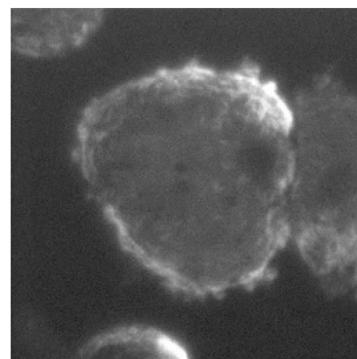
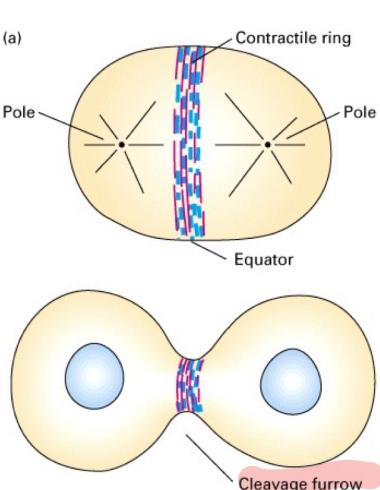


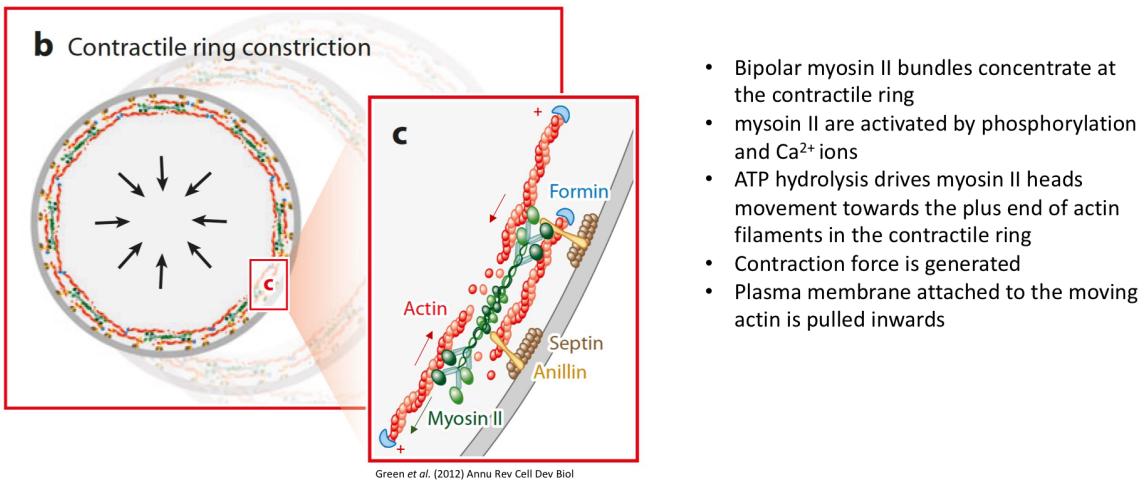
▼ Contractile - cytokinesis



Yumura et al. (2008) Traffic

- the contractile ring is a contractile bundle of actin filaments and myosin II similar to muscle
- bipolar myosin II filaments accumulate at the equatorial region in dividing cells and slide past two antiparallel actin filaments, similar to muscle sarcomere contraction

Contractile force within the contractile ring at cytokinesis is similar to muscle contraction, except the Z-disc is replaced by actin binding proteins within the plasma membrane



- Bipolar myosin II bundles concentrate at the contractile ring
- myosin II are activated by phosphorylation and Ca^{2+} ions
- ATP hydrolysis drives myosin II heads movement towards the plus end of actin filaments in the contractile ring
- Contraction force is generated
- Plasma membrane attached to the moving actin is pulled inwards

