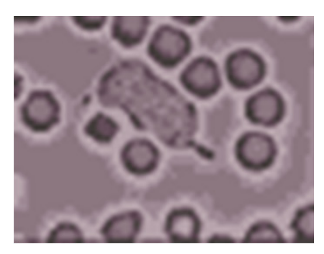
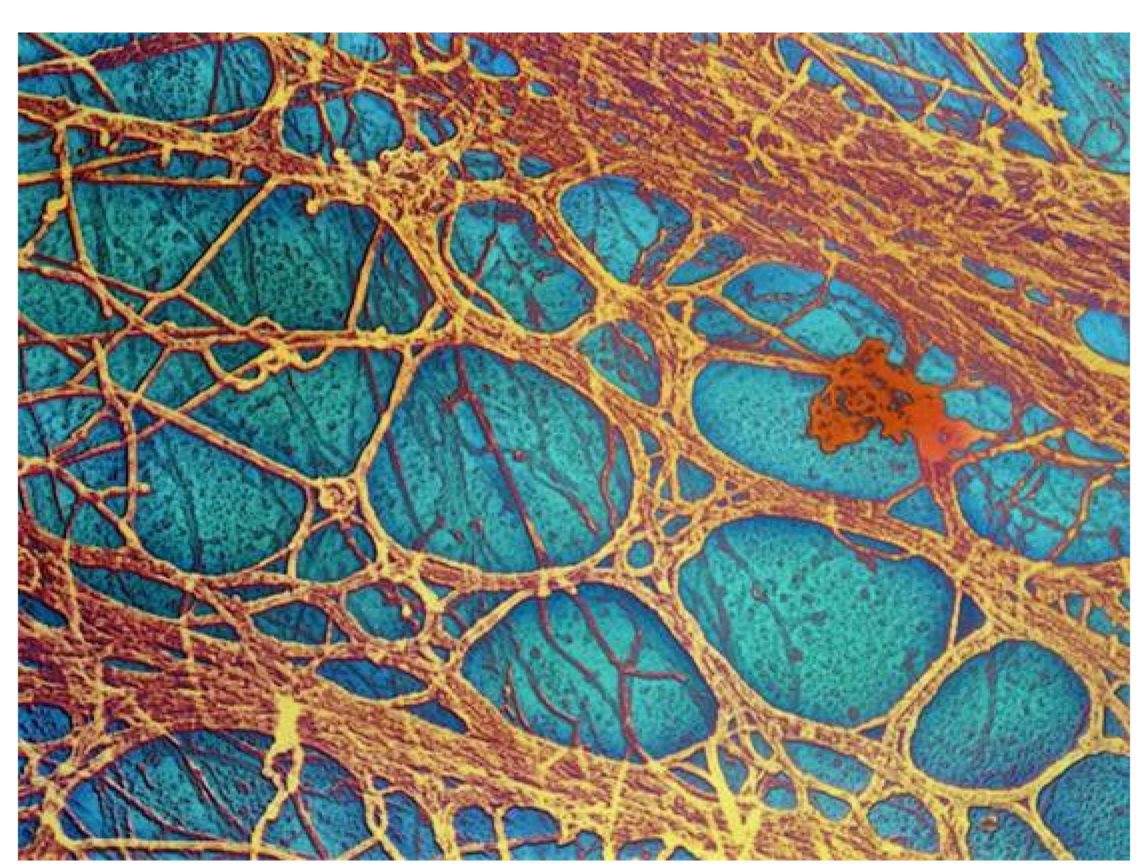
THE CYTOSKELETON



PART II: Microtubules and intermediate filaments in cell organization and movement



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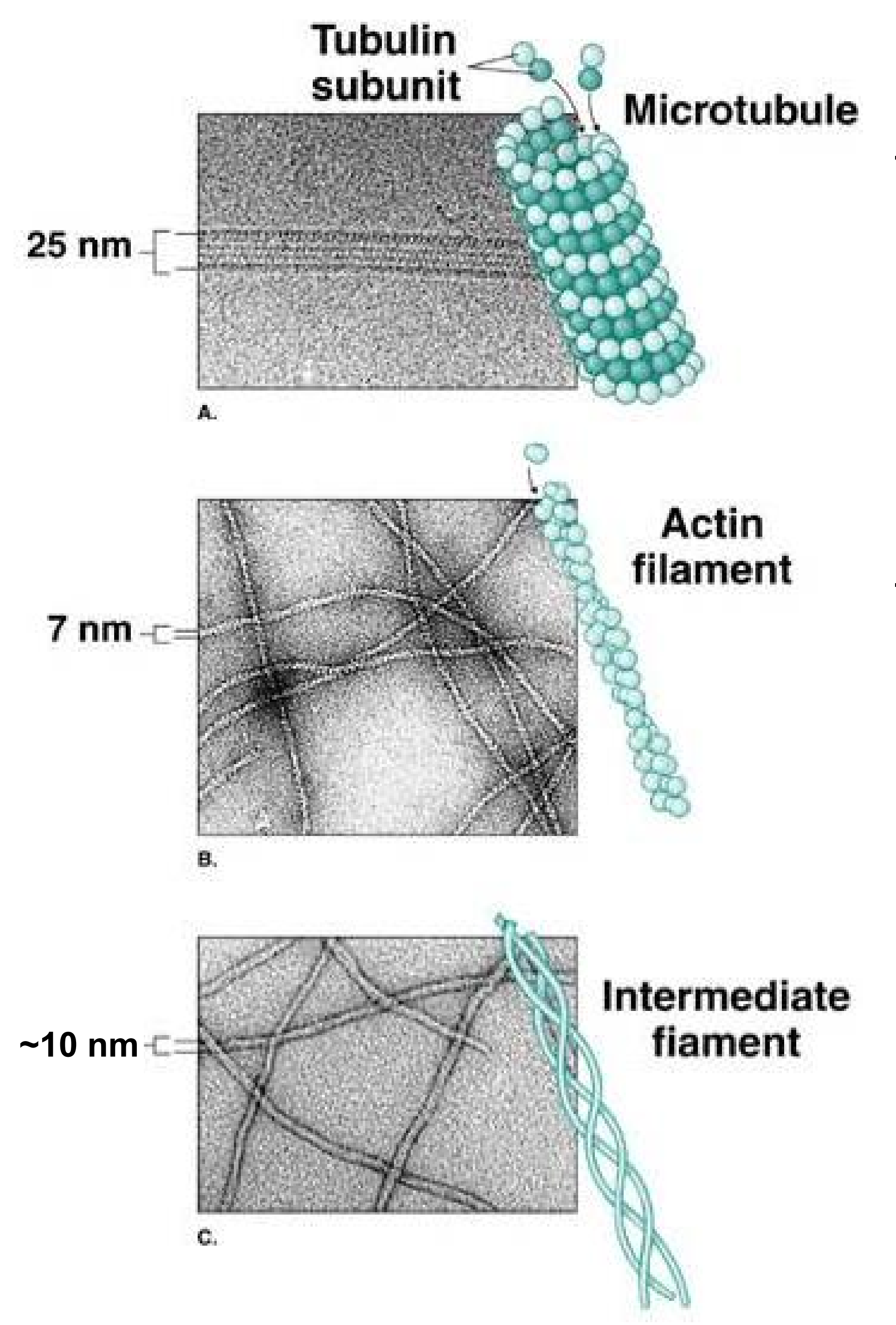


