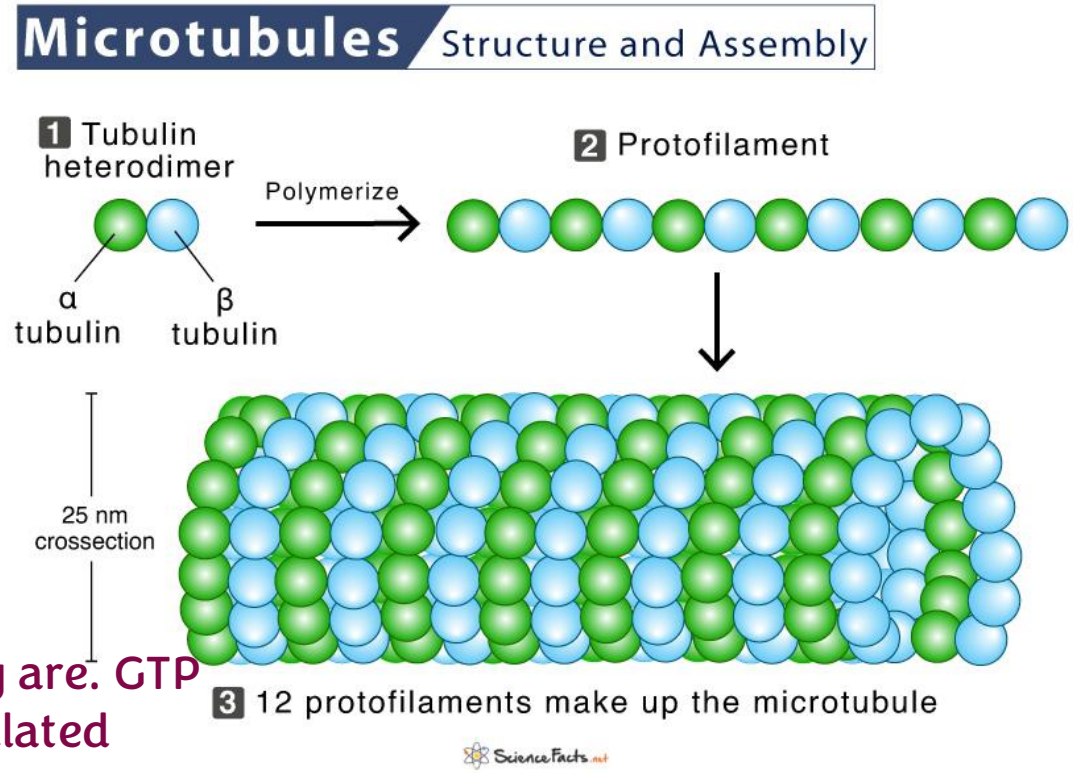
    Like, vesicles, lysosomes ,endosomes ,
    secretory vesicles

    Also determine the **location** of organelles(ER, Golgi..)
  - 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 th centrosome and it initiates microtubu assembly for chromosomal separatio
- The tubulin dimers polymerize to form protofilaments (a hollow core) of arrays of the tubulin dimers.
- Both α- and β-tubulin bind GTP.

A stretch fiber , would combine together forming microtubules that is hallow in the middle .



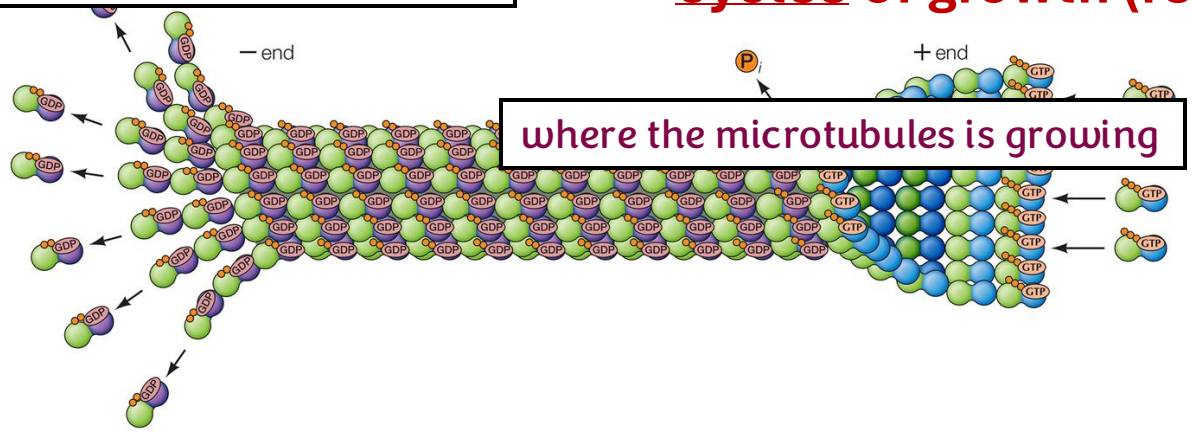
They are. GTP regulated

Treadmilling and dynamic instability

- Microtubules are polar like actin micro filaments 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.