▼ Contractile: stress fibres

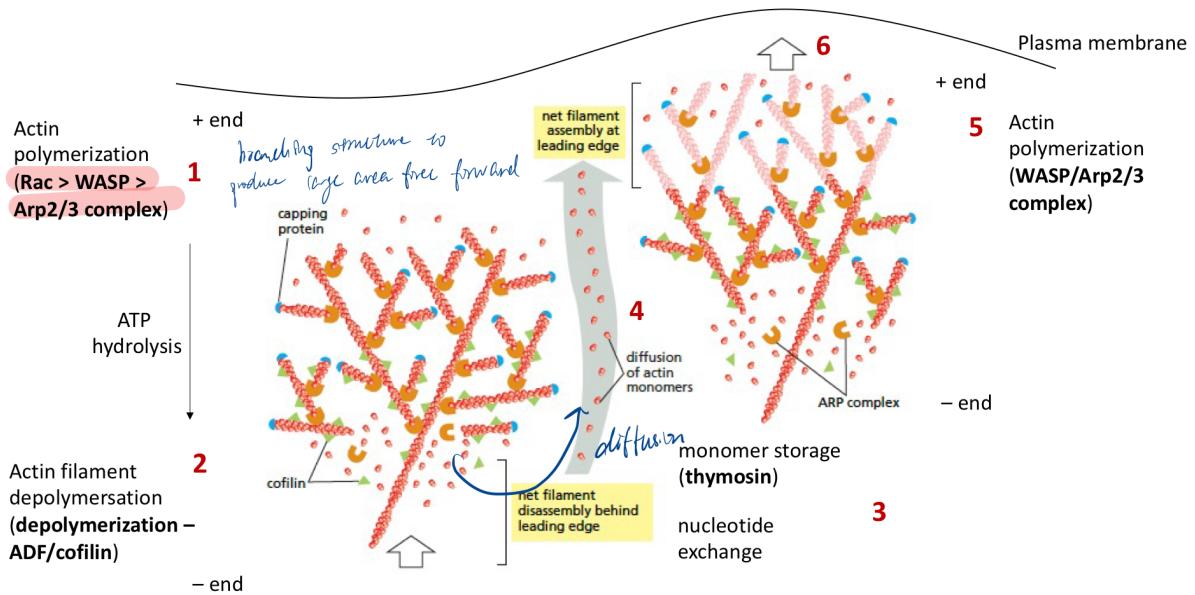
- focal adhesion - attach cell to extracellular matrix
- promote wound healing and regulate blood pressure in small capillaries

▼ Cell migration

CELL0023

actin nucleation-promoting factor Wiskott-Aldrich syndrome protein (WASP) contributes to maintenance of front-rear polarity by controlling localization and cellular levels of active Rac