Similar to F-actin, microtubules (MT) take part in intra- and extracellular movements:

- Beating of **flagella** and **cilia**
- Vesicle transport in the cytoplasm
- **Separation of chromosomes** (mitosis)
- Neuronal outgrowth of the axon

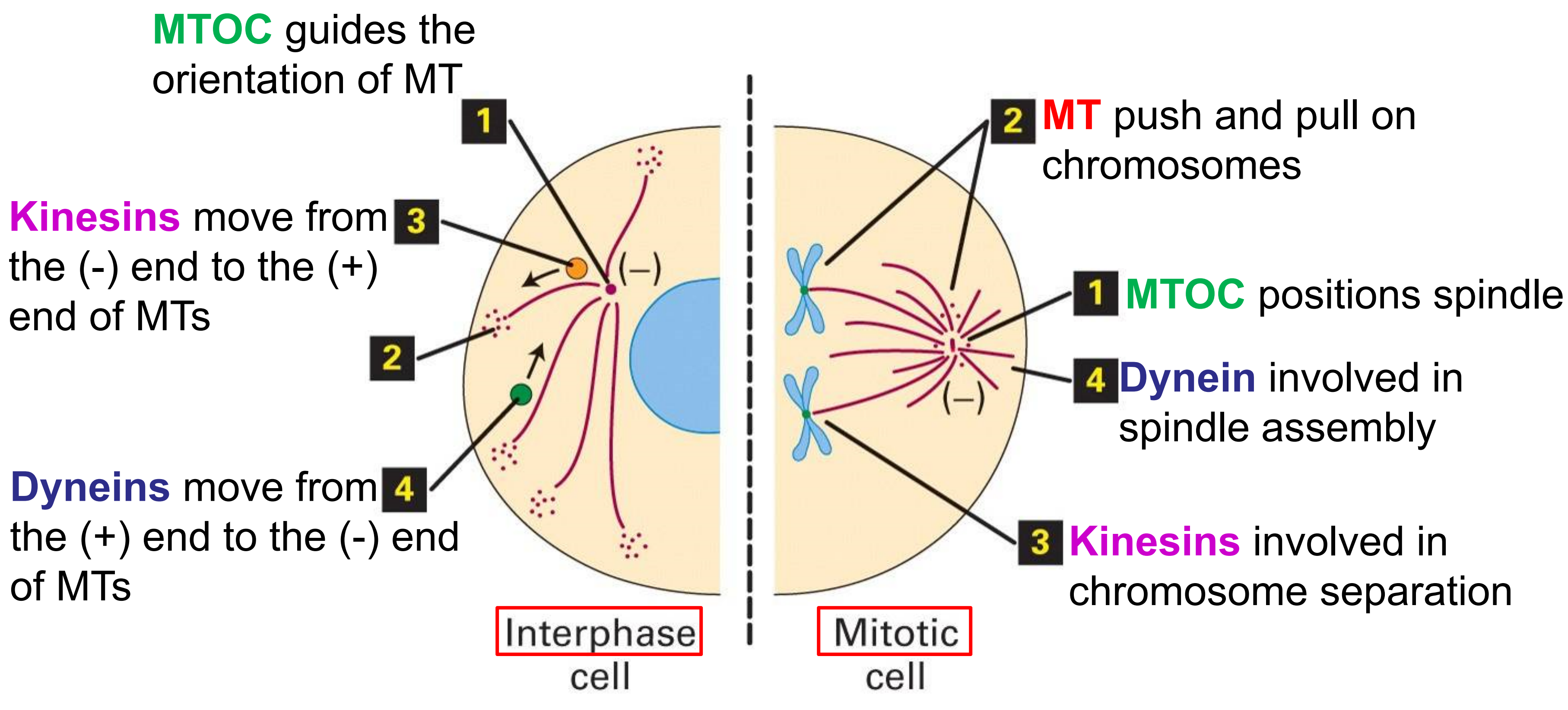
These motility events are based on:

- The biomechanical power of **tubulin** polymerization and depolymerization
- Microtubule-based **motors** (kinesins and dyneins)

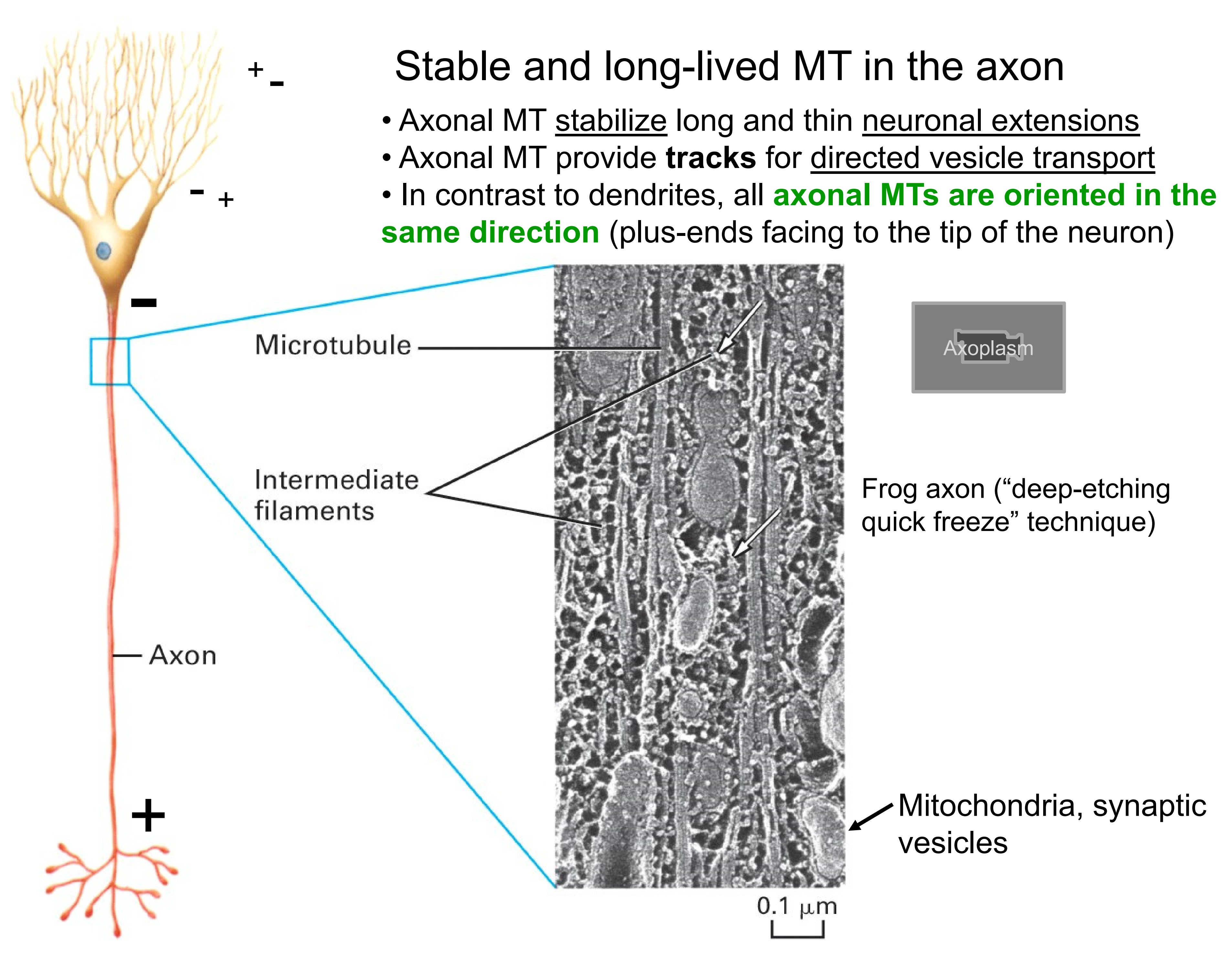
In addition, microtubules largely contribute to cell polarity thru the **MTOC**:

- MTOC = microtubule organizing center
- Located near the nucleus
- Specialized structure from which MT grow
- Determines the **orientation of MT**, direction of vesicle transport and orientation of organelles (ER and Golgi)

