The cytoskeleton

- Rearrange internal organization and polarity
- Move
- Pool chromosomes apart
- Divide
- Traffic organelles
- Support the fragile plasma membrane
- Provide mechanical link between organelles. Connect and stabilize organelles

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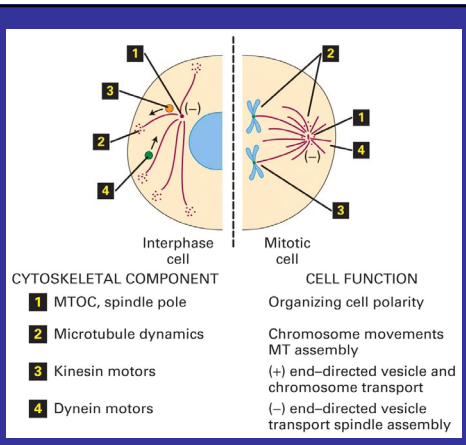
Molecular Cell Biology

Fifth Edition

Chapter 20:
Cytoskeleton II: Microtubules

The self assembly and dynamic cytoskeleton- A nano machines

- Intermediate filaments
- Microtubules
- Actin filaments
- These can operate only with accessory proteins



MTs Organization and Dynamics

Cylindrical tubes 25 nm in diameter

Length fraction of μm to hundreds of μm

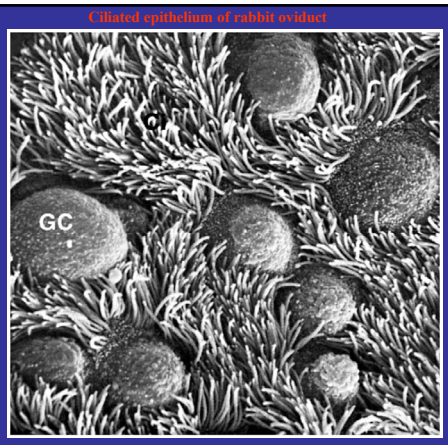
Cells contain two populations of MTs:

Stable MTs- long lived (Neurons –structure and tracks for vesicle transport, flagella, cilia)

Unstable – short lived in mitosis the spindle shape apparatus

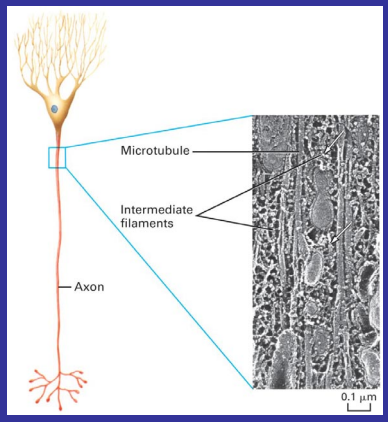
The cytoskeletal polymers are formed by weak noncovalent interactions – assembly or disassembly occurs without the formation of covalent modifications

Ciliated epithelium of rabbit oviduct



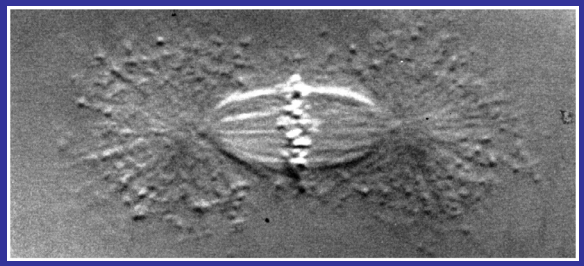
Stable MTs

Unstable MTs

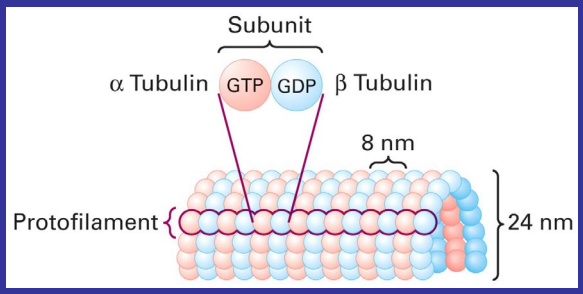


Structure assembly and disassembly of MTs

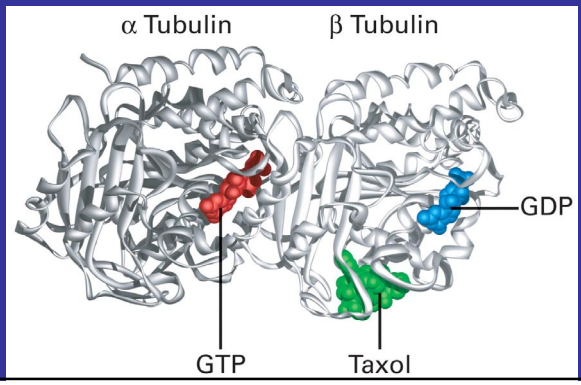
Mitotic apparatus



Protofilaments longitudinal interactions α- α; β- β
Cylindrical –tube, lateral interactions of 13 protofilaments
Because of the structure addition of new dimers takes place only at the +or – ends.

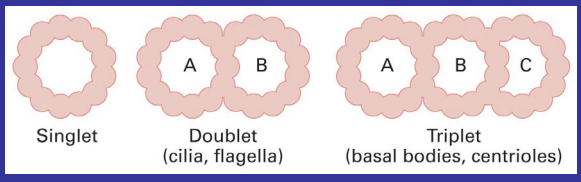


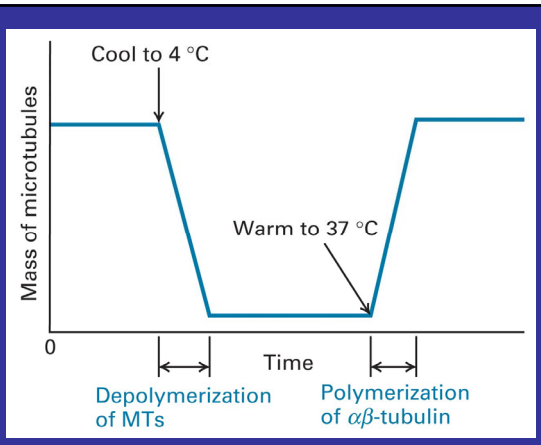
Building blocks are α and β-tubulin bound together by noncovalent bonds
55.000 MW. GTP-binding sites; α tubulin irreversibly, β tubulin binds GTP reversibly and serves as a GTPase. The GDP modulate the addition of tubulin subunits.



Heterodimeric tubulin subunits compose the wall of MT

- Building blocks are α and β-tubulin bound together by noncovalent bonds
- 55.000 MW
- γ tubulin
- GTP-binding sites; α tubulin irreversibly, β tubulin binds GTP reversibly and serves as a GTPase. The GDP modulate the addition of tubulin subunits.
- Protofilaments longitudinal interactions α– α; β– β
- Cylindrical –tube, lateral interactions of 13 protofilaments
- Because of the structure addition of new dimers takes place only at the +or – ends.
- The MTs have distinct polarity





MTs assembly and disassembly preferentially at the + end.

- Polymerization
- Temperature dependence 4o depolymerization 37o polymerization
- Polymerization dynamics: (1) [αβ]>Cc polymerization of the dimers into MTs.
- if [αβ]<Cc depolymerization (2) GTP/GDP modulate the Cc, (3)αβ>Cc at +end but is lower than the Cc of the-end, tread milling.

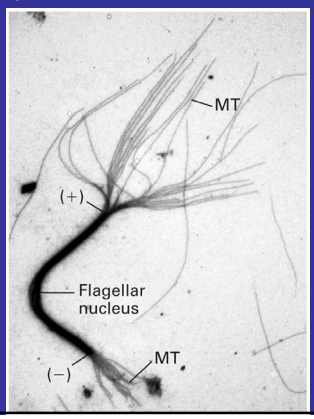
Stages in the assembly of MTs

- The free tubulin dimers have GTP bound to the exchangeable nucleotide binding site.
- The GTP on the β tubulin is hydrolyzed to GDP

If the rate of polymerization is faster than the hydrolysis a cap of GTP-tubulin is formed

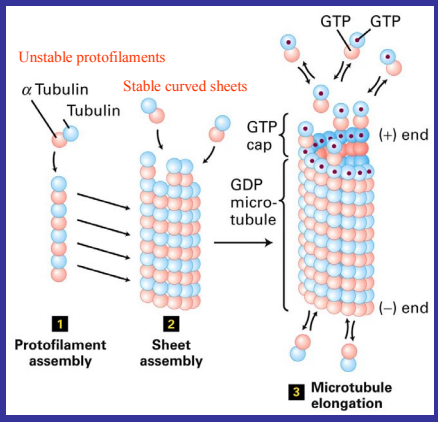
Polymerization is faster on the + end than the - end.

[αβ]>Cc polymerization of the dimers into MTs

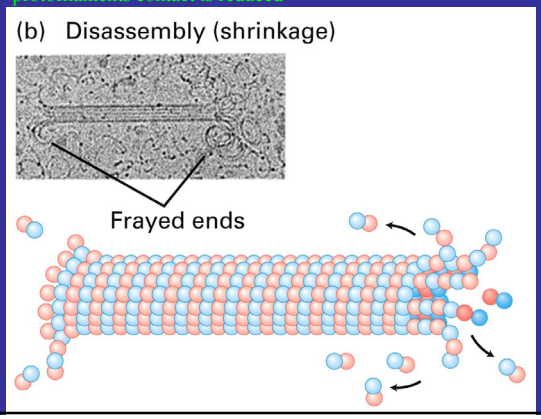


The Cc for assembly is 0.03μM
The intracellular concentration is 10-20μM
Polymerization is highly favored in cells

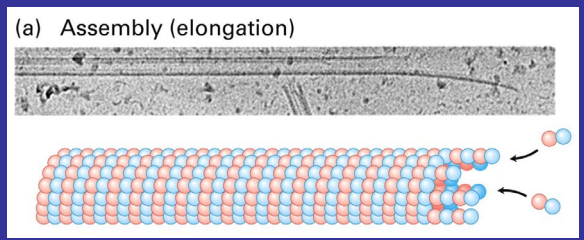
Assembly and disassembly mechanisms are different

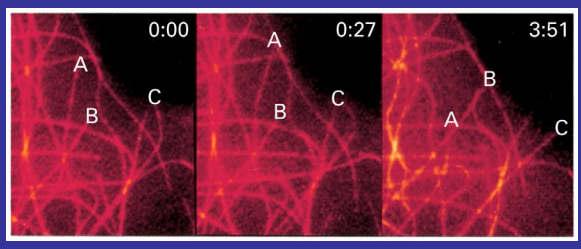


During disassembly the tips splays, suggesting that protofilaments contact is reduced



The plus end appears uneven as some protofilaments grow faster than the others,





Dynamic instability of MTs

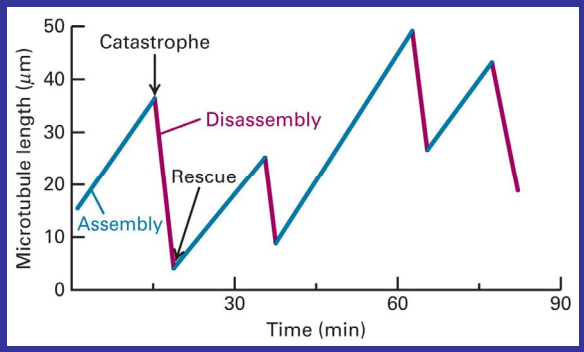
under given conditions all MTs should either grow or shrink!!!!!!

