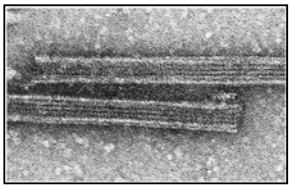
Microtubules

Microtubules

- Cylindrical stiff tubes 24 nm across
- Polymers of globular proteins - tubulins
- Dynamic system - assembly and disassembly
- Found in all eukaryotes

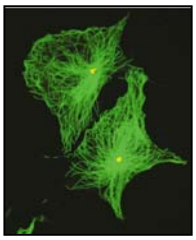


Microtubules - function

- Support for cellular components and tracks for several motor proteins
- Responsible for variety of movements
  - Beating of cilia and flagella
  - Transport of membrane vesicles
  - Extension of neuronal growth cone
  - Formation of mitotic spindle

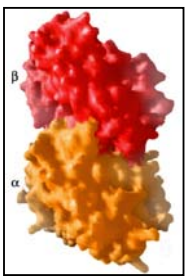
Organization within the cell

- Polarized
  - Plus and minus ends
- Have radial organization
  - Microtubule organizing center – centrosome or basal body
- Stability controlled by microtubule-associated proteins (MAPs)



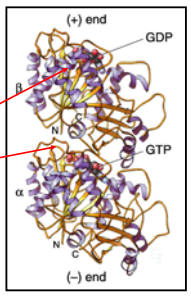
Tubulin

- Heterodimer of two different subunits - α and β
  - Products of separate genes but highly homologous
  - α and β subunits rarely dissociate
- γ subunit - not included in the filament, has a role in microtubule assembly



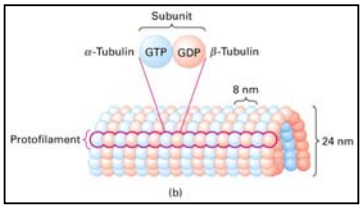
Tubulin

- Tubulin is a GTPase that binds and hydrolyses GTP
- Each tubulin binds 2 GTP
- Two binding sites
  - Exchangeable on β subunit
  - Irreversible on α subunit



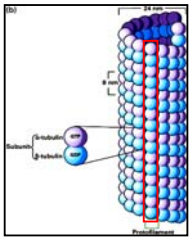
Structure of microtubules

- Cylinders of longitudinally oriented protofilaments



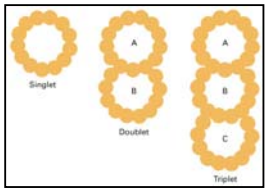
Protofilament

- Tubulins arranged end to end
- Dimeric subunit repeat every 8 nm
- Protofilaments have structural and functional polarity



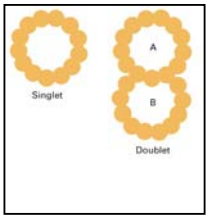
Arrangement of protofilaments in microtubules

- Protofilaments can be arranged in singlet, doublet or triplet microtubules
- Singlet
  - 13 protofilaments
  - Intracellular microtubules



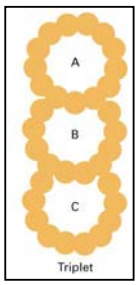
Singlet, doublet, and triplet microtubules

- Doublet
  - Singlet (A tubule) and one additional B tubule consisting of 10 protofilaments appended to its side
  - Cilia and flagella



Singlet, doublet, and triplet microtubules

- Triplet
  - A tubule plus additional B and C tubules of 10 protofilaments
  - Basal body and centrioles

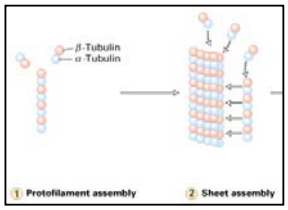


Microtubule assembly

- Microtubules assemble by polymerization of αβ subunits
- Addition and loss only at the ends
- Assembly of GTP bound tubulin is favored over GDP bound
- Assembly and disassembly depend on critical concentration of αβ subunits

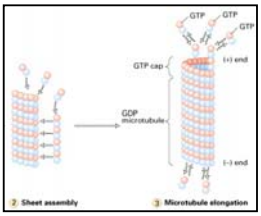
The steps of microtubule assembly

- Formation of protofilament
  - Free αβ- tubulin dimers associate longitudinally
- Sheet assembly
  - Lateral association of protofilaments



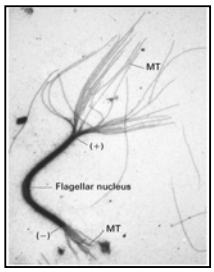
The steps of microtubule assembly

- Sheets wrap around to form a hollow tube
- Elongation by addition of subunits



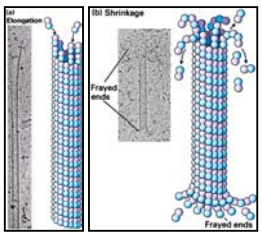
Microtubule assembly

- Addition preferentially at the plus end
  - Plus and minus ends look different
  - The ends of growing and shortening microtubules (assembly and disassembly) look different



Assembly and disassembly conditions

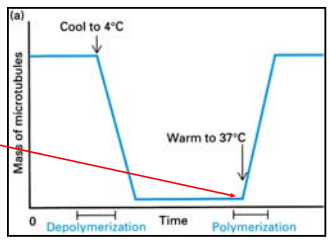
- Assembly
  - Relatively smooth ends
  - Some protofilaments are longer - elongate unevenly
- Disassembly
  - Ends are splayed
  - Frayed appearance