Molecular mechanism of protrusion

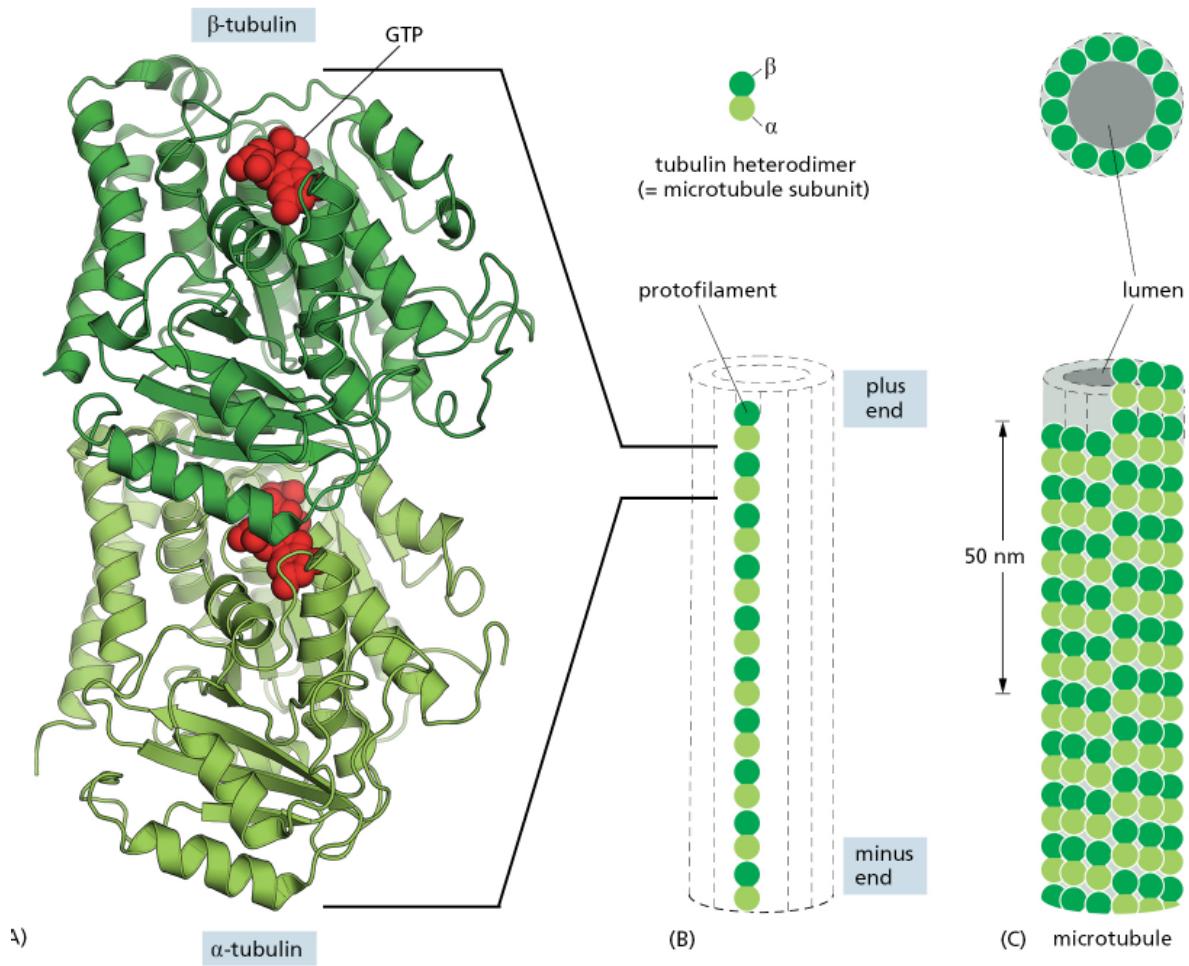


Microtubule

▼ Organisation from monomer

▼ monomer - tubulin

- heterodimer: alpha-tubulin and beta-tubulin
- each has a binding site for GTP; GTP binding to alpha-tubulin is never hydrolysed or exchanged; but **binding to beta-tubulin** can lead to activity change

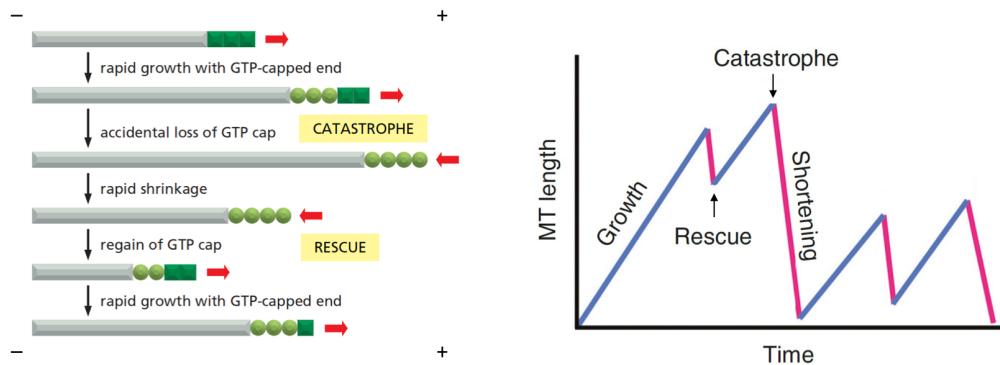


Each protofilament consists of many adjacent subunits with the same orientation. The microtubule is a stiff hollow tube formed from **13 protofilaments** aligned in parallel. The assembly of a microtubule generates two new types of protein-protein contacts. Along the longitudinal axis of a protofilament, the “top” of one β -tubulin molecule forms an interface with the “bottom” of the α -tubulin molecule in the adjacent heterodimer. This interface, which is very similar to the interface holding the α and β monomers together in the dimer subunit, has a high binding energy. Perpendicular to these interactions, neighboring protofilaments form lateral contacts, with the **main lateral contacts occurring between monomers of the same type (α - α and β - β)**