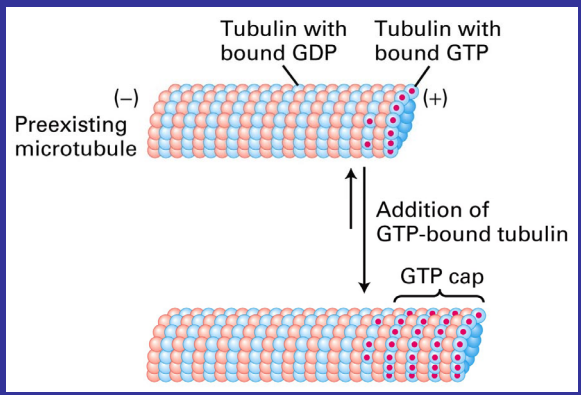
- Instead, at concentration near the Cc MTs grow and shrink !!
- Assembly; catastrophe, disassembly, rescue

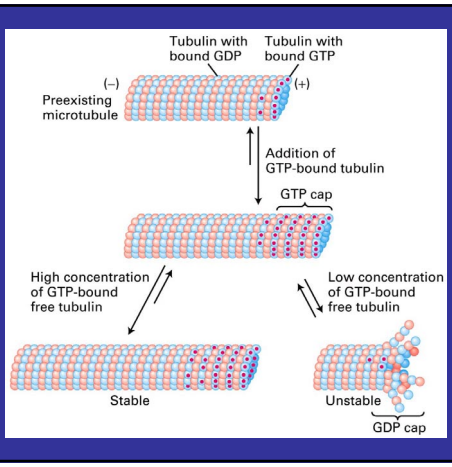
MTs instability

- Dissociation of GDP dimer is 4x larger than that of GTP dimer. Thus the +end is depolymerized if it is capped by GDP dimers
- This can happened when:
- The MT shrink rapidly
- When the polymerization is slow and the GTP is hydrolyzed to GDP

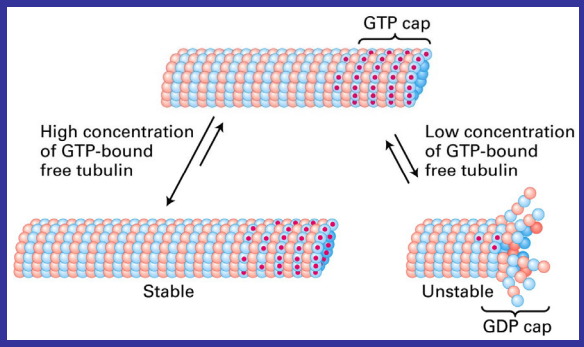
- GDP/GTP-binding site on β tubulin.
- off rate of GDP-tubulin > off rate of GTP-tubulin

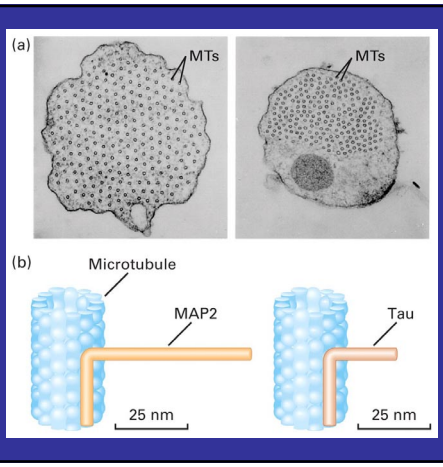






- **Proteins that regulate MTs dynamics, stability, and cross linking**





MAPs

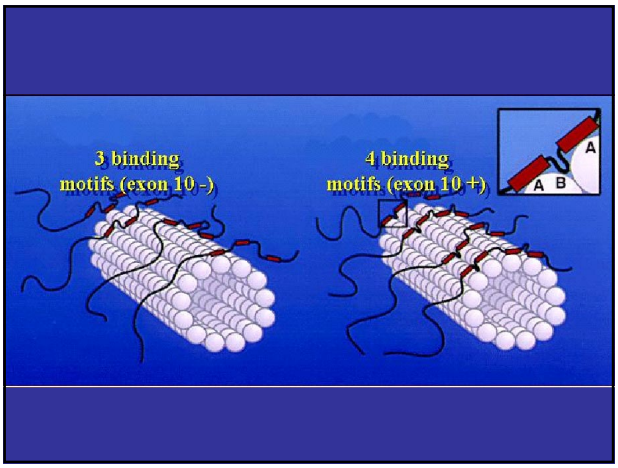
- stabilizes MTs- composed of a MT binding domain –positively charged 4 residue amino acid sequence that bind to tubulin.
- and acidic projection domain
- MAP1A MAP1B, MAP2 in dendrites only, MAP4 MTs stability, Tau axonogenesis, abbarent polymerization of tau -AD, CLIP170 cross link MTs to chromosomes

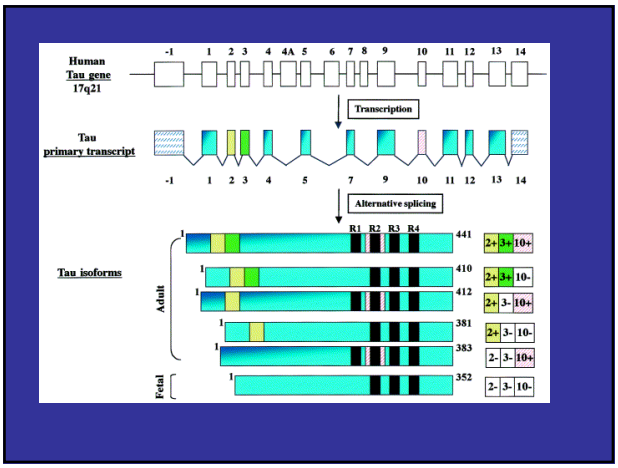
TABLE 20-1 Proteins That Modulate Microtubule (MT) Dynamics

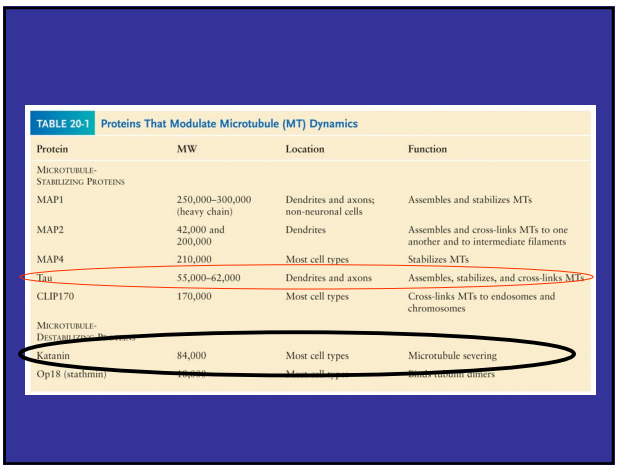
Protein	MW	Location	Function
MICROTUBULE-STABILIZING PROTEINS			
MAP1	250,000–300,000 (heavy chain)	Dendrites and axons; non-neuronal cells	Assembles and stabilizes MTs
MAP2	42,000 and 200,000	Dendrites	Assembles and cross-links MTs to one another and to intermediate filaments
MAP4	210,000	Most cell types	Stabilizes MTs
Tau	55,000–62,000	Dendrites and axons	Assembles, stabilizes, and cross-links MTs
CLIP170	170,000	Most cell types	Cross-links MTs to endosomes and chromosomes
MICROTUBULE-DESTABILIZING PROTEINS			
Katanin	84,000	Most cell types	Microtubule severing
Op18 (stathmin)	18,000	Most cell types	Binds tubulin dimers

Phosphorylation of MAPs

- Phosphorylated MAPs are unable to bind MTs, leading to MTs disassembly.
- MAP kinase participate in many signal-transduction events indicating that MAP are targets of many extracellular signals
- MAp4 is phosphorylated by cyclin-dependent kinase (CDK) cell cycle







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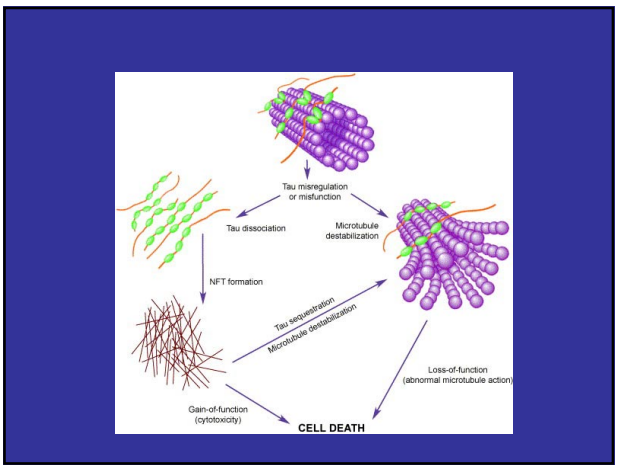
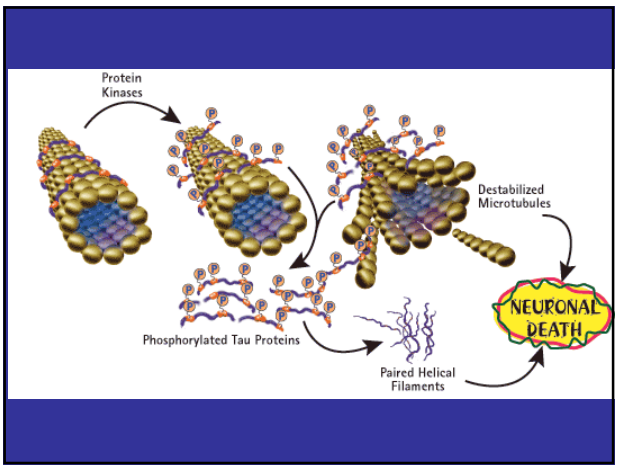
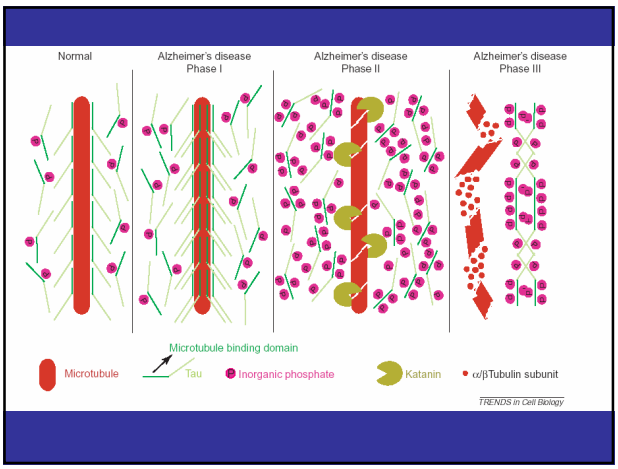
Op18 (stathmin)	[illegible]	[illegible]	Binds tubulin dimers