ATP binding isn't important for the actin proteins to grow , but it **stabilizes** the structure.
But here , GTP binding in plus+ end is **important** for **growing** a microtubules .

where the microtubules shrinkage ,get breaking up



where the microtubules is growing

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

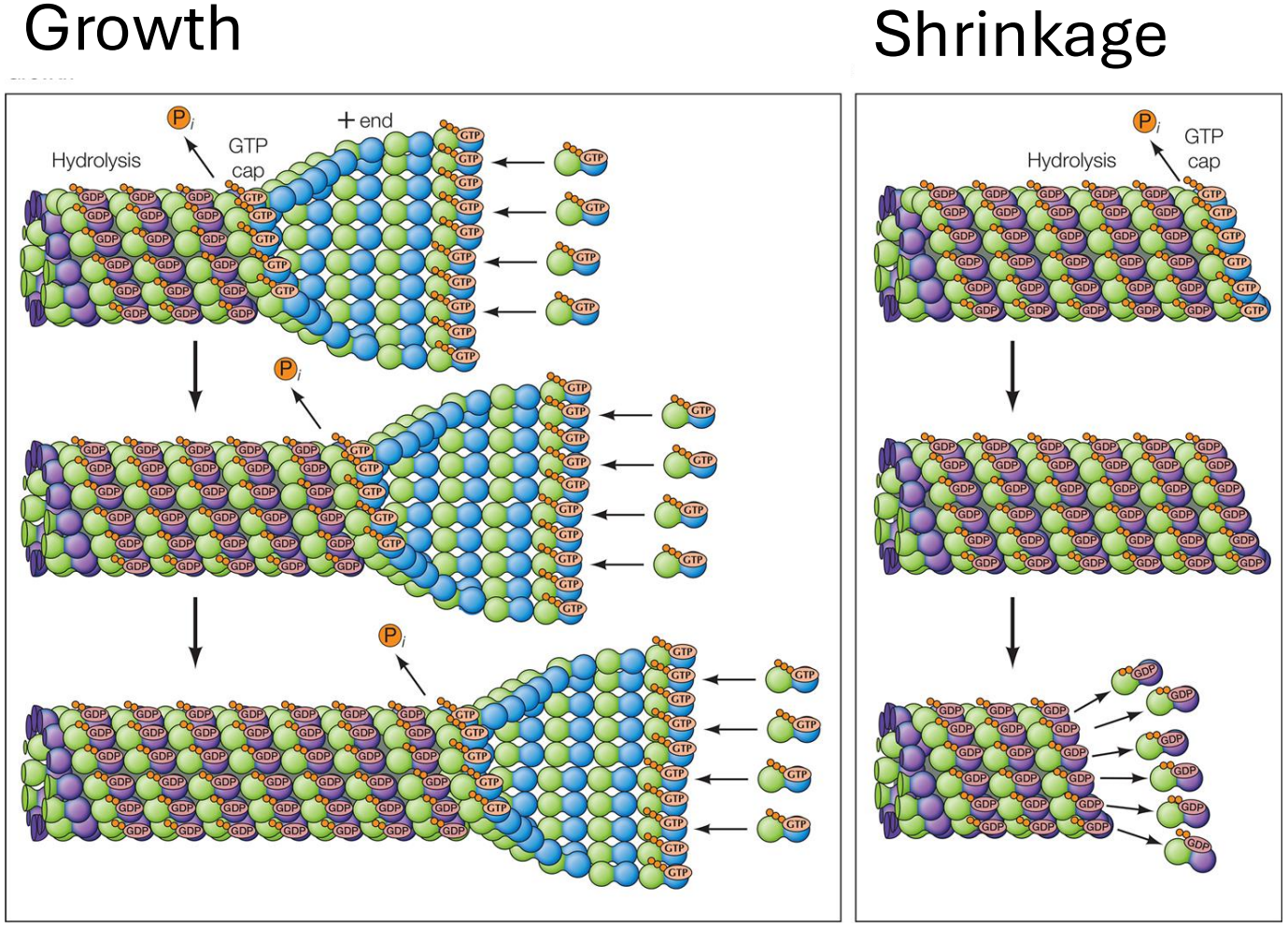
binding GTP to B- tubulin make it hydrolysis this destabilizing to the microtubules so at the minus – end we have breaking up and dissociation of dimers which have dynamic instability alternates between shrinkage and growth not always the cell has growth and shrinkage with each other (so it can be extend or growth without dissociation in other end vise versa) called treadmiling
Sometimes we have shrinkage more than growth depend on the need of the cell.

The reason behind dynamic instability

- Dynamic instability results from GTP hydrolysis of β-tubulin during polymerization, which reduces its binding affinity for neighboring molecules.

GTP hydrolysis occurs only in β-tubulin, not in α-tubulin

- 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.