Microtubule action in the **interphase** cell and in **mitotic** cells

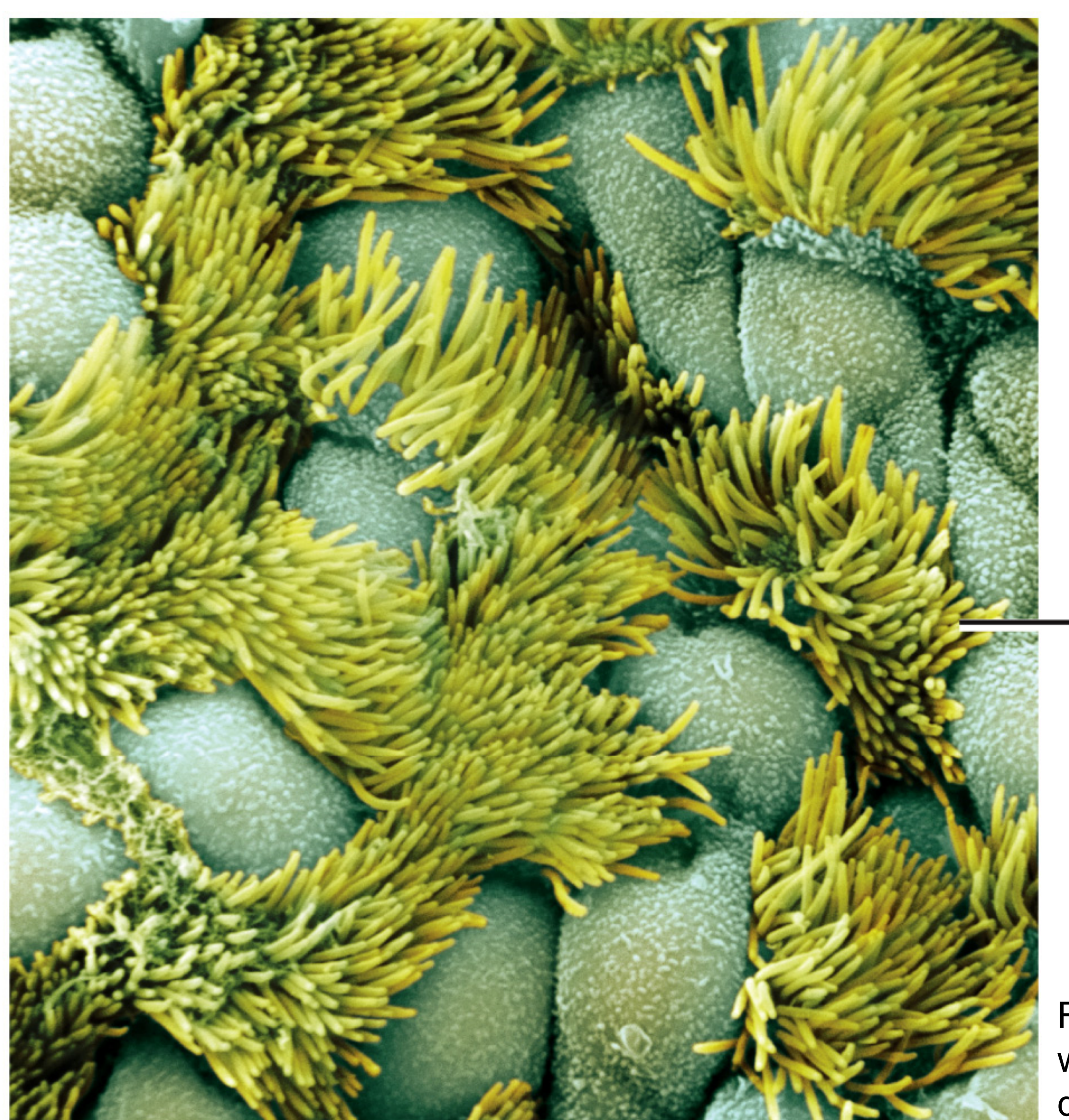


Stable long-lived MTs ⇔ **Unstable** and short-lived MTs


Stable and long-lived MT in the axon
• Axonal MT stabilize long and thin neuronal extensions
• Axonal MT provide tracks for directed vesicle transport
• In contrast to dendrites, all axonal MTs are oriented in the same direction (plus-ends facing to the tip of the neuron)
+
-
-
+
-
+
Microtubule
Intermediate filaments
Axon
Axoplasm
Frog axon ("deep-etching quick freeze" technique)
Mitochondria, synaptic vesicles
0.1 µm

Stable (long-lived) microtubules

Cilia and flagella are extensions of the plasma membrane formed by **thick bundles of microtubules** which move rhythmically