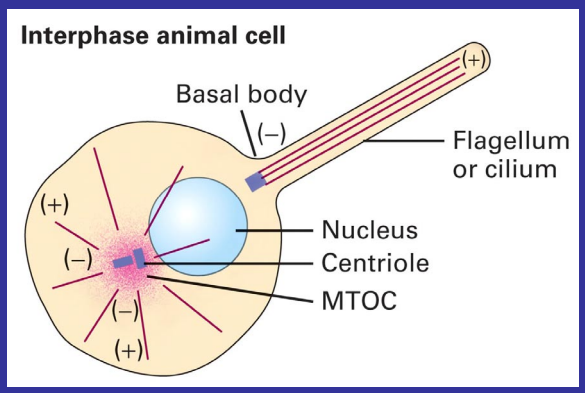


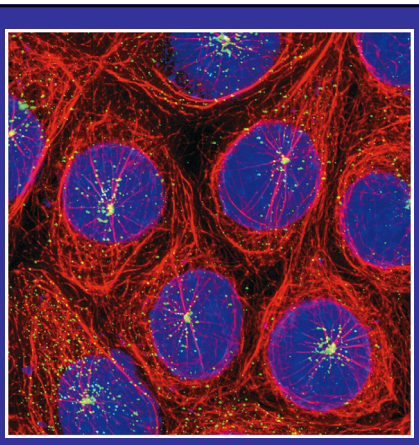


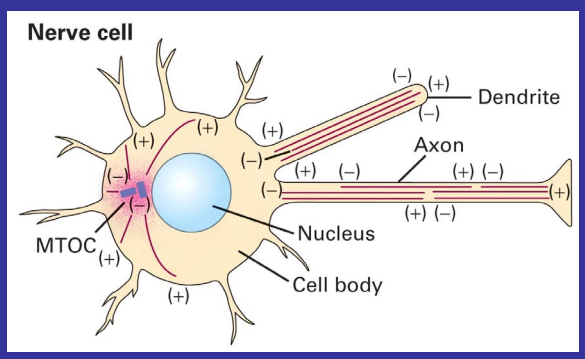
Microtubules organizing center- MTOC

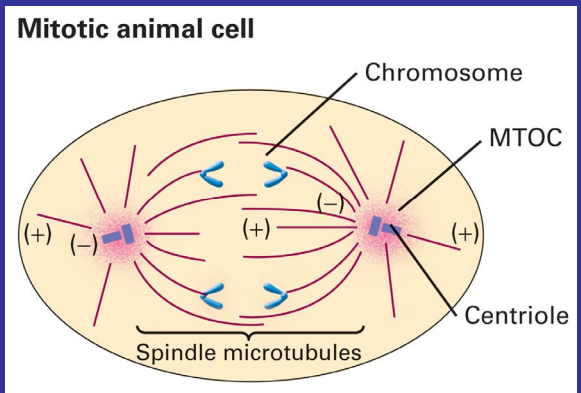
- MTs polarity is fixed in a characteristic organization
- This orientation is determined by the centrosome which function as an MTOC

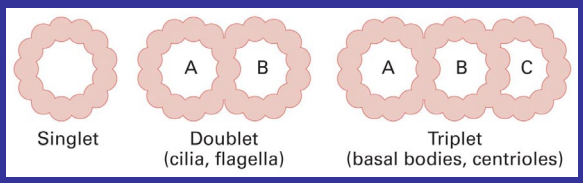
Colchicine/taxol









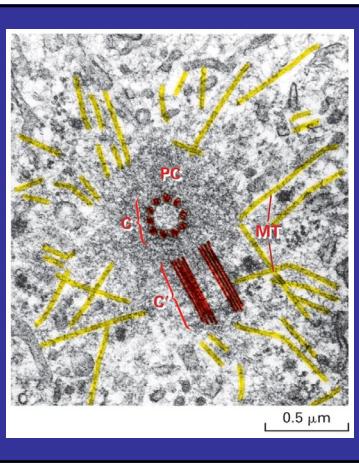


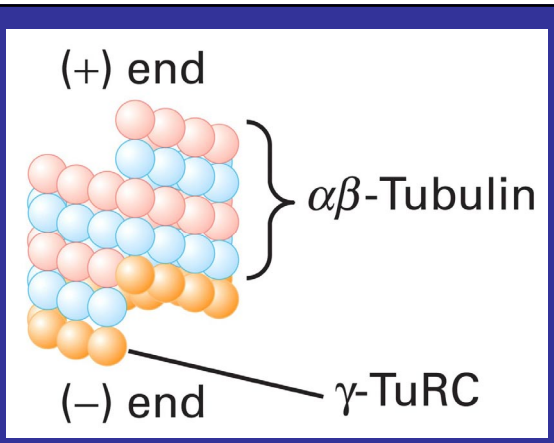
Structure of the MTOC

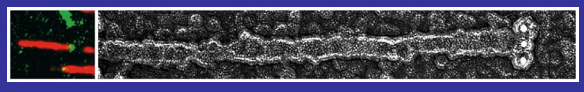
- A pair of two orthogonal centrioles
- Pericentriolar Matrix containing γ-tubulin and pericentrin
- The minus ends of MTs are embedded in the PC matrix
- **Injection of antibodies against γ-tubulin blocks MTs assembly**

The γ-tubulin ring complex

- The γ-tubulin ring complex directly nucleate MTs assembly at **sub Cc levels**



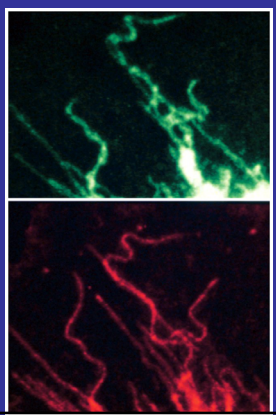




Colocalization of ER and MTs

Dissociation of the MTs leads to dissociation of the ER network.

The Golgi is associated with the MTOC. Dissociation of MTOC MTs leads to "breakdown of the Golgi



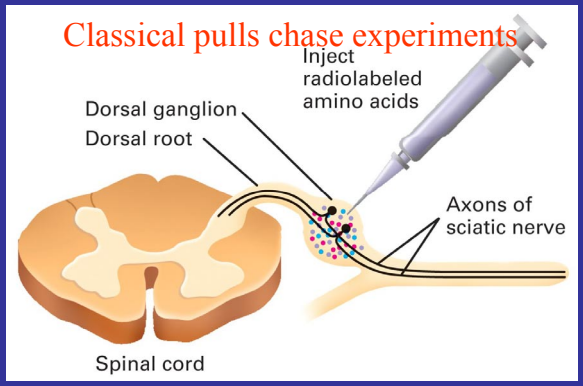
ER

MTs

MTs based intracellular movements

- Organelles
- Cilia flagella

Classical pulls chase experiments



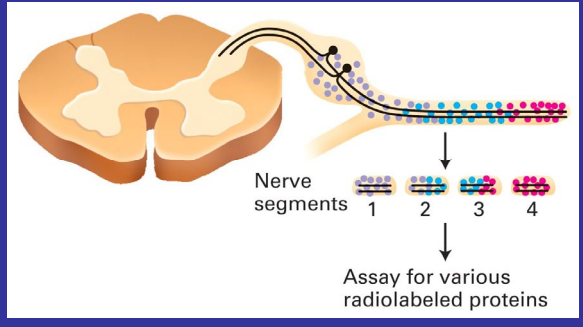
Kinesin and Dynein – Molecular motors

- Intracellular transport is driven by molecular motors
- First discovered in neurons – the question was **how can neurons with long axons survive**
- Radiolabeleing

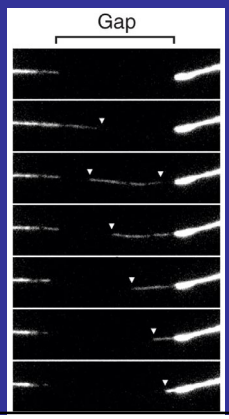
Transport rates

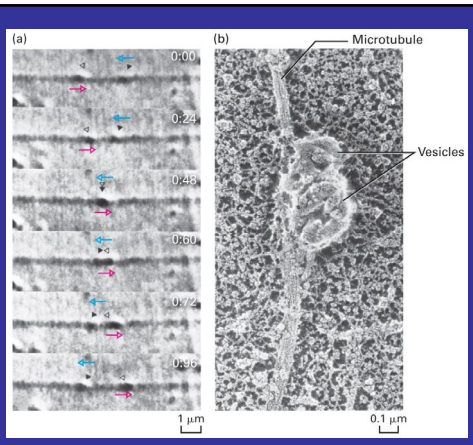
- Fast transport-250mm/day about 3μm/sec (vesicles mRNAP)
- Slow transport mm/day (tubulin subunits; neurofilaments)**?????!!!!!!!!**

- There are no fundamental differences between the fast and the slow transport as they are all driven by molecular motors. The differences are a mater of saltatory transport/ continuous



GFP tagged neurofilaments exhibit periodic pauses





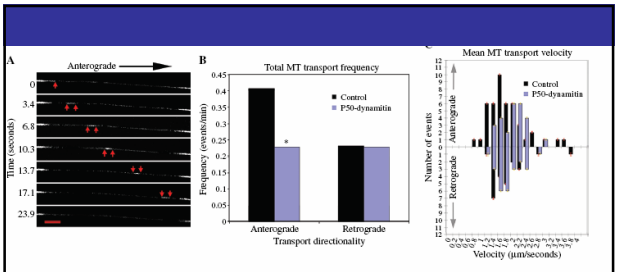
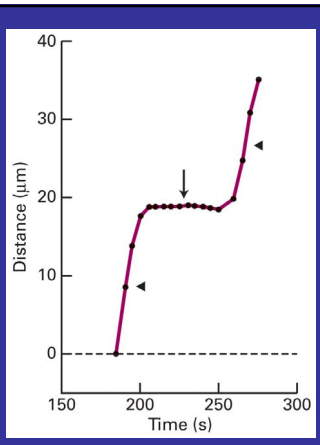


Figure 3: P50-dynamitin overexpression reduces anterograde microtubule transport frequency. (A) Time-lapse images reveal a microtuble moving in the anterograde direction through the photobleached region. Red arrows mark the leading and trailing ends of the microtubule. Subsequent staining for myc confirmed that this axon was from a neuron overexpressing P50-dynamitin. (B) The frequency (events/min) of anterograde microtubule transport was significantly reduced in the axons of P50-dynamitin-myc-expressing neurons (chi-square test, *, $p < 0.001$) as compared to control axons, with no significant difference observed in retrograde transport frequency (chi-square test, $p > 0.05$). (C) Histogram depicting the mean velocities of microtubule movements in the axons of control (velocity ± SEM = 1.70 ± 0.105 μm/second (anterograde); 1.70 ± 0.116 μm/second (retrograde)] and P50-dynamitin-myc-expressing neurons [velocity ± SEM = 1.90 ± 0.098 μm/second (anterograde); 1.77 ± 0.103 μm/second (retrograde)]. Comparison of these two groups showed no statistically significant variation in microtubule transport velocity regardless of movement directionality (two-tailed t-test, anterograde $p > 0.05$, retrograde $p > 0.05$).