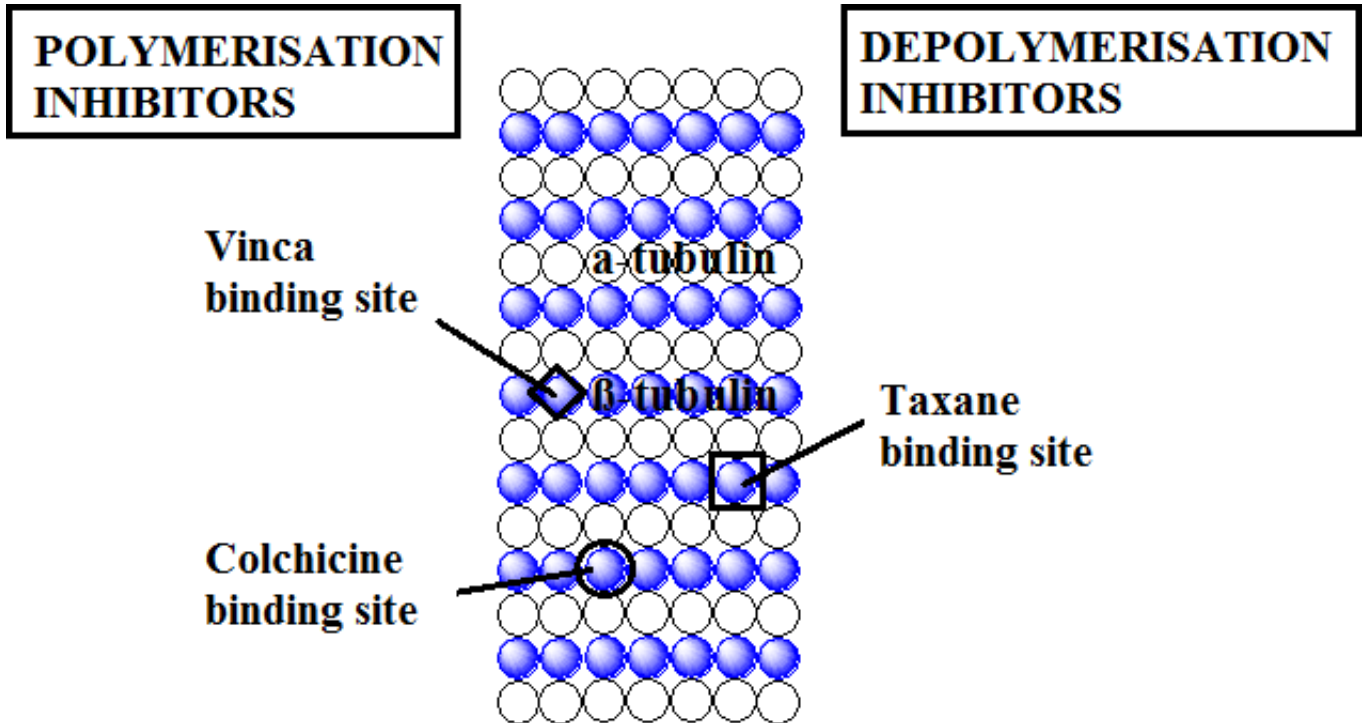
Drugs

Can inhibit growth or inhibit shrinkage

We need to know the name

- 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.

Treat cancer patients



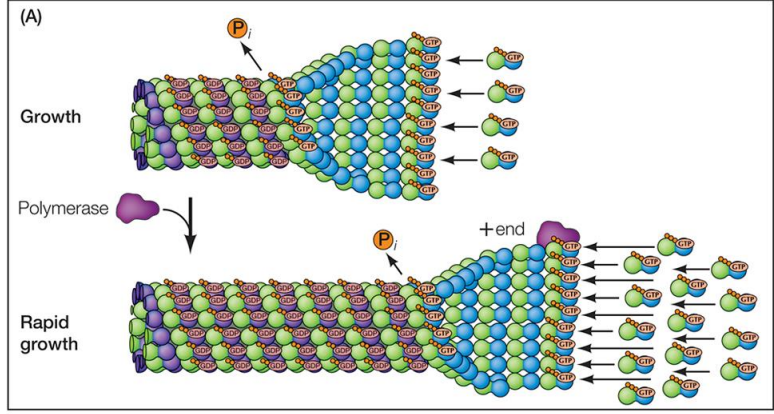
Regulatory proteins

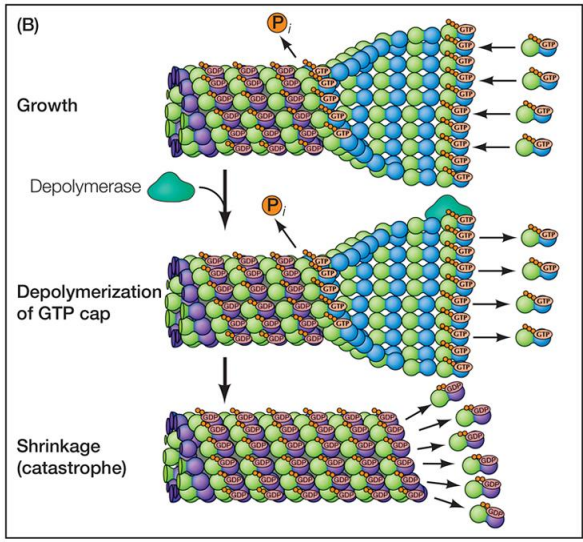
- Microtubule-associated proteins (MAPs) regulate the dynamic behavior of microtubules by
  1. Regulating:
     A. growth or polymerization (by polymerases) or
     B. shrinkage or depolymerization (by depolymerases) at the plus ends of microtubules,
  2. Suppressing microtubule catastrophe and promoting rescue:
     - CLASP (close on the microtubules prevent the catastrophe then start growth) proteins rescue microtubules from catastrophe.