▼ Dynamic Instability

- rescue: change from shrinkage to growth

- catastrophe: change from growth to shrinkage
- GTP hydrolysis, which occurs only within the β -tubulin subunit of the tubulin dimer, **proceeds very slowly in free tubulin subunits** and is **greatly accelerated when they are incorporated into microtubules**. After GTP hydrolysis, a free phosphate group is released, leaving the GDP bound to β -tubulin within the microtubule lattice. Thus, as in the case of actin filaments, two different types of microtubule structures can exist, one in the T form bound to GTP and one in the D form bound to GDP. Because some of the energy of phosphate bond hydrolysis is stored as elastic strain in the polymer lattice, **the free-energy change for dissociation of a subunit from the D-form polymer is more favorable** (negative) than the free-energy change for dissociation of a subunit from the T-form polymer. This makes the **ratio of koff/kon for GDP-tubulin [which is equal to its critical concentration, $C_c(D)$]** much higher than that of GTP-tubulin. As a result, under physiological conditions, **the T form tends to polymerize and the D form tends to depolymerize**.
- Suppose that the concentration of free tubulin is intermediate between the critical concentration for a T-form end and the critical concentration for a D-form end (*that is, above the concentration necessary for T-form assembly, but below that for the D form*). Now, any end that happens to be in the T form will grow, whereas any end that happens to be in the D form will shrink. On a single microtubule, **an end might grow for a certain length of time in a T form, but then suddenly change to the D form and begin to shrink rapidly. At some later time, it might regain a T-form end and begin to grow again. This rapid interconversion between a growing and shrinking state, at a uniform free tubulin concentration, is called dynamic instability** (Figure 16–39 and Figure 16–40A; see Panel 16–2). The change from growth to shrinkage is called a catastrophe, while the change from shrinkage to growth is called a rescue.

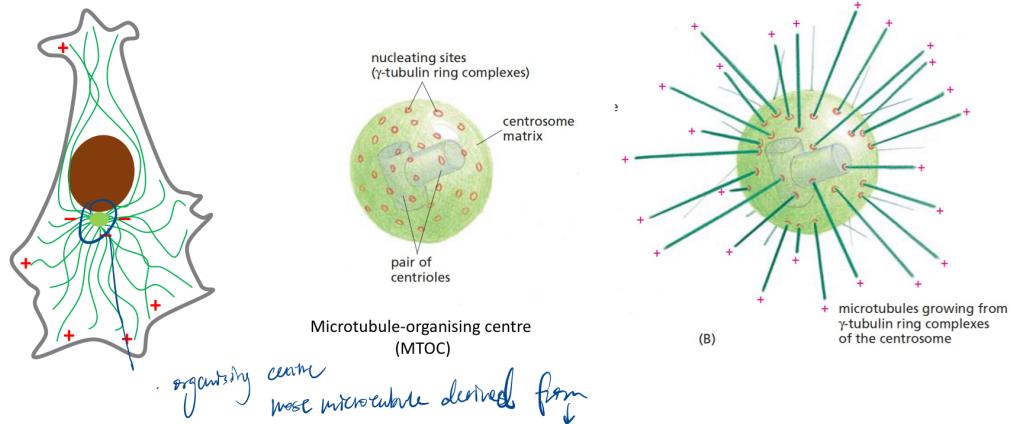


- GTP-cap is lost if polymerisation (= incorporation of new GTP-tubulin subunits) is slower than GTP hydrolysis
- Loss of GTP-cap causes catastrophe and disassembly (= depolymerisation and shrinkage of the microtubule filament)
- Re-gaining of the GTP-cap is termed rescue and allows re-growth of filament
- Both polymerisation (elongation) and depolymerisation (shrinkage) occur on the same end (plus end)

This constant fluctuation between growth and shrinkage at the plus end is termed *dynamic instability*