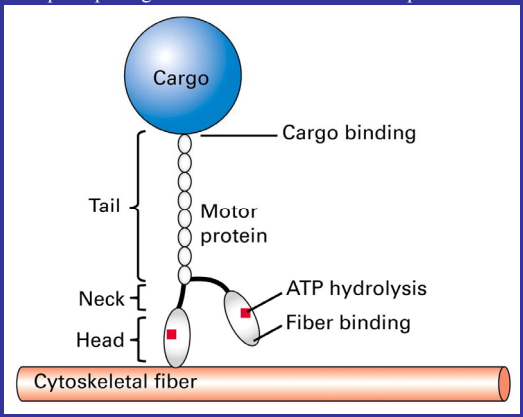
Kinesin I anterograde vesicles transport in axons

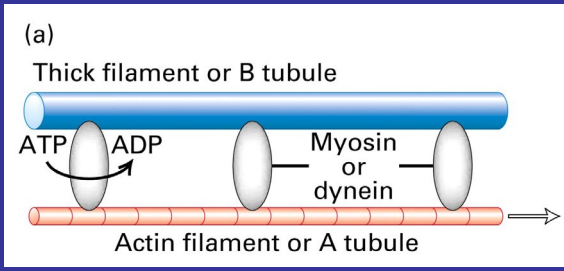
- In vitro experiments show that:
- Vesicles move in the presence of ATP
- AMPPNP a non hydrolysable analog of ATP leads to binding of vesicles to the MTs but not motility
- This led to the discovery of the molecular motors

Discovery of molecular motors

- MTs assembled in vitro (purified, stabilized by taxol)
- Vesicles + ATP= no motility
- Cytoplasm from squid (without MTs and vesicles) motility was observed
- Conclusion – a soluble factor underlie motility

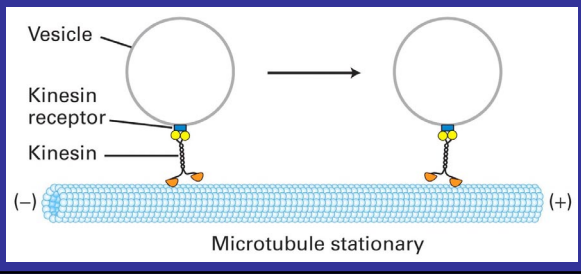
The principal organization of a molecular motor protein



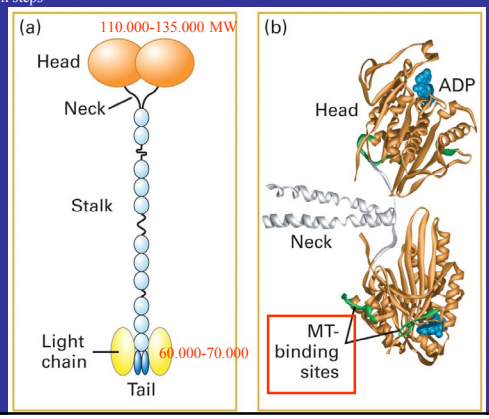


Kinesin I is a dimer of two heavy chains complexed to light chains
total MW 380.000
The head domain binds to MT and ATP.
The tail domain binds to the cargo through the light chain

Kinisin 1 moves cargo from the minus to the plus end



Current models suggest that ATP hydrolysis by kinisin causes movement of the flexible neck.
In 8 nm steps



The functional differences are mainly related to the nature of the tail domain which determine the cargo

TABLE 20-2 Functional Classes of Microtubule Motor Proteins

Class	Common Members	Cargo	Direction of Movement*
Cytosolic motors	Kinesins (I, KIF1A, KIF1B)	Cytosolic vesicles/organelles	(+)
	Cytosolic dynein	Cytosolic vesicles/organelles	(–)
	Kinesin II	Cytosolic vesicles/organelles	(+)
Mitotic motors	Kinesin BimC (bipolar)	Spindle and astral MTs	(+)
	Chromokinesins	Chromosomes (arms)	(+)
	MCAK	Kinetochores	(+)
	CENP-E	Kinetochores	(+)
	Kinesin Ncd	Spindle and astral MTs	(–)
	Cytosolic dynein	Kinetochores, centrosomes, cell cortex near spindle poles	(–)
Axonemal motors	Outer-arm and inner-arm dyneins†	Doublet microtubules in cilia and flagella	(–)

*Movement of motor protein toward the (+) end or (–) end of microtubules.
† Outer-arm dyneins have three heavy chains, and inner-arm dyneins have two heavy chains.

+ and – end directed kinesin motors

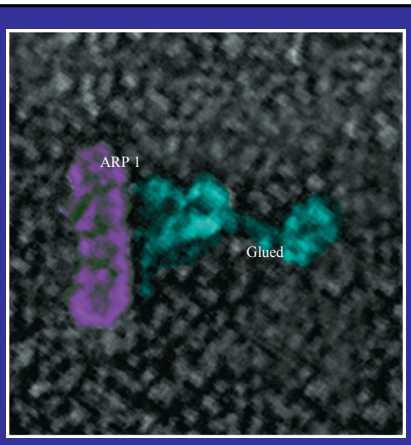
- 10 different kinesin subfamilies are known
- They differ mainly in the location of the heavy chain: N-terminal centrally located (M-type) are plus end directed
- C-terminal , C-type minus end directed.
- Most kinesin have two heavy chain but some one or 4.
- Some are bipolar
-

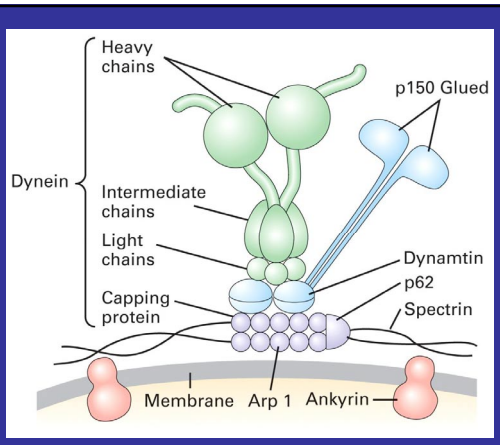
Dynein and dynectin heterocomplex

- Dynein are very large molecular complexes **exceeding 1.000.000 MW**
- Dynein requires dynectin for functioning
- Dynectin is a complex including glued –MT binding, Arp1 which bind spectrin and dynamatin which interact with dynein

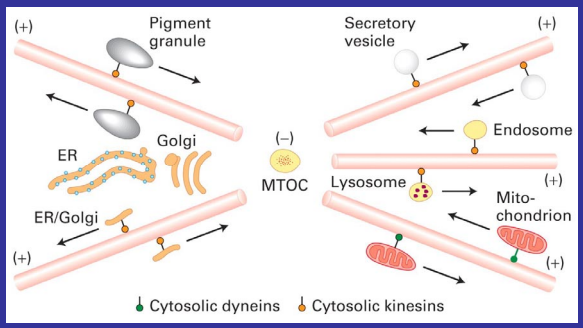
Direction of movement is dependent on the properties of the neck not the motor

- Replacement of the neck reverses the directionality

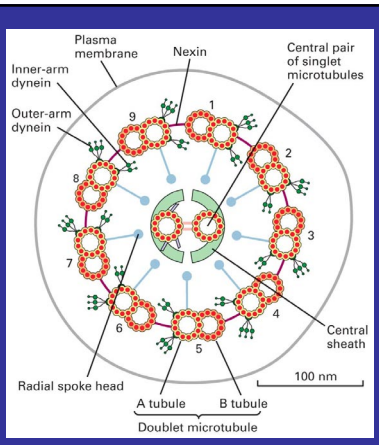




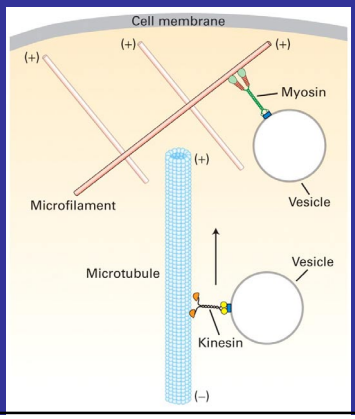
Bidirectional transport of organelles

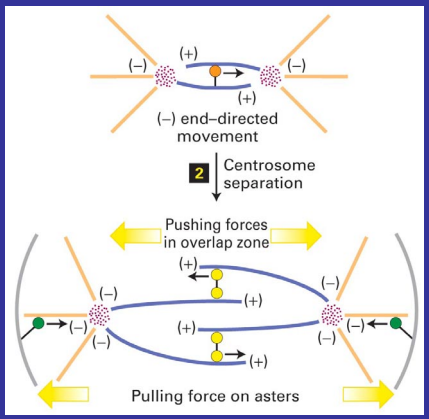


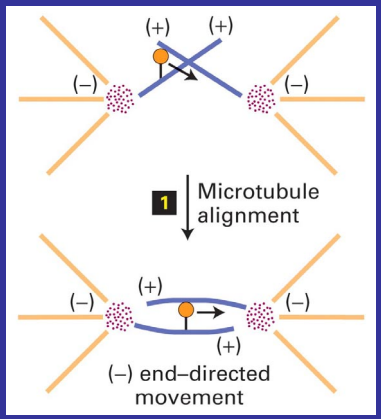
- **One model propose that dynein generate the force to move vesicles but remains tethered to the MTs through dynactin**