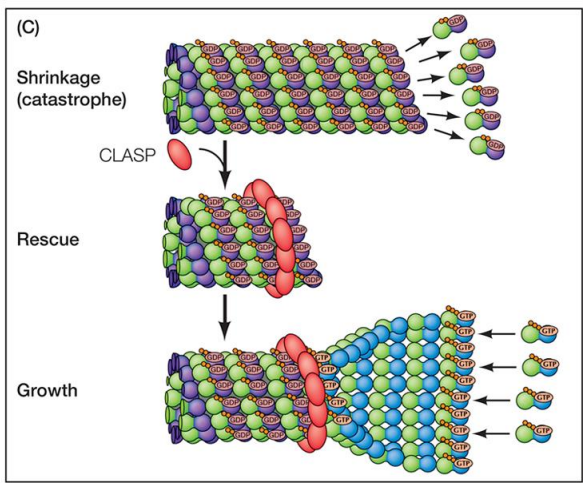
Catastrophe كارثة: Microtubules become quick depolymerisation from the(+ end) .

Rescue: stop the quick depolymerisation and start again polymerisation

Catastrophe = growth →shrinkage
Rescue = shrinkage →growth

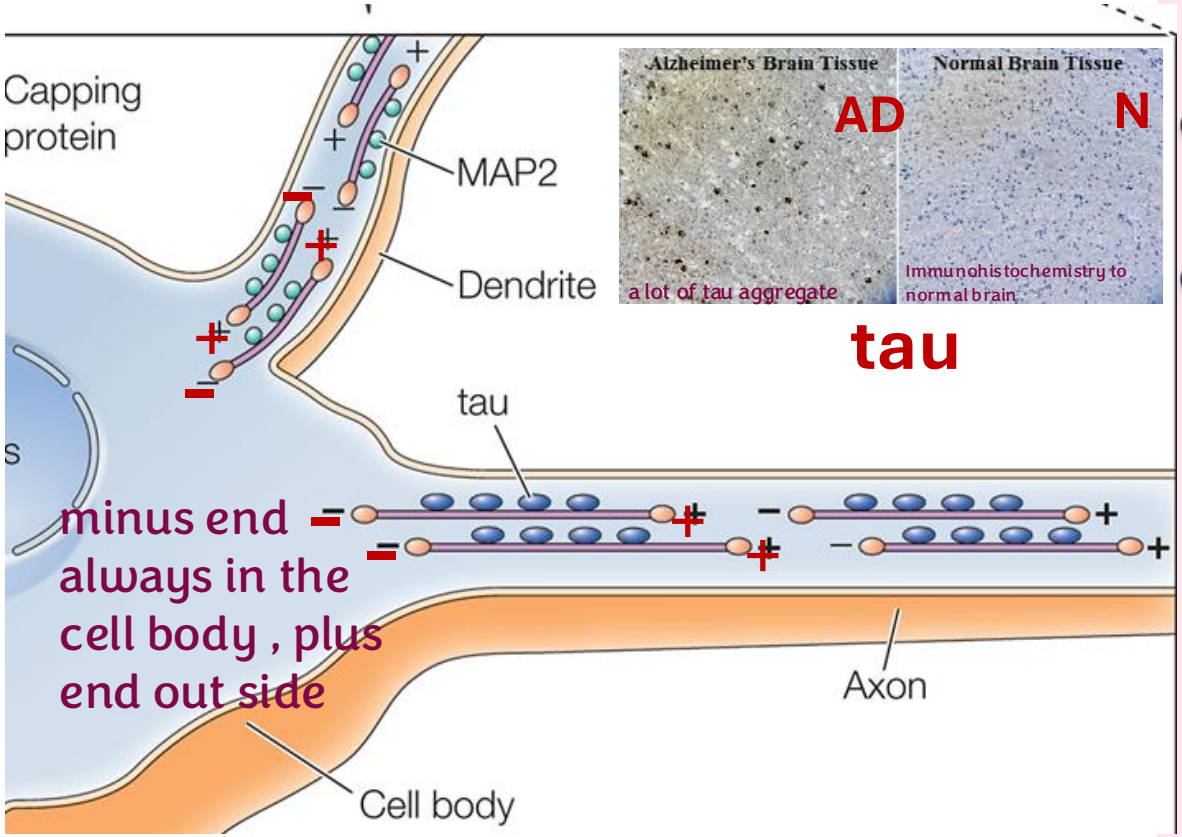






Organization of microtubules within cells Example: neuron

- 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. In one direction
  - Axons contain tau is a microtubules binding protein, which is the main component of lesions found in the brains of Alzheimer's patients it accumulate inside the cell making cell toxicity and death and aggregates to the out .