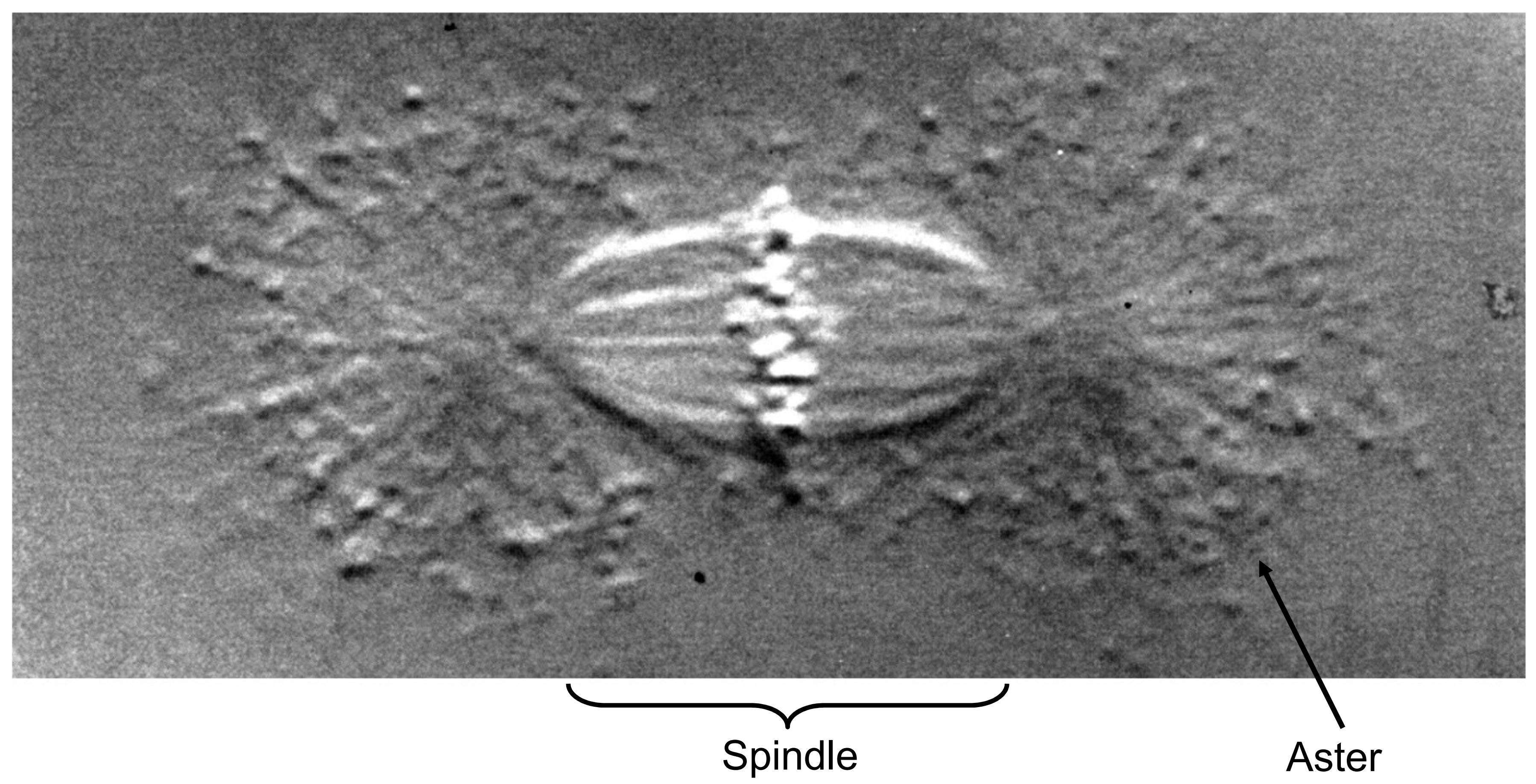


- Flagella enable a **sperm** to swim
- Cilia move material across epithelial surfaces (e.g., mucus in trachea)
- Cilia move **eggs** thru an oviduct

Rabbit oviduct epithelium covered with beating cilia to move eggs down the fallopian tube

Unstable (short-lived) microtubules

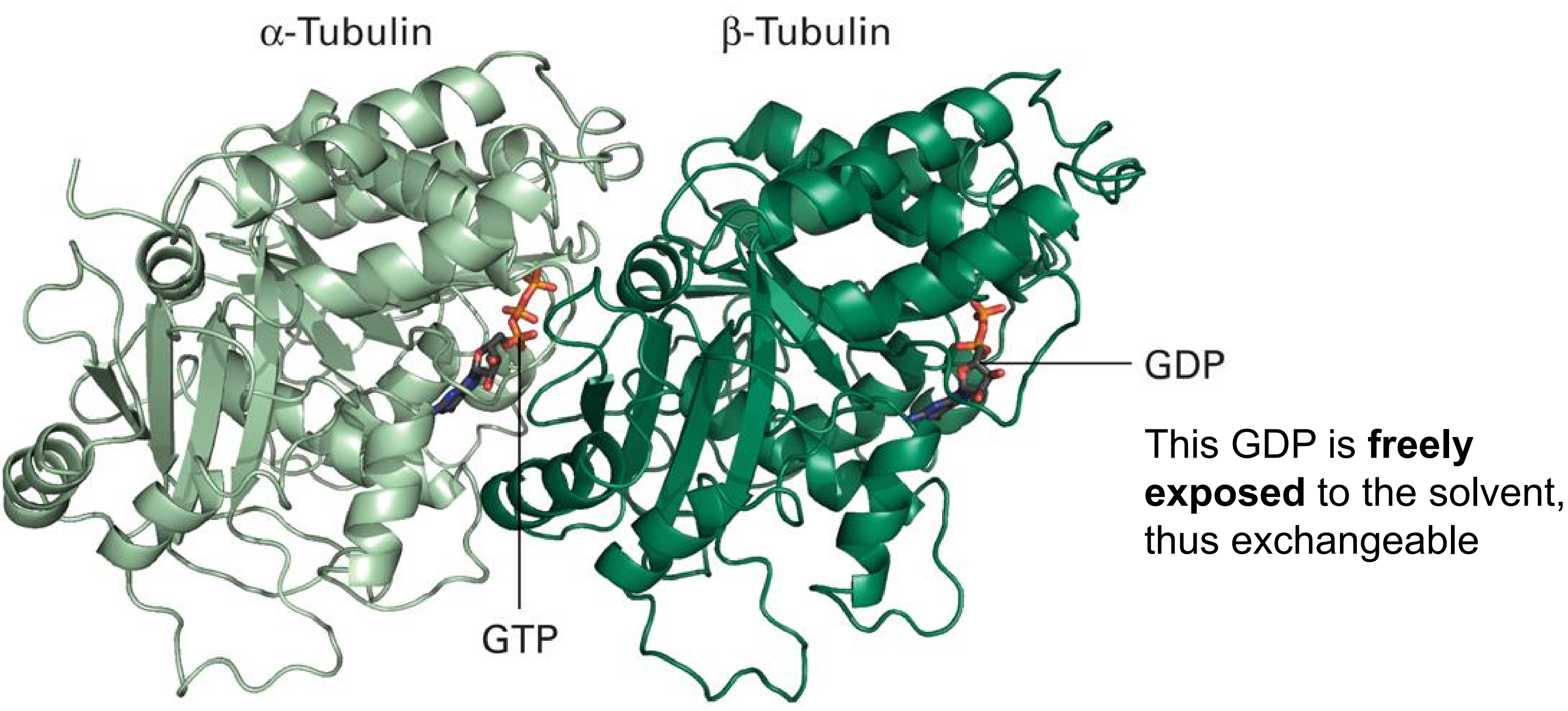
Found in structures with the need to assemble and disassemble quickly:
Cytosolic MT disassemble during **mitosis** and the material is used to form the **spindle**-shaped apparatus which organizes and separates the chromosomes