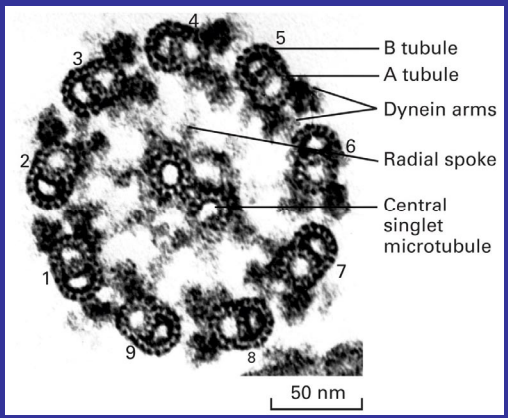


Cooperation of molecular motors

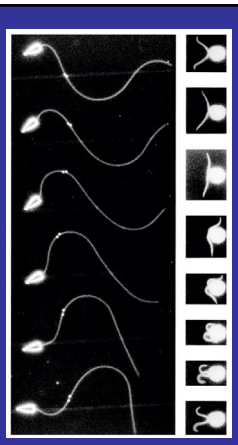


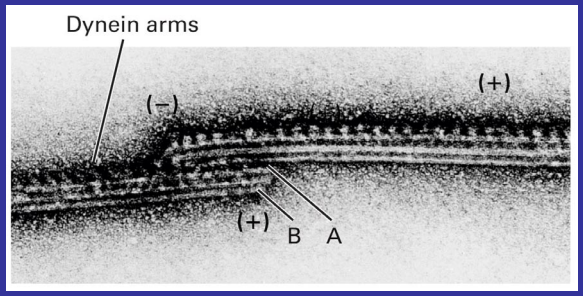


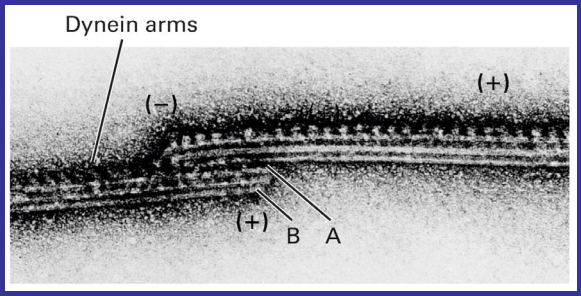




The end of meeting #1







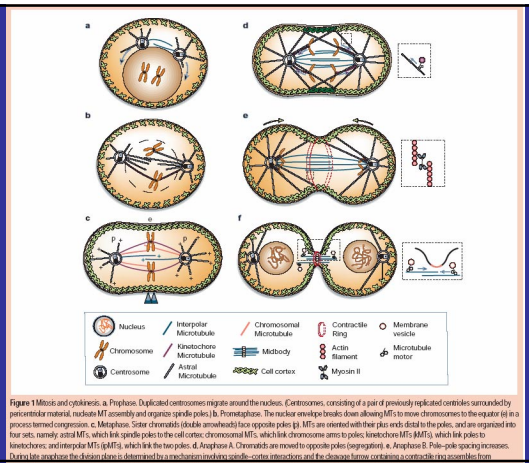
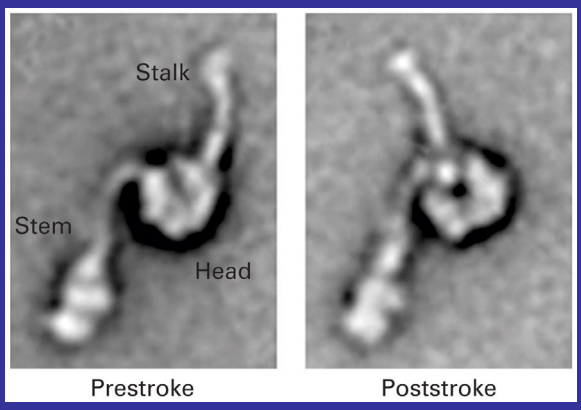
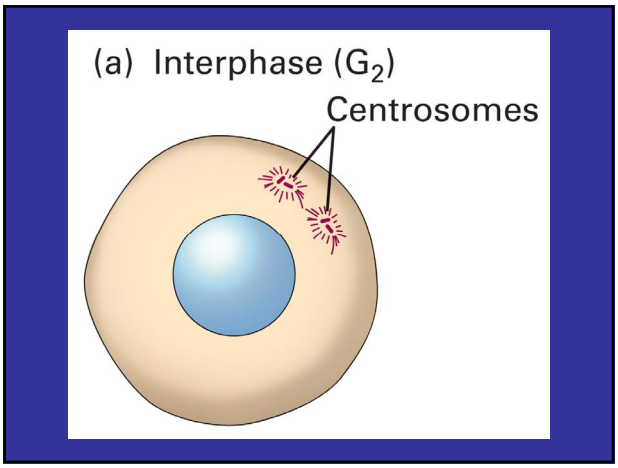
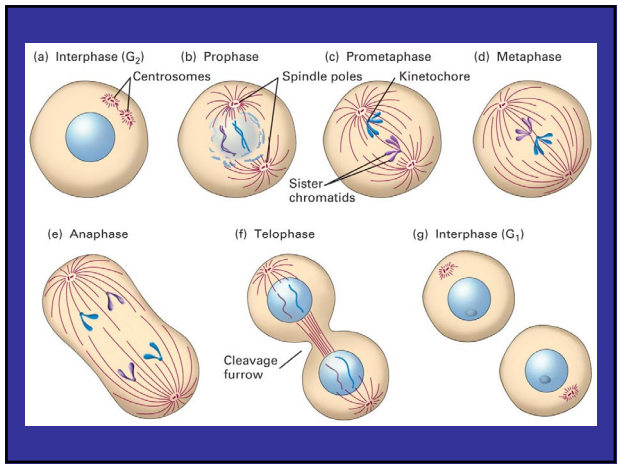
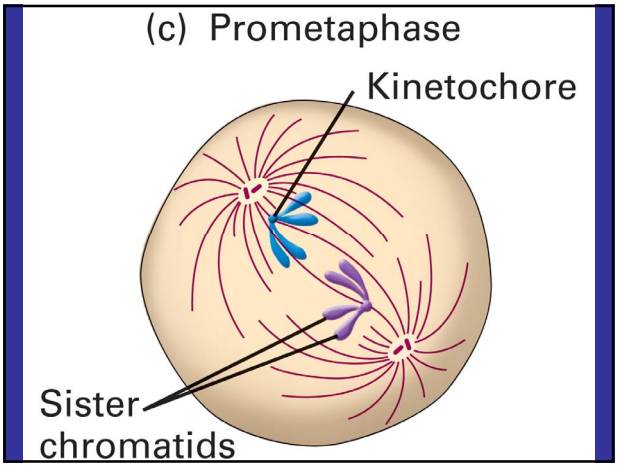


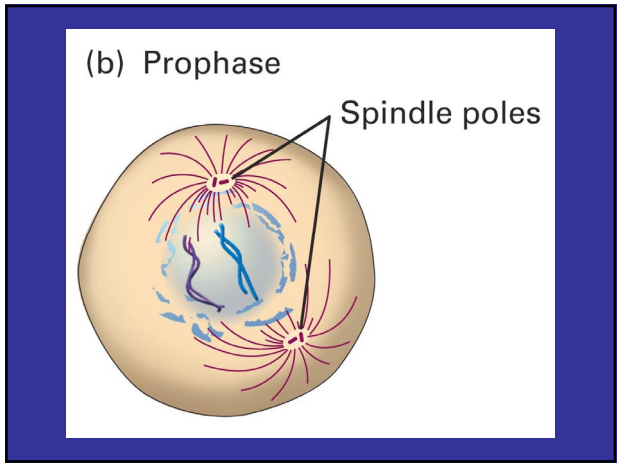
Figure 1 Mitosis and cytokinesis. a. Prophase. Duplicated centrosomes migrate around the nucleus. (Centrosomes, consisting of a pair of previously replicated centrioles surrounded by pericentriolar material, nucleate MT assembly and organize spindle poles.) b. Prometaphase. The nuclear envelope breaks down allowing MTs to move chromosomes to the equator (e) in a process termed congression. c. Metaphase. Sister chromatids (double arrowheads) face opposite poles (p). MTs are oriented with their plus ends distal to the poles, and are organized into four sets, namely: astral MTs, which link spindle poles to the cell cortex; chromosomal MTs, which link chromosome arms to poles; kinetochore MTs (kMTs), which link poles to kinetochores; and interpolar MTs (ipMTs), which link the two poles. d. Anaphase A. Chromatids are moved to opposite poles (segregation). e. Anaphase B. Pole–pole spacing increases. During late anaphase the division plane is determined by a mechanism involving spindle–cortex interactions and the cleavage furrow containing a contractile ring assembles from

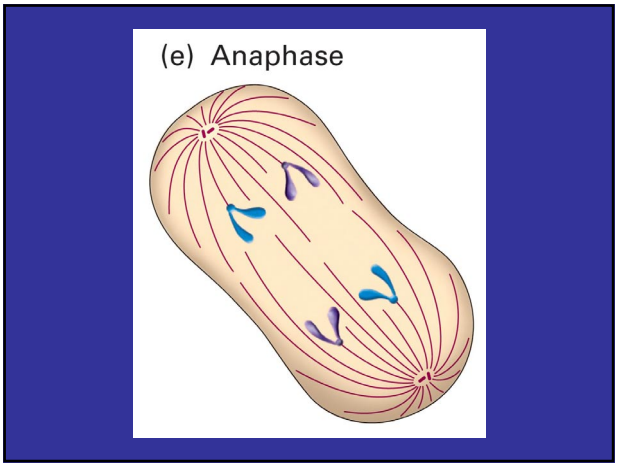


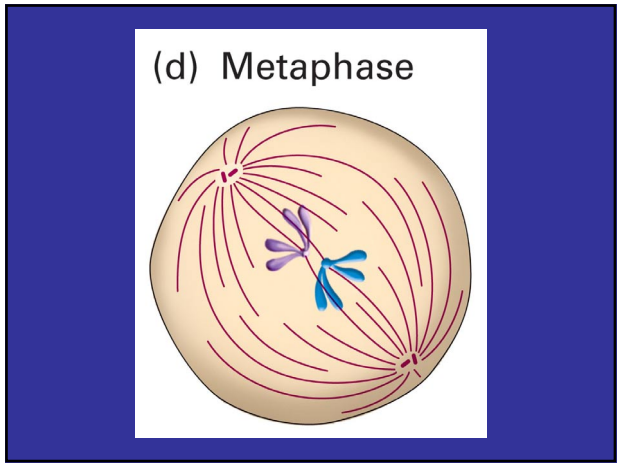

(a) Interphase (G_2)
Centrosomes

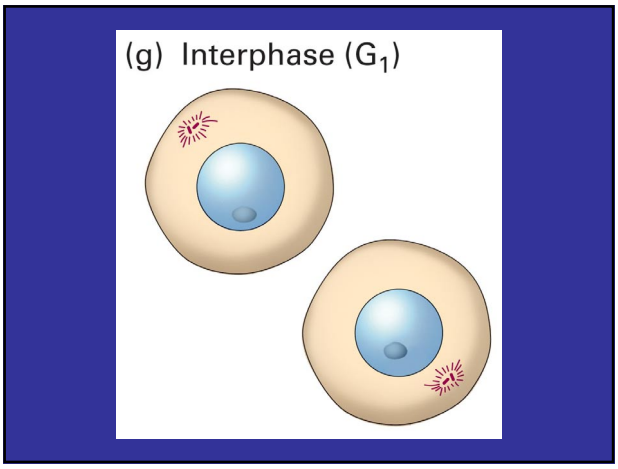

(a) Interphase (G_2)
Centrosomes
(b) Prophase
Spindle poles
(c) Prometaphase
Kinetochore
(d) Metaphase
Sister chromatids
(e) Anaphase
(f) Telophase
Cleavage furrow
(g) Interphase (G_1)