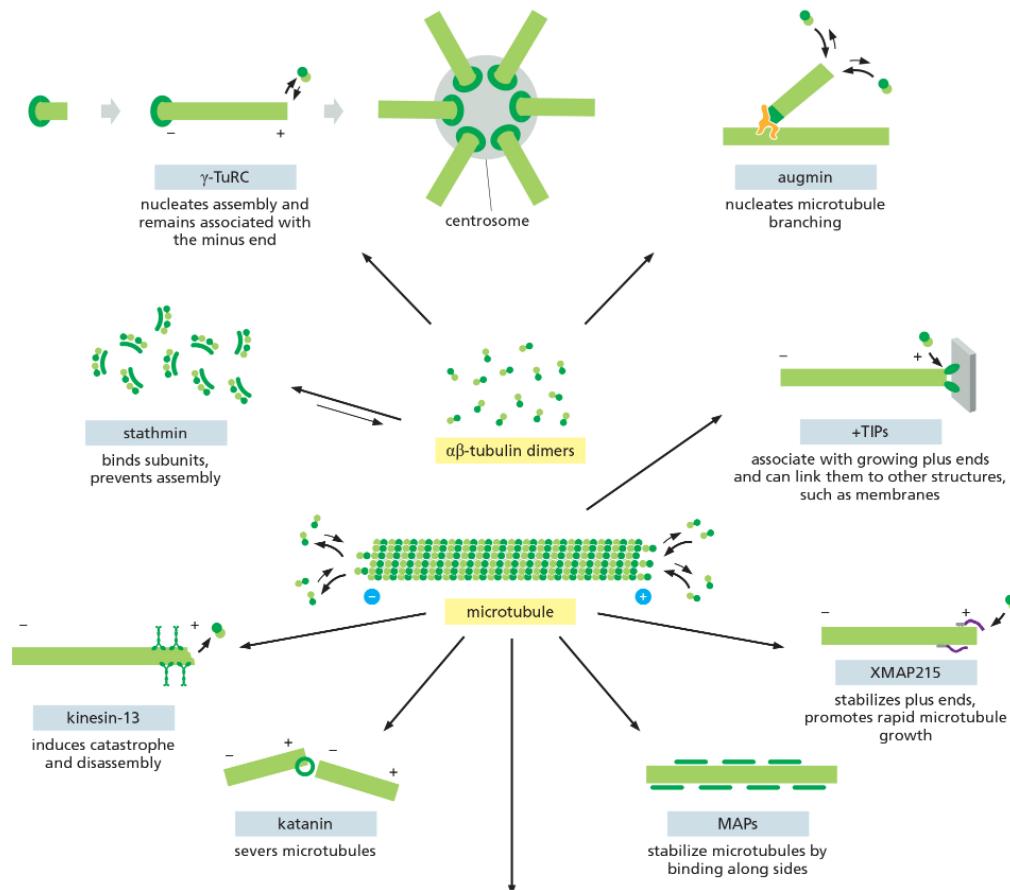
▼ Nucleation of microtubules

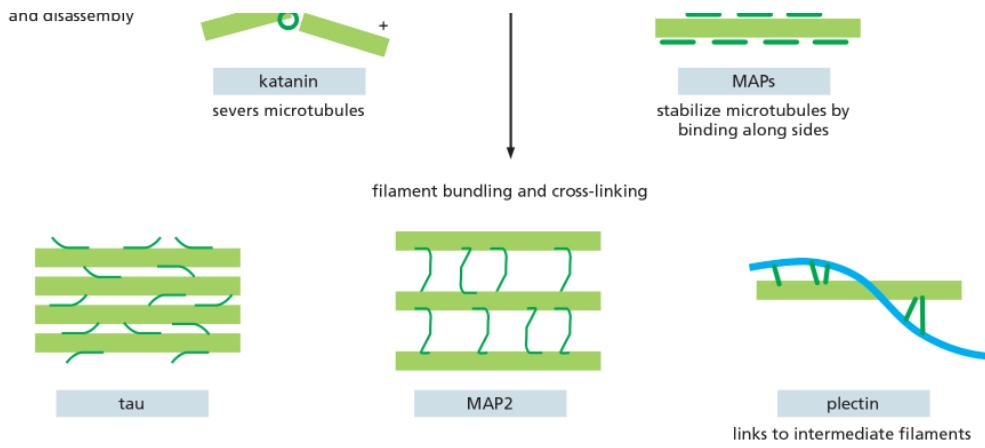
- microtubule-organising centre (MTOC) — enriched with **gamma-tubulin**
 - **nucleation depends on the gamma-tubulin ring complex (γ -TuRC)** : γ -tubulin + accessory proteins
- centrosome: is a cellular organelle **found in animal cells**, typically located near the nucleus — serves as the main microtubule-organizing center (MTOC) of the cell
 - a centrosome typically recruits more than 50 copies of γ -TuRC
 - it is the γ -TuRC not centrioles that is responsible for nucleation of microtubules
 - contains two centrioles arranging at a particular angle
 - While the centrosome is the primary MTOC in animal cells, other structures can serve this function in different organisms or cell types.
 - In plant cells, for example, the MTOC is often associated with the nuclear envelope or the Golgi apparatus.