Isolated mitotic spindle apparatus in DIC microscopy

Structure and dynamics of microtubules

- MT are build by heterodimeric tubulin subunits composed of α**-** and **β-tubulin**
- 55 kDa (compare G-actin: 43 kDa)
- **γ-tubulin** is an isomer used to seed/nucleate polymerization of αβ-tubulin
- Bacterial GTPase (**FtsZ**) exhibits high **homology to tubulin** (tubulin ancestor)
- α-tubulin binds GTP irreversible and **cannot hydrolize it**
- β-tubulin binds GTP reversible and **can hydrolyze it** like a common GTPase

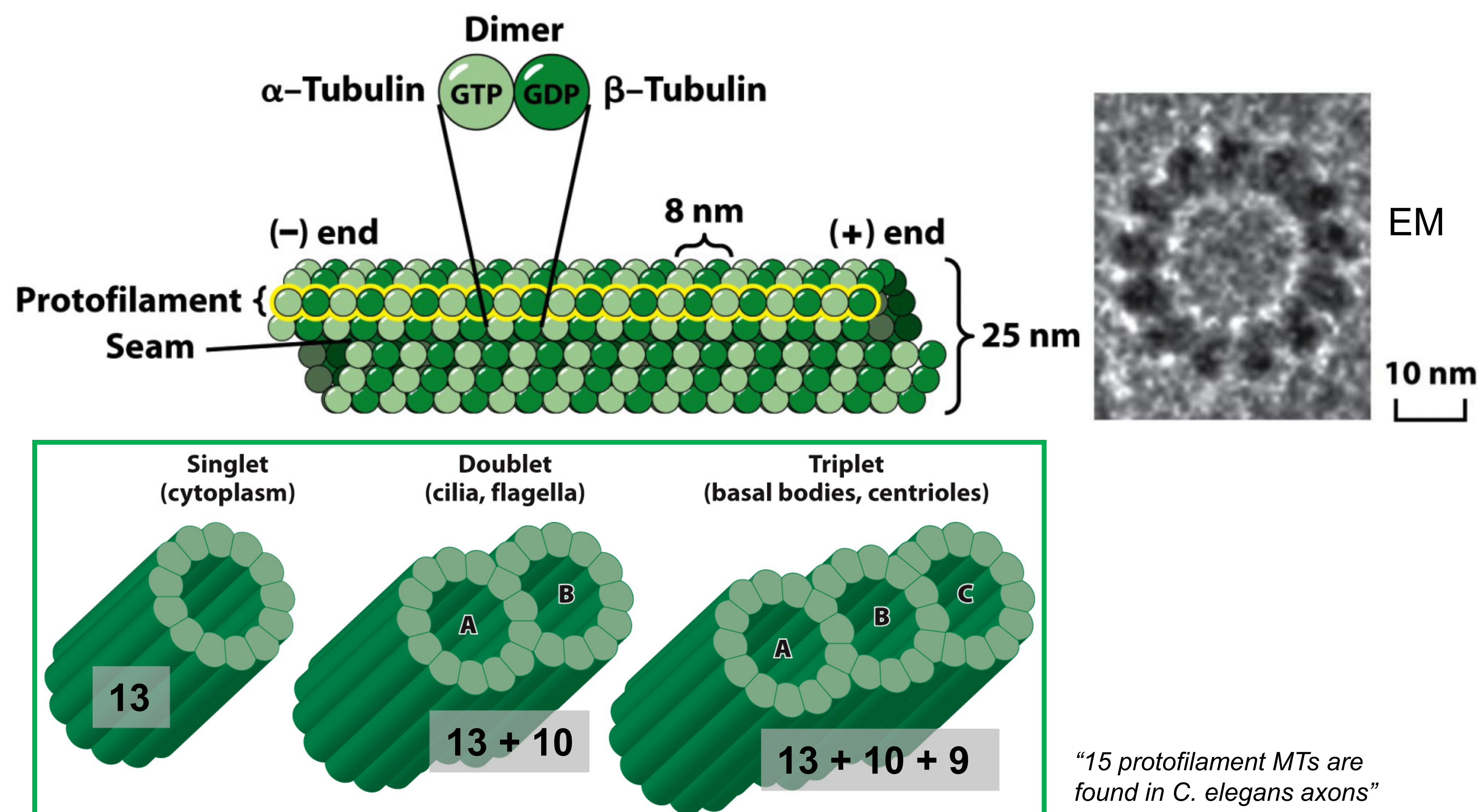


This GDP is **freely exposed** to the solvent, thus exchangeable

This GTP is **trapped** at the interface between α- and β- tubulin (thus not exchangeable)