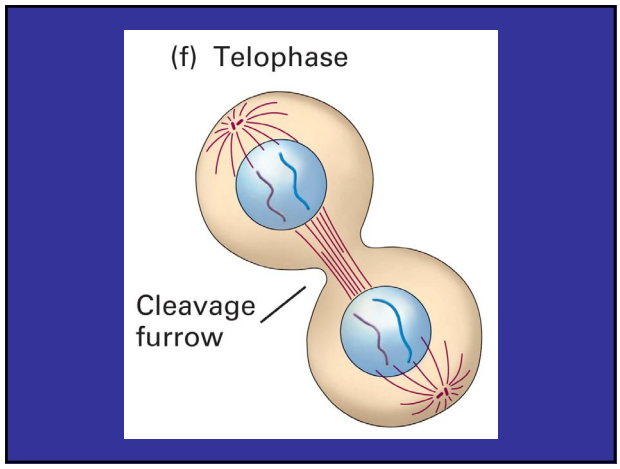

(c) Prometaphase
Kinetochore
Sister chromatids

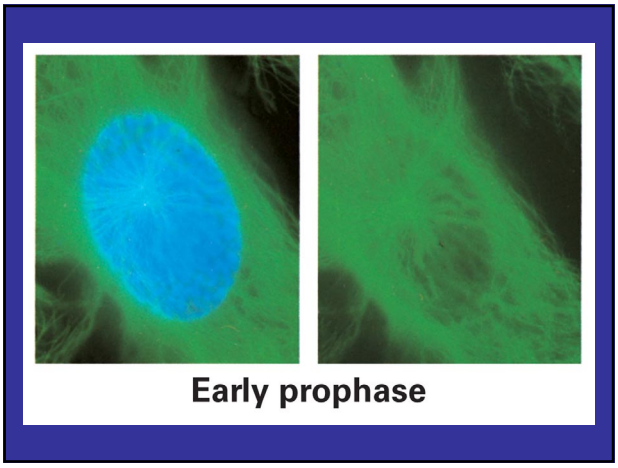

(b) Prophase
Spindle poles

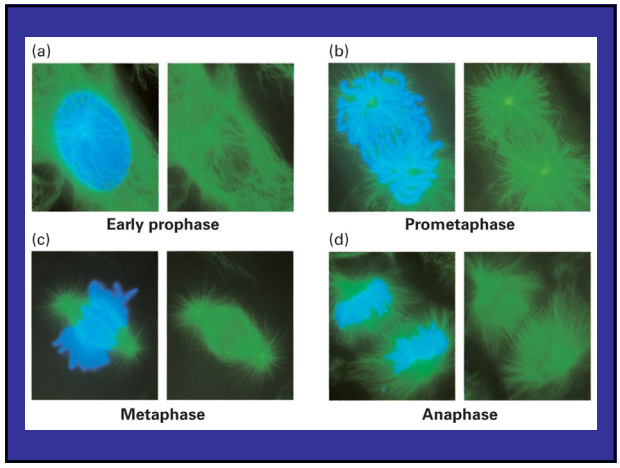

(e) Anaphase

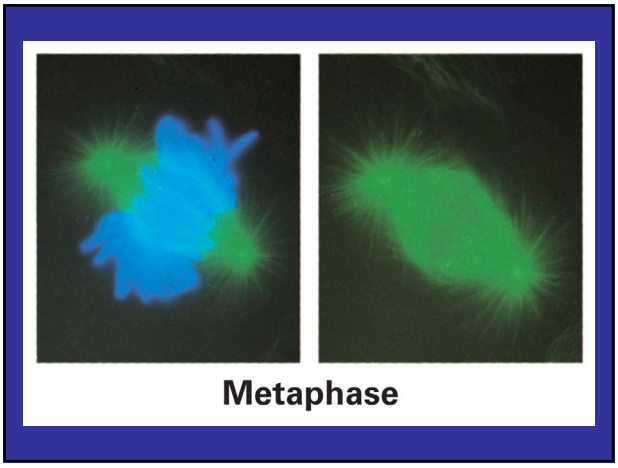

(d) Metaphase

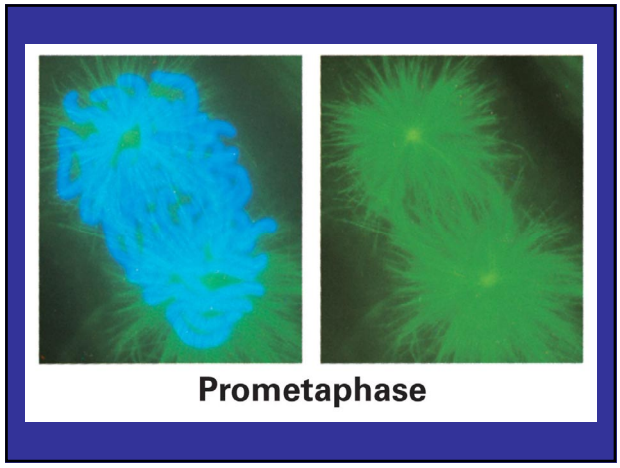

(g) Interphase (G_1)