- The majority of microtubules in cells nucleate from the centrosome
- A gamma-tubulin ring complex (TURC), not the centrioles, serves to nucleate cytoplasmic microtubules

▼ microtubule binding proteins



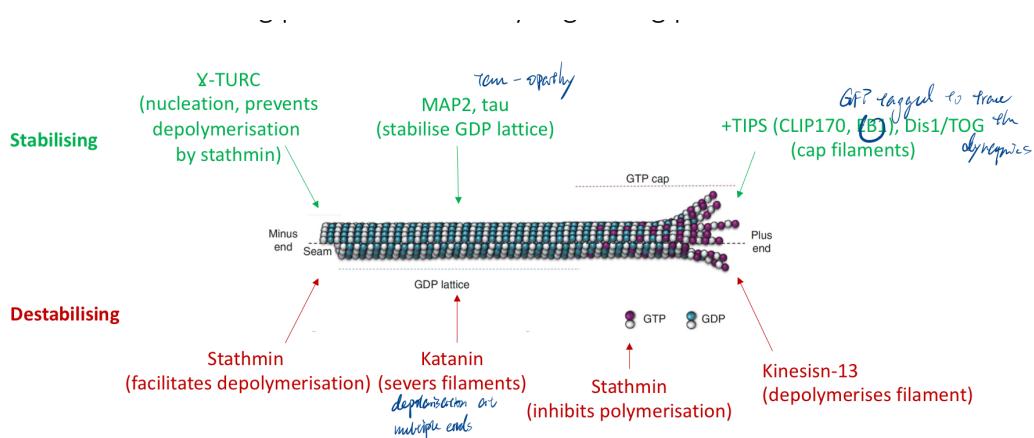


▼ Stabilising

- γ -TuRC: nucleation & prevent depolymerisation (stathmin)
- MAPs, MAP2 & Tau
- TIPs

▼ Destabilising

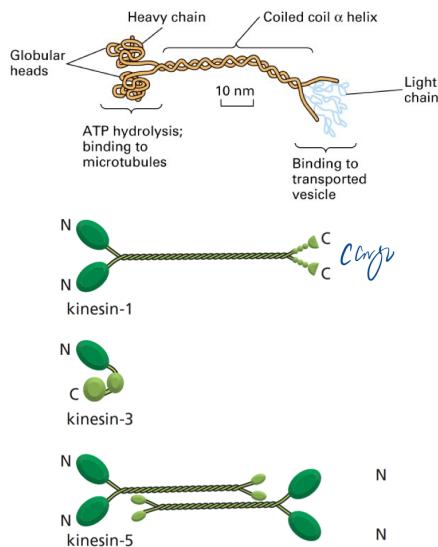
- Stathmin
- Katanin
- **Kinesin-13**



- The minus end is anchored in the centrosome via γ -TURC which stabilizes the minus-end
- Some proteins bind to the side of microtubules to stabilize or sever microtubules
- The plus ends of MTs can be captured and stabilized, to make MT plus-ends less dynamic
- Catastrophes can be enhanced to make MT plus-ends more dynamic