Microtubules are generally composed of 13 protofilaments

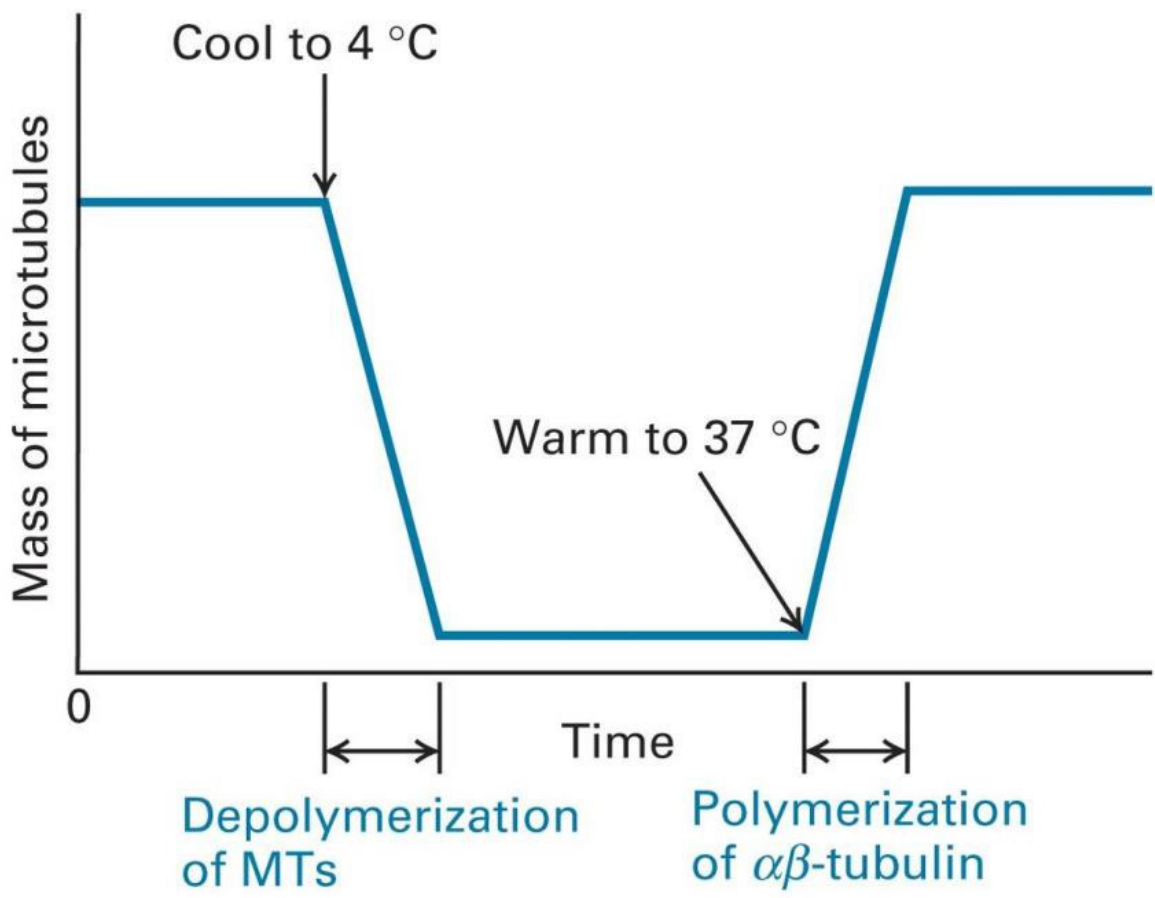
• Tubulin heterodimers polymerize into **protofilaments** which longitudinally associate to form the **hollow MT cylinder**
• The 8 nm distance between the subunits reflects exactly the **kinesin step size**
• The **plus-end** of the polar MT contains **β-tubulin** with its exchangeable GTP
• Except at the **seam**, α-tubulin and β-tubulin from other protofilaments are in contact



"15 protofilament MTs are found in C. elegans axons"

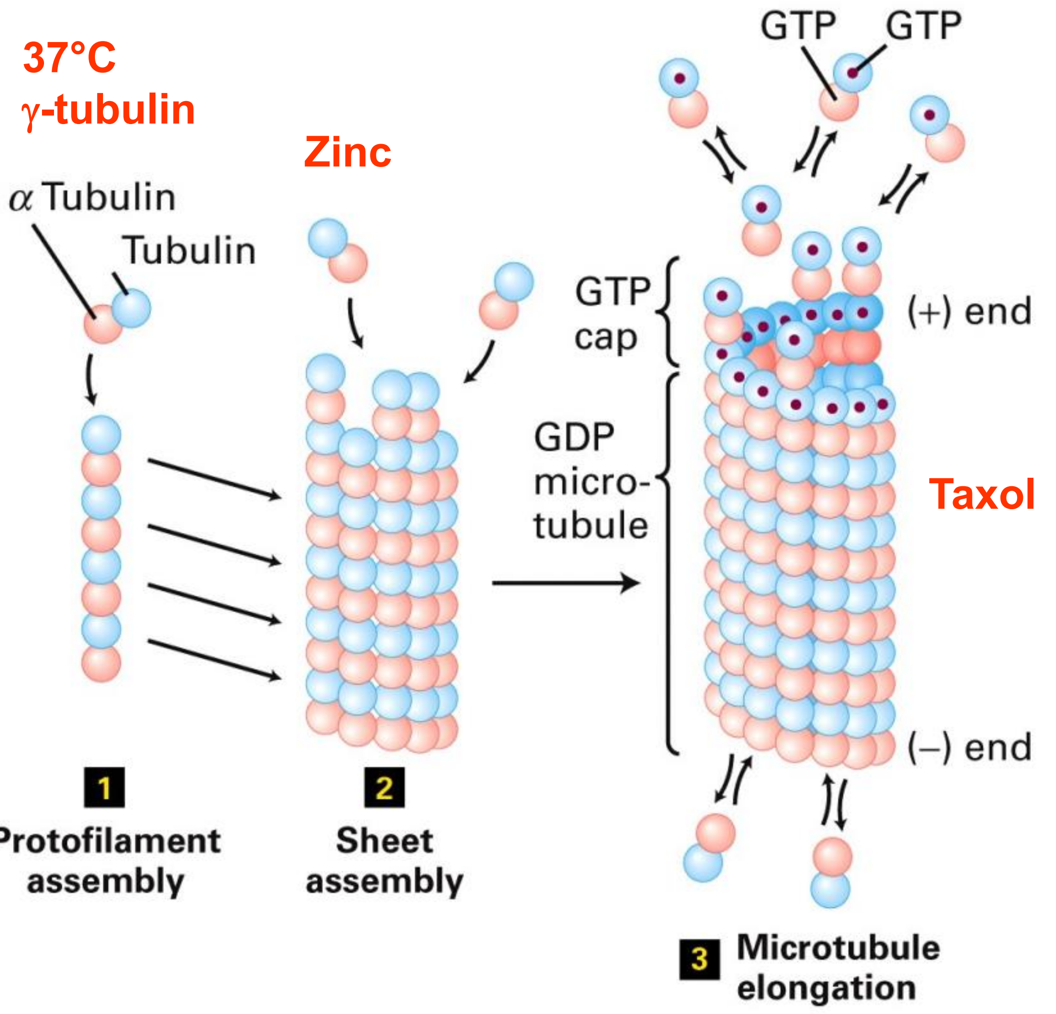
Microtubule polymerization is temperature dependent