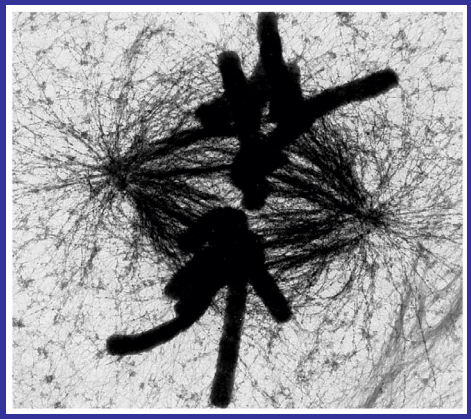

(f) Telophase
Cleavage furrow

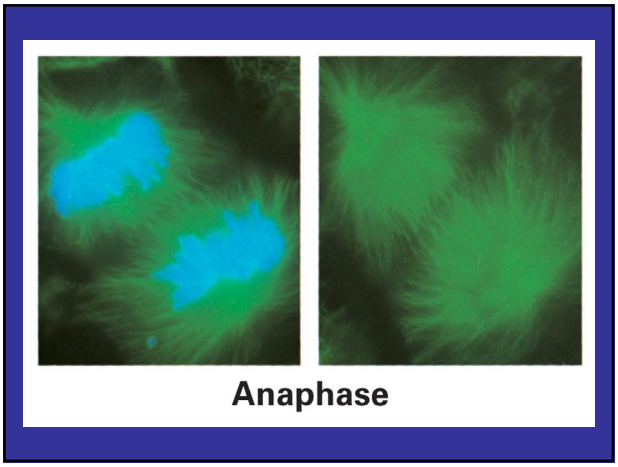

Early prophase

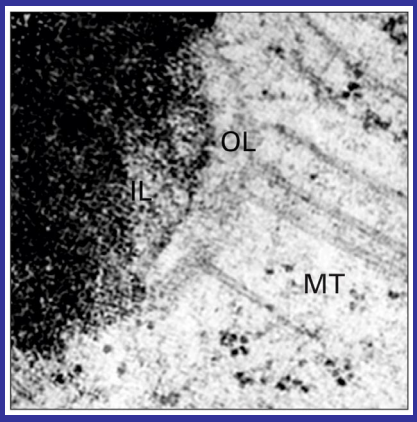

(a)
(b)
Early prophase
Prometaphase
(c)
(d)
Metaphase
Anaphase

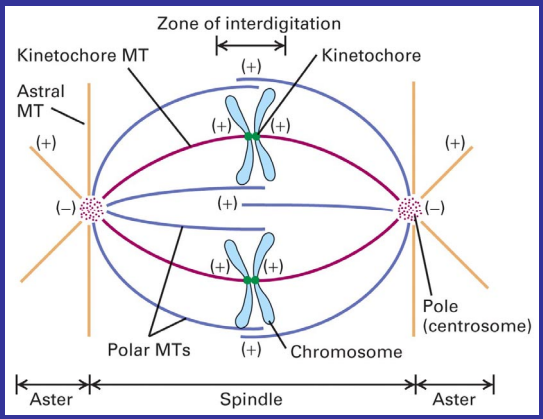

Metaphase

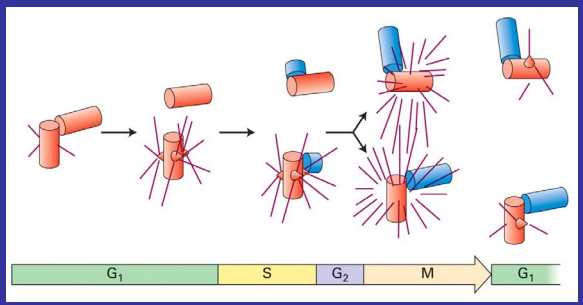

Prometaphase

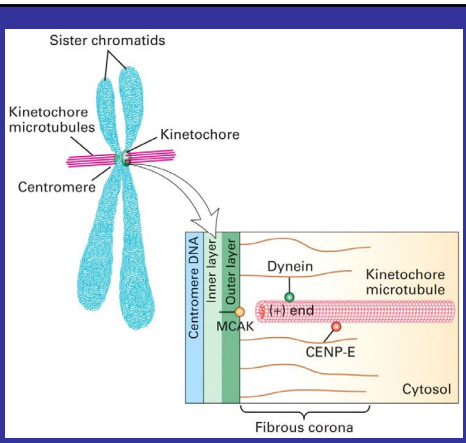


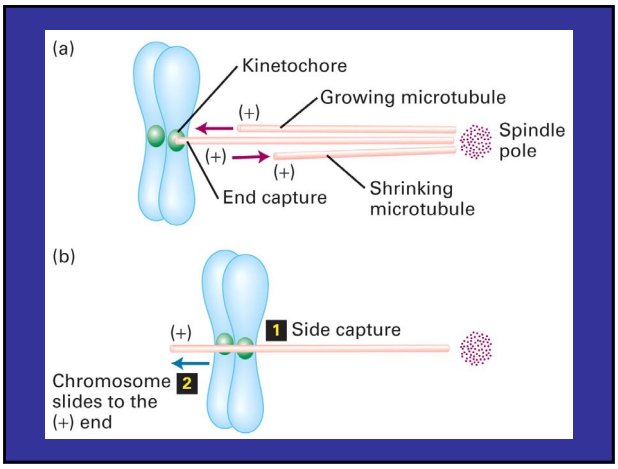

Anaphase

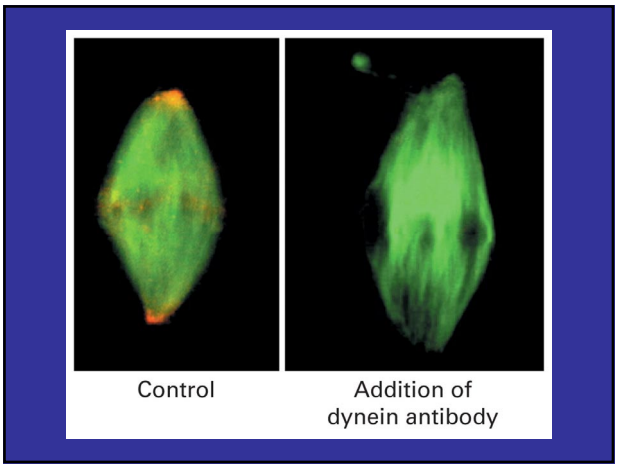

OL
IL
MT

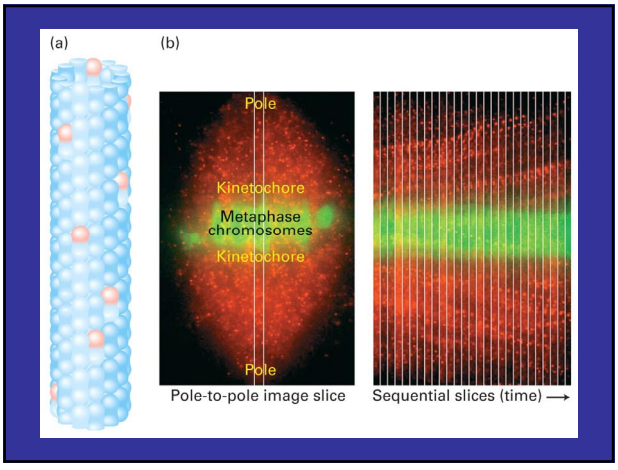

Zone of interdigitation
Kinetochore MT
Kinetochore
Astral MT
(+)
(–)
Pole (centrosome)
Polar MTs
Chromosome
Aster
Spindle
Aster

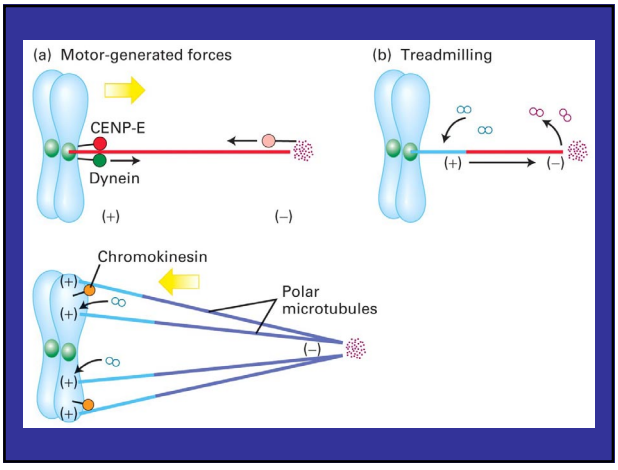

G1
S
G2
M
G1

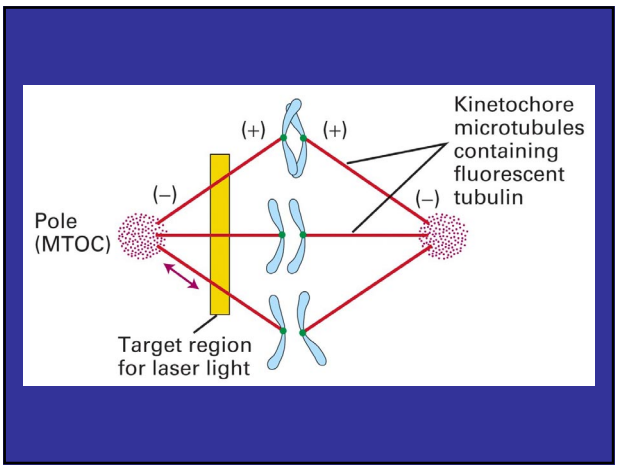

Sister chromatids
Kinetochore microtubules
Kinetochore
Centromere
Centromere DNA
Inner layer
Outer layer
Dynein
Kinetochore microtubule
(+) end
MCAK
CENP-E
Cytosol
Fibrous corona

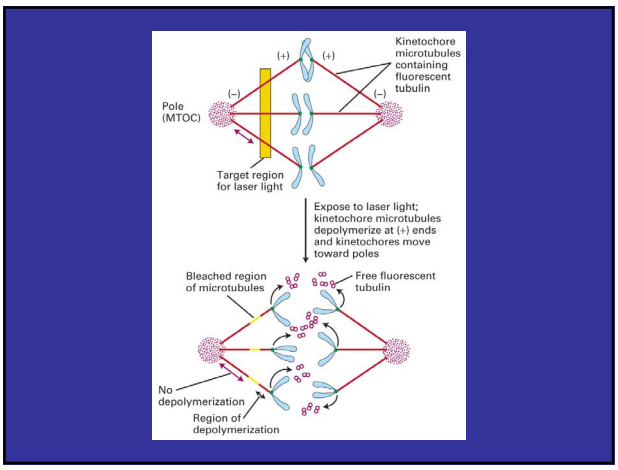

(a)
Kinetochore
Growing microtubule
(+)
Spindle pole
(+)
(+)
End capture
Shrinking microtubule
(b)
(+)
1 Side capture
Chromosome 2 slides to the (+) end

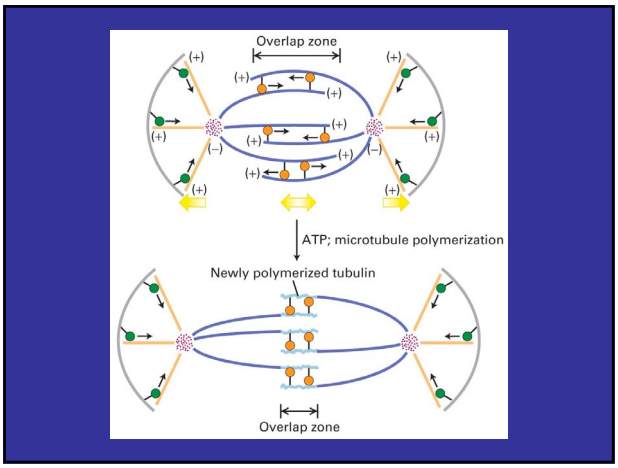

Control
Addition of dynein antibody

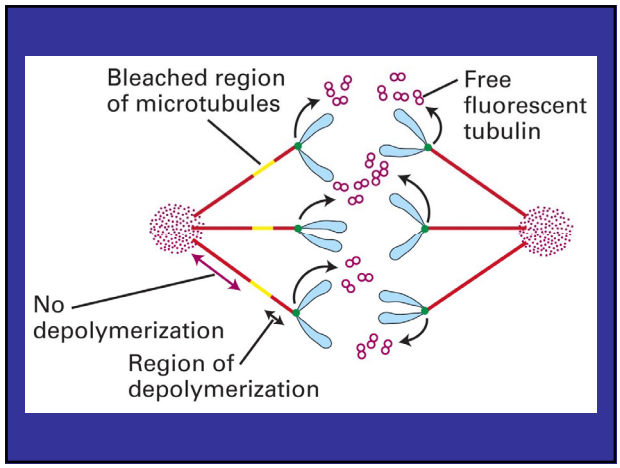

(a)
(b)
Pole
Kinetochore
Metaphase chromosomes
Kinetochore
Pole
Pole-to-pole image slice
Sequential slices (time)

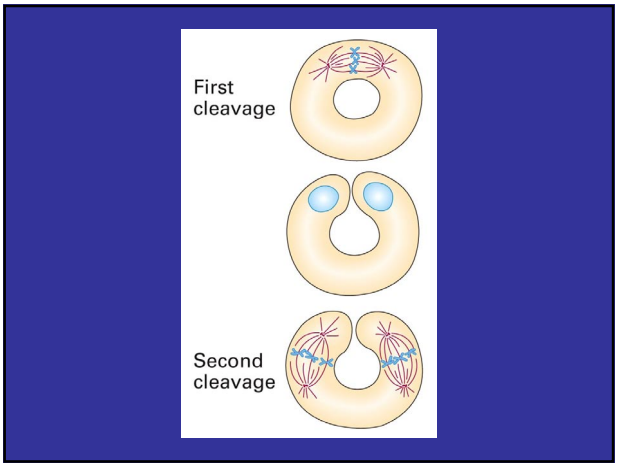

(a) Motor-generated forces
(b) Treadmilling
CENP-E
Dynein
(+)
(–)
(+)
(–)
Chromokinesin
Polar microtubules
(+)
(+)
(–)
(+)
(+)

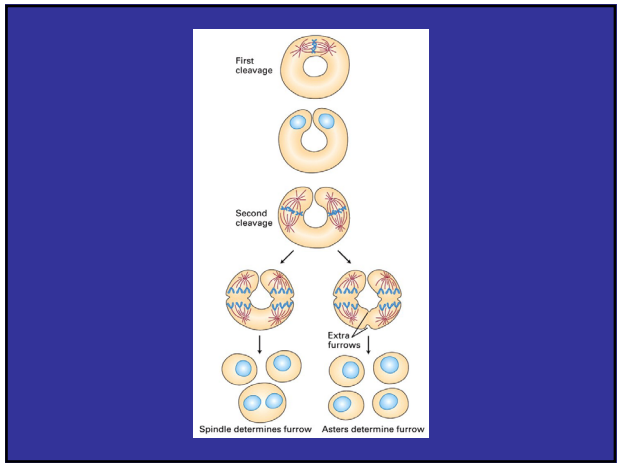

(+)
(+)
Kinetochore microtubules containing fluorescent tubulin
(–)
(–)
Pole (MTOC)
Target region for laser light