Microtubules-motor proteins

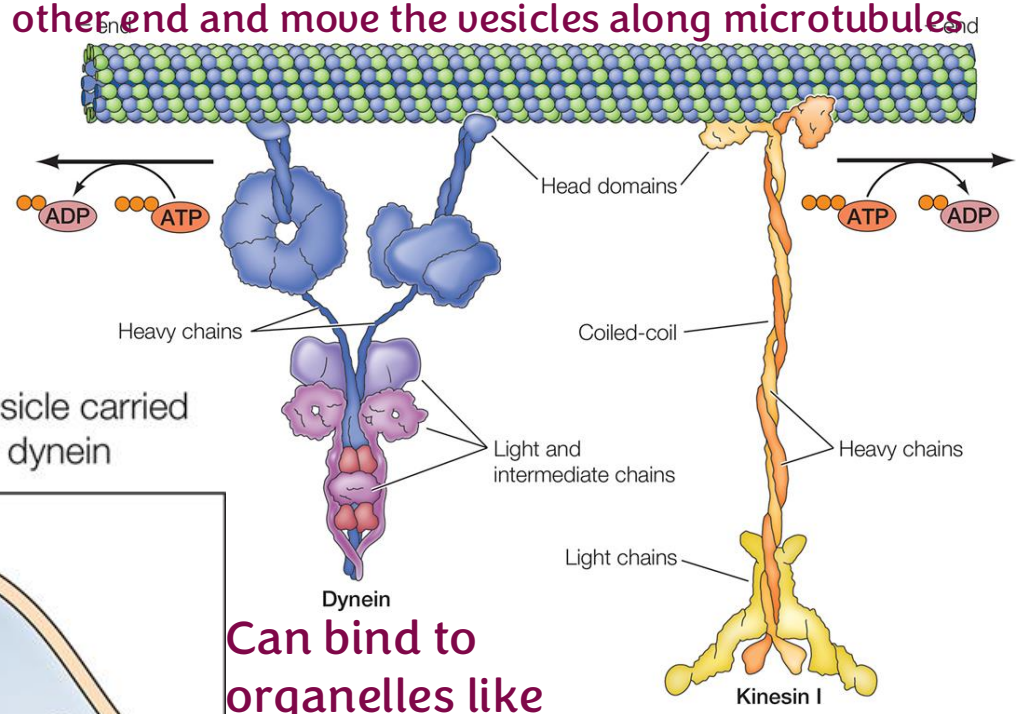
e.g., kinesin and dynein

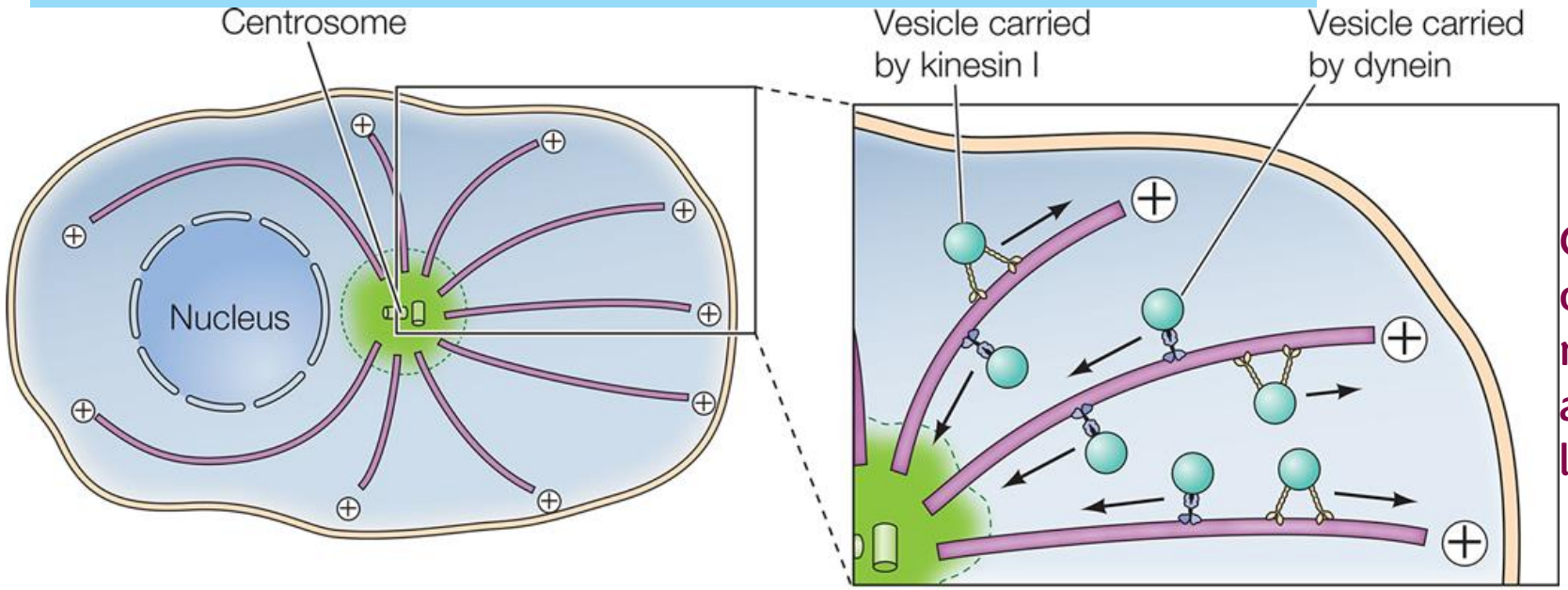
- Microtubules-motor proteins use ATP to move along microtubules in opposite directions.
  - Kinesin moves toward the plus end.
  - Dynein moves toward the minus end.