Microtubule polymerization

- Temperature dependent

polymerization in the presence of GTP

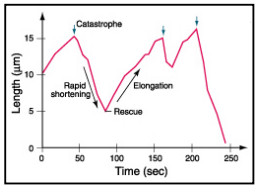


Drugs that disturb microtubule polymerization

- Drugs that disrupt microtubule assembly have antimitotic effect
- Taxol and vinblastine
  - Stabilize microtubules
  - Block cell division
  - Anti-cancer
  - High concentrations promote depolymerization - vinblastine paracrystals

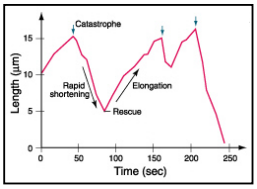
Dynamic instability of microtubules

- Intrinsic property of microtubules
- Random oscillation between periods of growth and shortening
  - Microtubule grows until it reaches the point when it starts to shrink
  - And than shrinks until it starts to grow again



Dynamic instability of microtubules

- The transitions are random events
  - Catastrophe and rescue
- Microtubule shortens much more rapidly than it elongates

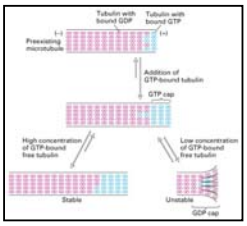


The GTP cap model of dynamic instability

- A microtubule is stable when capped with GTP bound tubulins
- A microtubule becomes unstable when capped with GDP tubulins

The GTP cap model of dynamic instability

- After incorporation - GTP hydrolysis on b tubulin
  - This creates a core of less stable GDP bound tubulin
- Loss of the cap is thought to cause the catastrophe

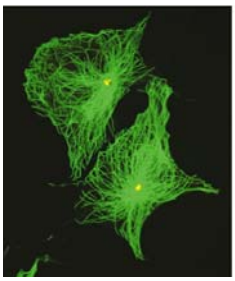


Microtubule Organizing Centers (MTOC)

- Microtubule assemble from microtubule organizing centers (MTOC)
- Minus end is adjacent to MTOC, plus is a distal end
- Primary MTOC
  - Centrosome
  - Basal body

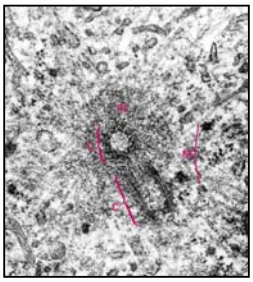
Microtubule Organizing Centers (MTOC)

- Hub-and-spoke array
- Nucleate and organize the assembly of cytosolic microtubules
- Usually perinuclear



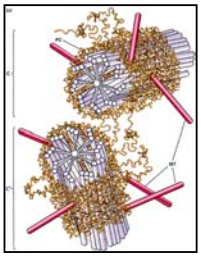
Centrosome

- A pair of centrioles at the right angle
- and pericentriolar matter



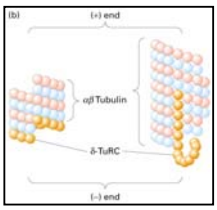
Centriole

- A pinwheel arrangement of triplet microtubules
- Does not make direct contact with the ends of cytosolic microtubules



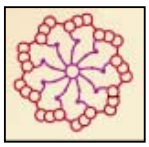
Pericentriolar matter

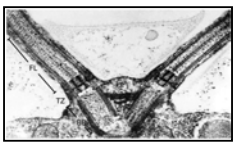
- Lattice of microtubule-associated proteins
- Contain pericentrin and γ tubulin
- γ tubulin ring complex nucleates the microtubule assembly



Basal bodies

- MTOC for cillia and flagella
- Cyllindrical structure of 9 triplet microtubules
- Two basal bodies form the point of "V"





Microtubule associated proteins (MAPs)

- Regulate microtubule assembly and architecture
  - Can stabilize
  - Destabilize or sever microtubules
  - Also cross link microtubules with plasma membrane or other cytoskeletal filaments
- Each group consist of many different proteins

Microtubule Stabilizing MAPs

- Bind along the end or cap a microtubule
- Tau family, MAP2, MAP4
- 3-4 repeats in the microtubule-binding domain separated by flexible linkers
- Each repeat binds to tubulin

Microtubule Stabilizing MAPs

- MAP2
  - Found only in dendrites
  - Forms cross-bridges between microtubules and intermediate filaments
- MAP4
  - Neuronal and non-neuronal cells
  - Regulates microtubule stability during mitosis

Microtubule Stabilizing MAPs

- Tau
  - Axons and dendrites
  - Cross-links microtubules into intermediate filaments
- In Alzheimer disease tau forms paired helical filaments

Microtubule Destabilizing MAPs

- **Op18/stathmin** increases depolymerization by binding tubulin dimers
- Enhances dynamic instability

Microtubule Severing MAPs

- **Katanin** severs microtubules into short fragments
- Creates additional ends for depolymerization
- Acts opposite to taxol

Regulation of MAPs

- Through second messengers that can activate kinases
- Phosphorylated MAPs do not bind to microtubules