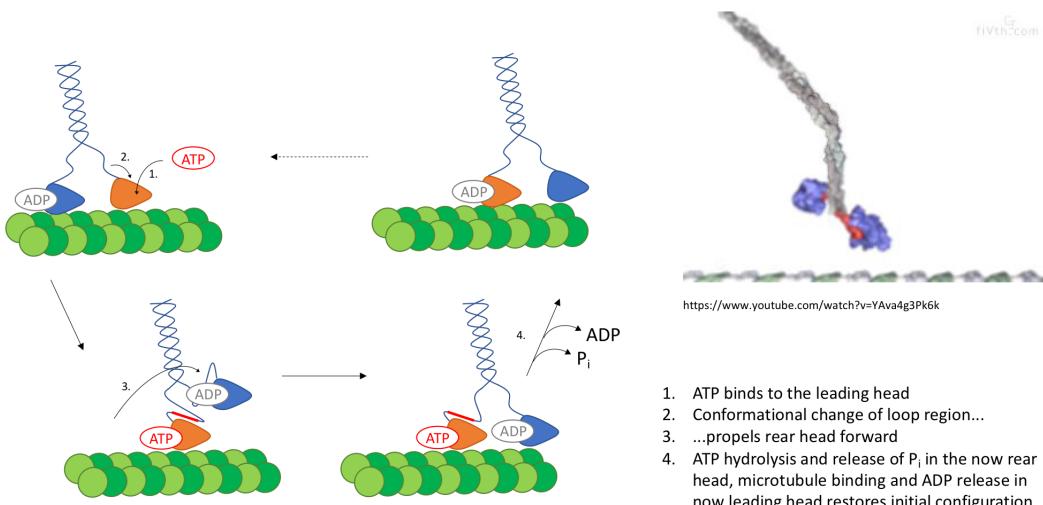
▼ Microtubule motor proteins

- +ve end (periphery) : kinesins
- -ve end (retrograde) : dyneins
- Globular head domain → binds microtubules (ATP hydrolysis driven)
- Tail domain → cargo binding



- Globular head domain (=motor domain) binds microtubules and ATP and drives movement (8 nm per step)
- Tail domain facilitates dimerization to homo- and/or heterodimers and cargo binding
- Differences in tail domain account for cargo specificity (e.g. Kinesin 13A for AP-1 and Mannose-6-Phosphate receptor at the Golgi and Kinesin 17 for NMDA receptor in dendrites)
- Not all kinesins act as motors, but rather as regulators of microtubule dynamics