Disclaimer : Exceptions

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

Attach with vesicles at one end and attach to tubule in other end and move the vesicles along microtubules



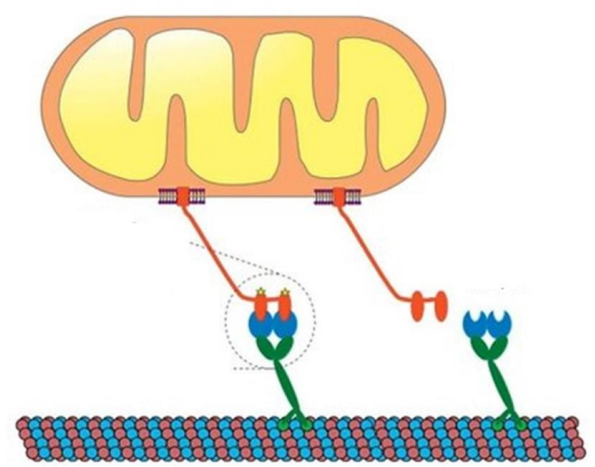


Can bind to organelles like mitochondria and molecules like mRNA.

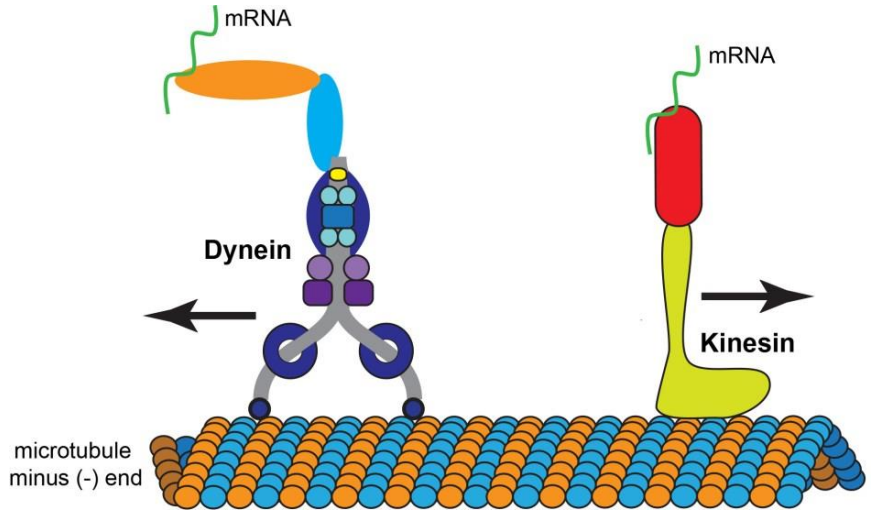
Translation of mRNA occurs at specific locations within the cell

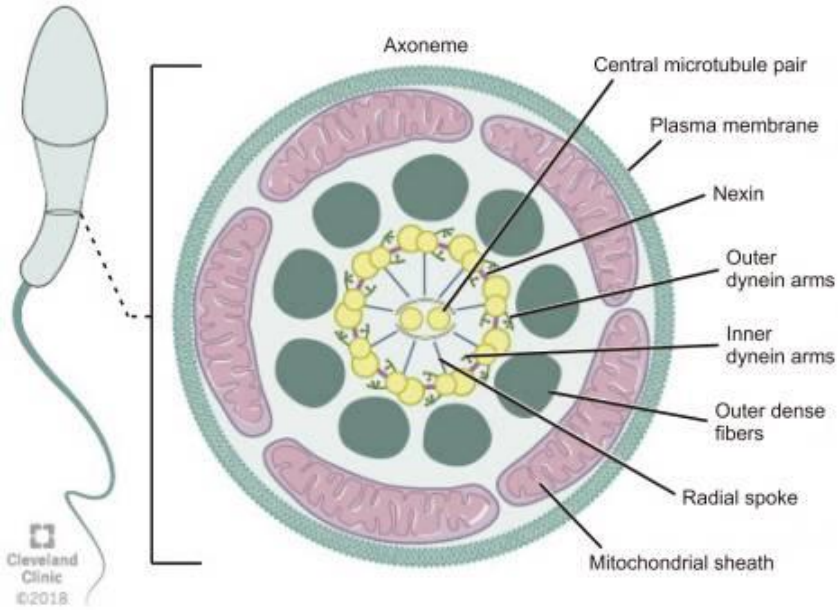
Other functions of microtubules

- 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.