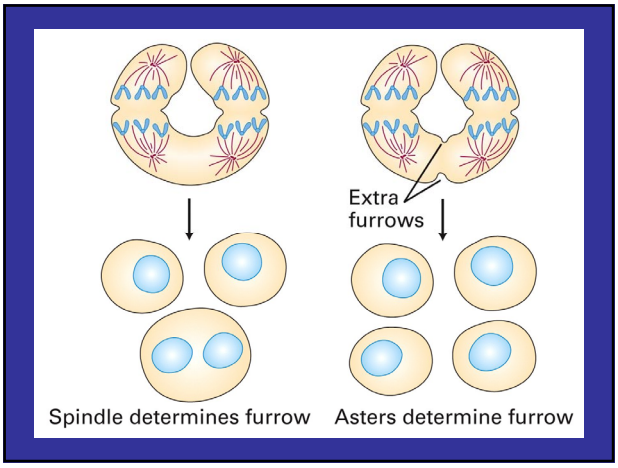

(+)
(+)
Kinetochore microtubules containing fluorescent tubulin
(–)
(–)
Pole (MTOC)
Target region for laser light
Expose to laser light; kinetochore microtubules depolymerize at (+) ends and kinetochores move toward poles
Bleached region of microtubules
Free fluorescent tubulin
No depolymerization
Region of depolymerization

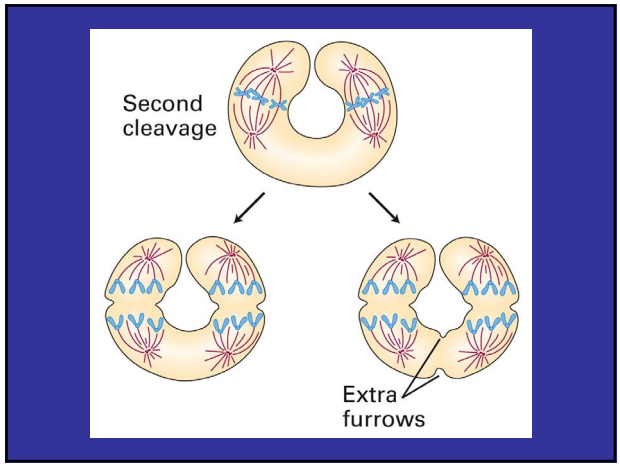

Overlap zone
ATP; microtubule polymerization
Newly polymerized tubulin
Overlap zone

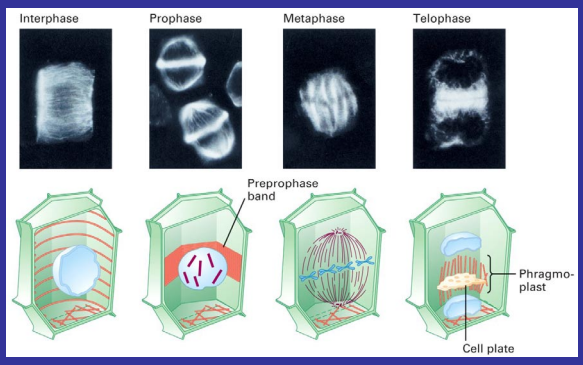

Bleached region of microtubules
Free fluorescent tubulin
No depolymerization
Region of depolymerization

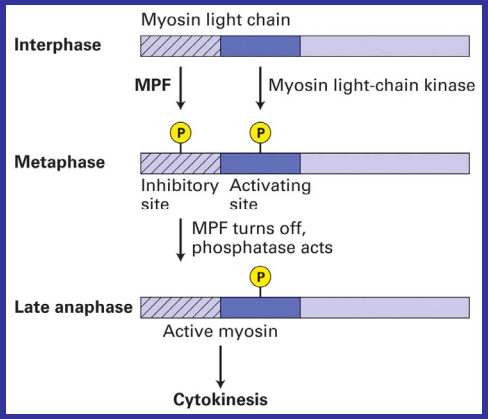

First cleavage
Second cleavage


First cleavage
Second cleavage
Extra furrows
Spindle determines furrow
Asters determine furrow


Extra furrows
Spindle determines furrow
Asters determine furrow

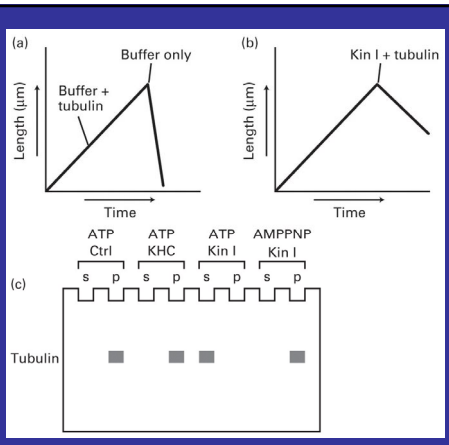

Second cleavage
Extra furrows





Heterodimeric tubulin subunits compose the wall of MT

- Building blocks are α and β-tubulin
- 55.000 MW
- γ tubulin
- GTP-binding sites. α tubulin irreversibly, β tubulin binds it reversibly and serves as a GTPase. The GDP modulate the addition of tubulin subunits.
- Protofilaments longitudinal interactions
- Cylindrical –tube, lateral interactions of 13 protofilaments



The Cc for assembly is 0.03μM
The intracellular concentration is 10-20μM
Polymerization is highly favored in cells

Assembly and disassembly mechanisms are different

MTs assembly and disassembly preferentially at the + end.

- Polymerization
- Temperature dependence 4o depolymerization 37o polymerization
- Polymerization dynamics: (1) αβ>Cc polymerization of the dimers into MTs, if <Cc depolymerization (2) GTP/GDP modulate the Cc, (3)αβ>Cc at +end and< from the –end, treadmill.

MAPs

- stabilizes MTs- composed of a MT binding domain –positively charged 4 residue amino acid sequence that bind to tubulin.
- and acidic projection domain
- MAP1A MAP1B, MAP2 in dendrites only, MAP4 MTs stability, Tau axonogenesis, abbarent polymerization of tau -AD, CLIP170 cross link MTs to chromosomes

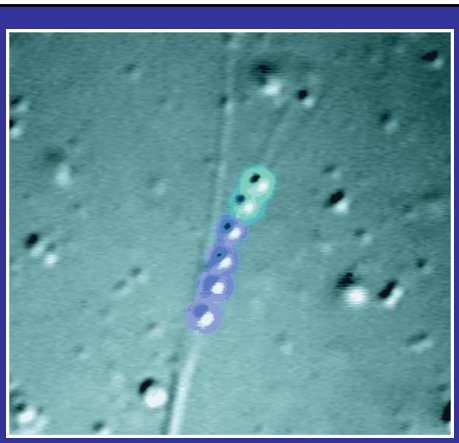
Dynamic instability of MTs

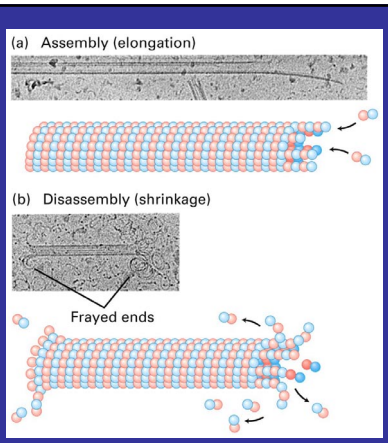
- At concentration near the Cc MTs grow and shrink !!
- Assembly; catastrophe, disassembly, rescue
- GDP/GTP-binding site on β tubulin.
- off rate of GDP-tubulin > off rate of GTP-tubulin

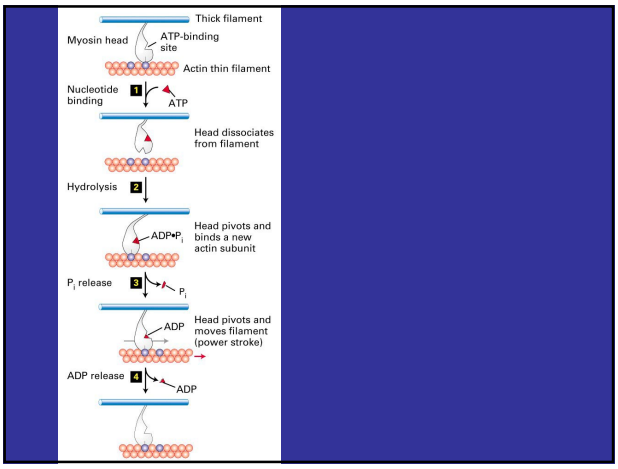
Colchicine/taxol

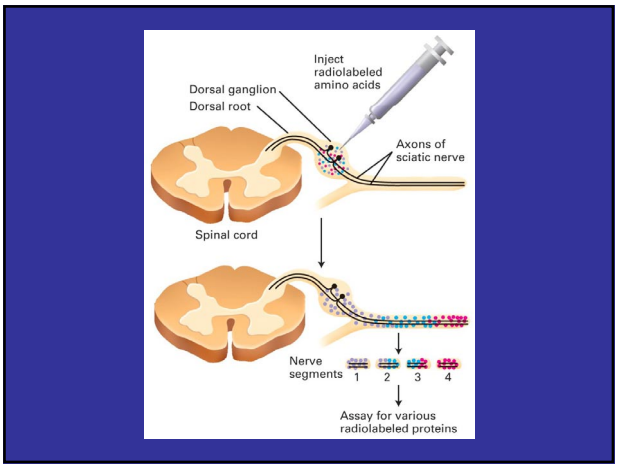
Phosphorylation of MAPs

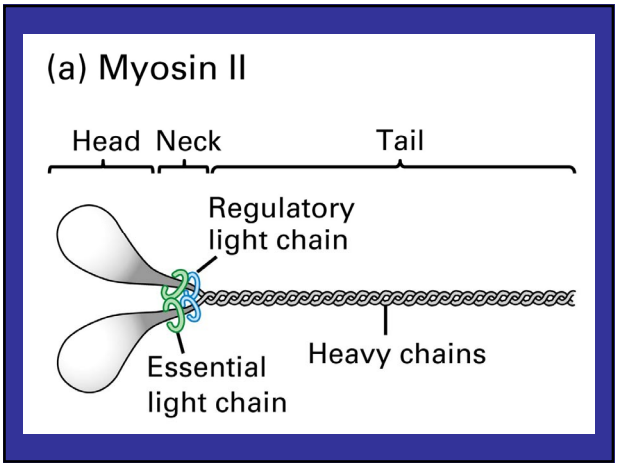
- Phosphorylated MAPs are unable to bind MTs, leading to MTs disassembly.
- MAP kinase participate in many signal-transduction events indicating that MAP are targets of many extracellular signals
- MAp4 is phosphorylated by cyclin-dependent kinase (CDK) cell cycle






Thick filament
Myosin head
ATP-binding site
Actin thin filament
Nucleotide binding
1
ATP
Head dissociates from filament
Hydrolysis
2
ADP•Pi
Head pivots and binds a new actin subunit
Pi release
3
Pi
ADP
Head pivots and moves filament (power stroke)
ADP release
4
ADP


Inject radiolabeled amino acids
Dorsal ganglion
Dorsal root
Axons of sciatic nerve
Spinal cord
Nerve segments
1
2
3
4
Assay for various radiolabeled proteins


(a) Myosin II
Head
Neck
Tail
Regulatory light chain
Essential light chain
Heavy chains