▼ Kinesin movement

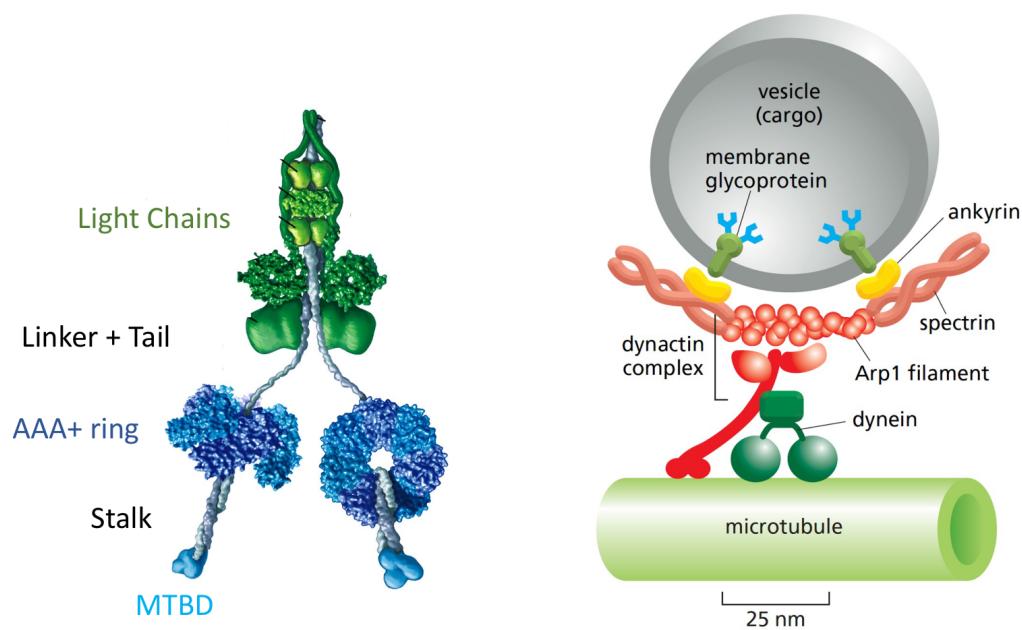


1. ATP binds to the leading head
2. Conformational change of loop region...
3. ...propels rear head forward
4. ATP hydrolysis and release of P_i in the now rear head, microtubule binding and ADP release in now leading head restores initial configuration

- the driving force of kinesins: The attachment of ATP on leading head →
- propel the rear head forward (ATP hydrolysis detaches the rear head from microtubule)
- now the new leading and rear head have the original configuration

▼ Dynein movement **towards -ve end**

- can form both homo- and heterodimers
- do not bind cargo directly, but via dynein-associated proteins creating complexes e.g. dynactin complex



- short-distance transport is accomplished by actin/myosin — at cell cortex
 - also endo- and exo-cytosis
- long distance (axonal transport) — dynein/kinesin

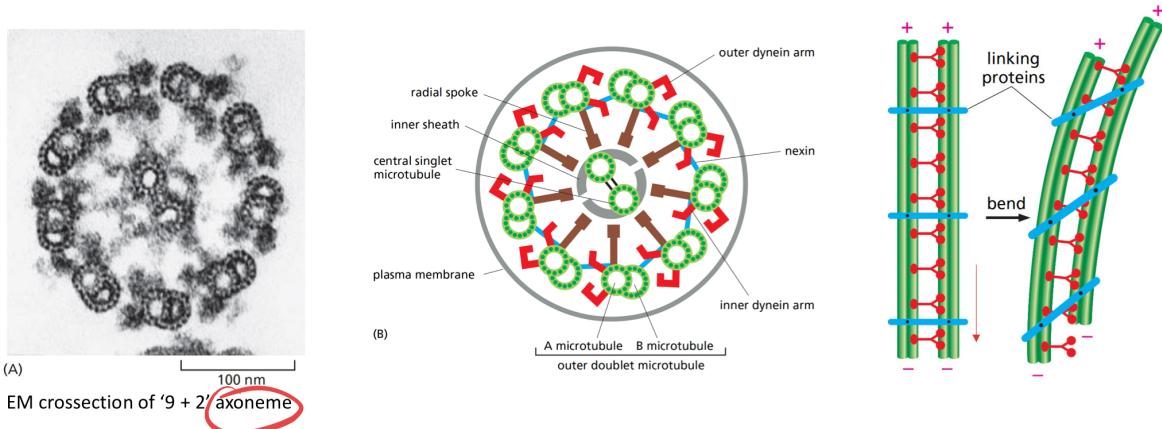
▼ Other Microtubule cellular function — cilia & flagella

- ciliated cells in lungs (trachea bronchi)
- epithelia of uterine tubes & upper respiratory tract (nose)
- flagella → locomotion of sperms

▼ Cilia beating model - **axoneme**

- what is axoneme: microtubules and associated proteins arranged in a regular pattern
- 9+2 structure: 9 microtubule doublets (one complete one half) forming a ring + a pair of single microtubules

- Common structure of motile eukaryotic flagella and cilia
- Axonemal dynein form bridges between neighbouring microtubule doublets
- Molecules of axonemal dynein form bridges between adjacent microtubule doublets around the circumference of the axoneme (Figure 16–59). When the motor domain of this dynein is activated, the dynein molecules attached to one microtubule doublet (see Figure 16–60) attempt to walk along the adjacent microtubule doublet, tending to force the adjacent doublets to slide relative to one another, much as actin thin filaments slide during muscle contraction. However, the presence of other links between the microtubule doublets prevents this sliding, and the dynein force is instead converted into a bending motion (Figure 16–60). Mti



- Dynein movement along cross-linked microtubule doublets causes cilia bending
- Defects in axoneme architecture can cause severe diseases including chronic lung disease or infertility (*male factor*)