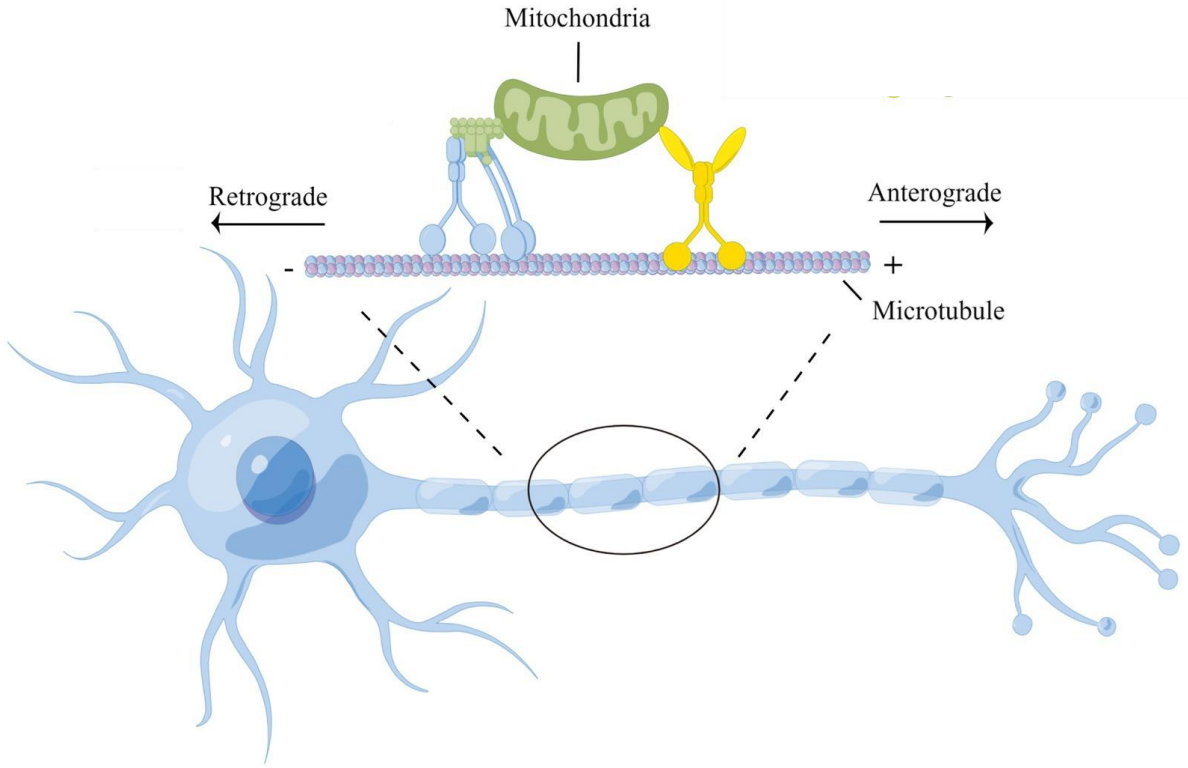
Any mutation in kinesin or dynein can lead to infertility





Neuronal axonal transport and diseases

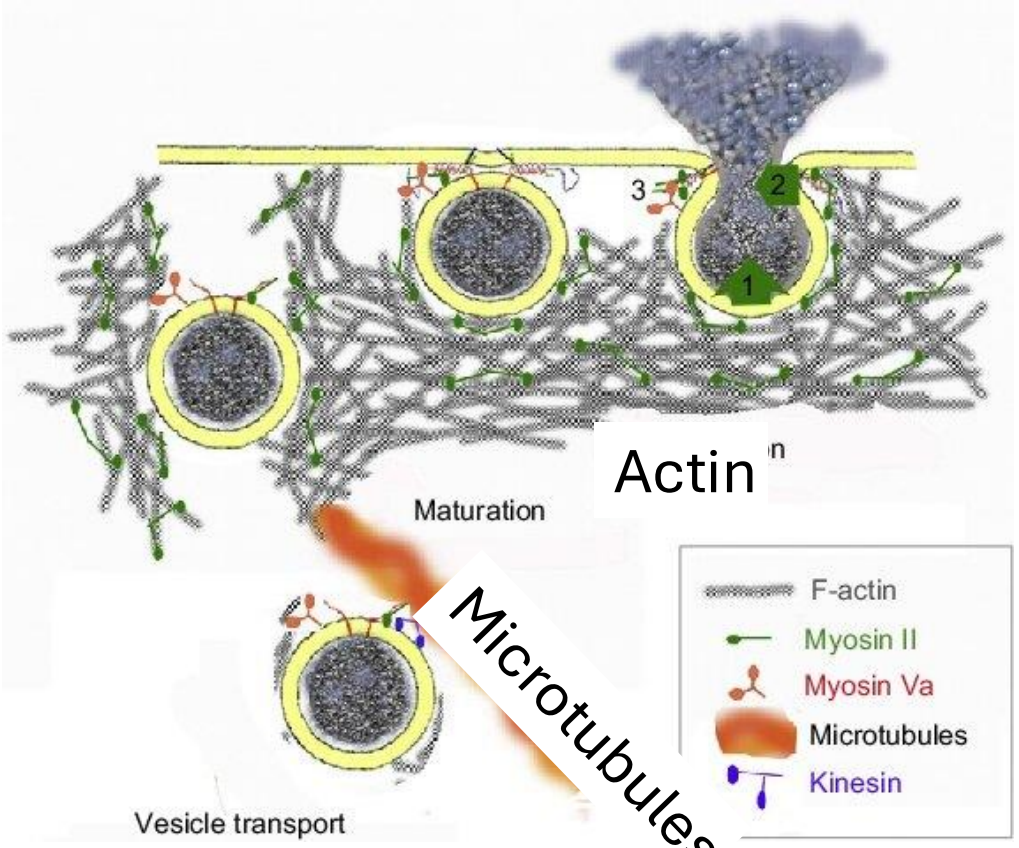
Mutants of dynein and kinesin proteins reduce the ability of neurons to move organelles and proteins in neuron's leading to neurodegeneration such as in amyotrophic lateral sclerosis (ALS; loss of muscle control), Alzheimer's disease (dementia) and Charcot-Marie-Tooth disease.



Mutations in these vesicular binding proteins certain diseases can be cause (multi symptomatic) and many neuron affected .

“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.



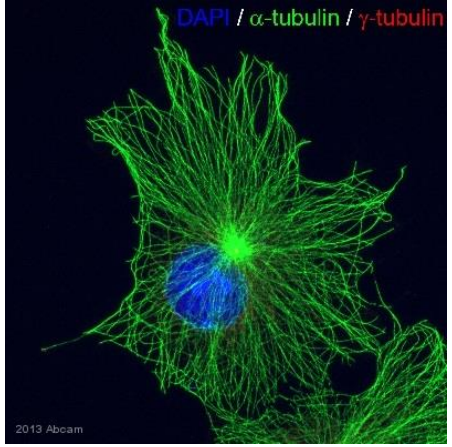
Actin in neurons close to the membrane
Microtubules moving the vesicles to actin cytoskeleton Between actin and microtubules there is a gab waiting the signal(calcium ion) and other gab between actin and plasma membrane.,When signal inter the cell the actin cytoskeleton make a conformational change and interact with microtubules (bridge) and interact with plasma membrane then the vesicles will jump from microtubules to the actin and reach the plasma membrane make a fusion and release of neurotransmitters.

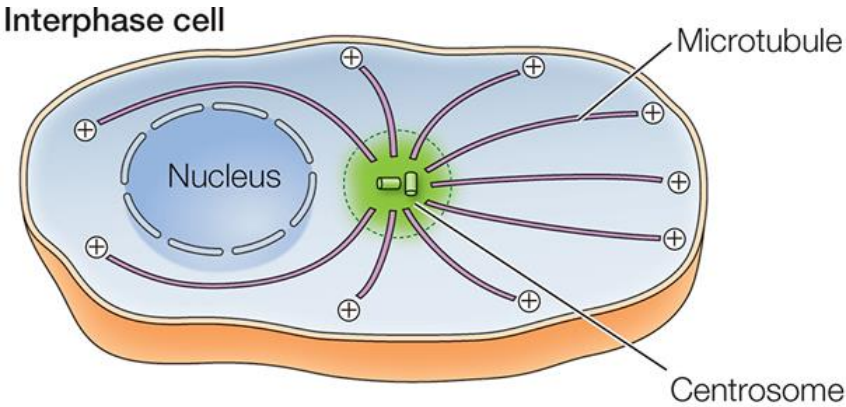
Centrosome

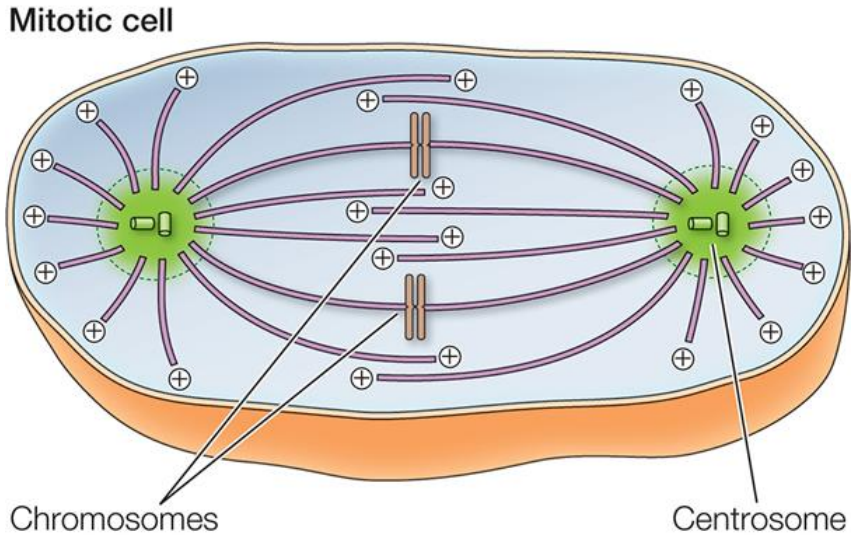
A microtubule-organizing center

- 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.

alpha and beta tubules present in all cells but gamma tubule present in the dividing cell which responsible to formation a centrosome for the mitosis.







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