- In contrast to F-actin, MT polymerization is **temperature dependent**
- Similar to F-actin, MT polymerize when a certain **critical concentration** of free tubulin subunits is reached
- MT depolymerize when the conc. of free tubulin is below Cc = 0.03 μM (cell: 10-20 μM)
- Cc differs depending on bound GTP or GDP at the plus- or minus-end (similar to actin)



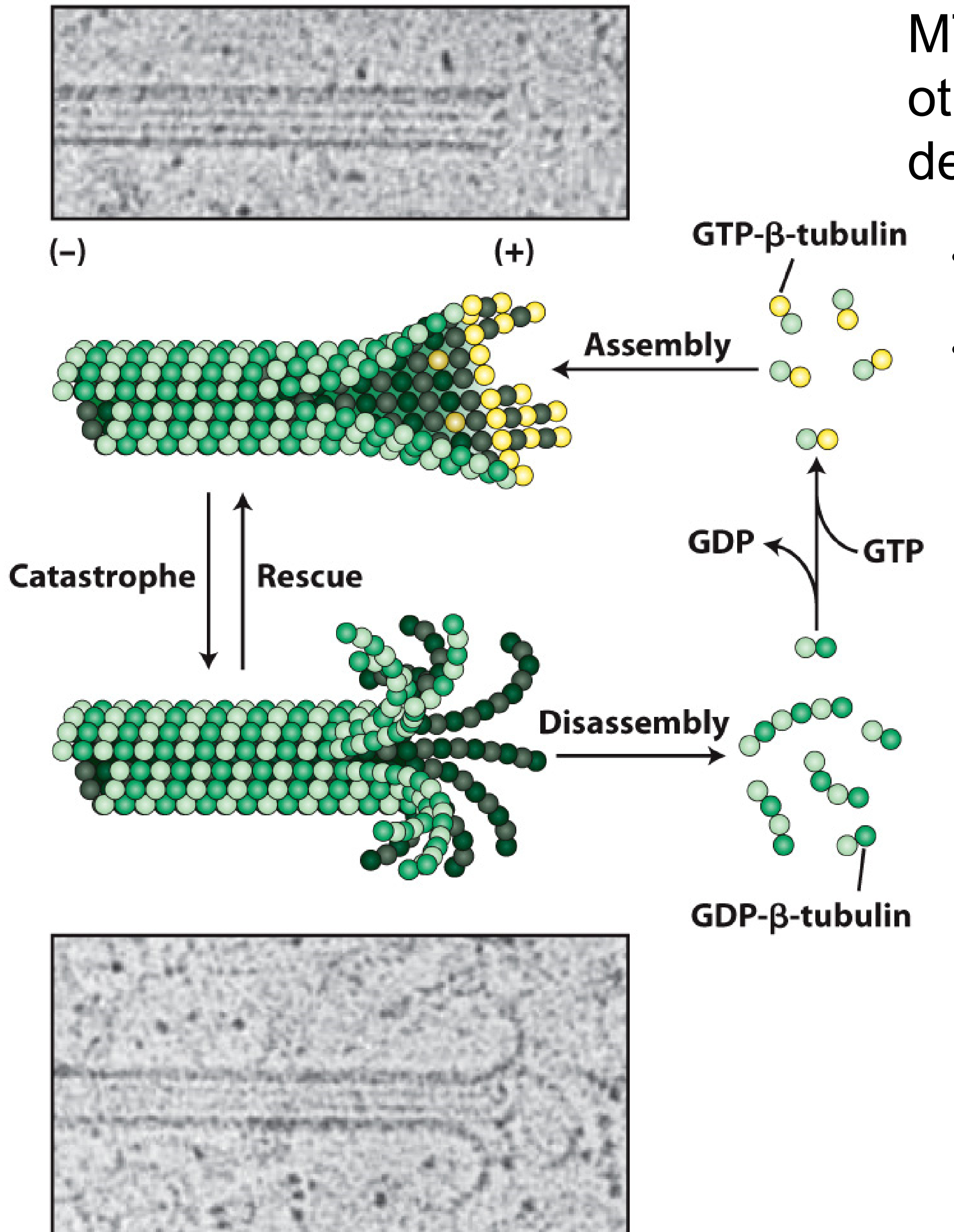
The 3 steps of microtubule assembly

1. Free $\alpha\beta$-tubulin dimers polymerize *longitudinally* into **protofilaments**
2. Unstable protofilaments associate *laterally* into more stable **sheets**
3. Sheet of 13 protofilaments closes at the MT seam. MT grows by the addition of GTP-tubulin subunits to its plus-end.



Growth at plus-end is twice as fast as at the minus-end; in addition, GTP hydrolysis is a slow process that's why a (protective) **GTP-cap** forms

MT ends look different from each other during polymerization and depolymerization



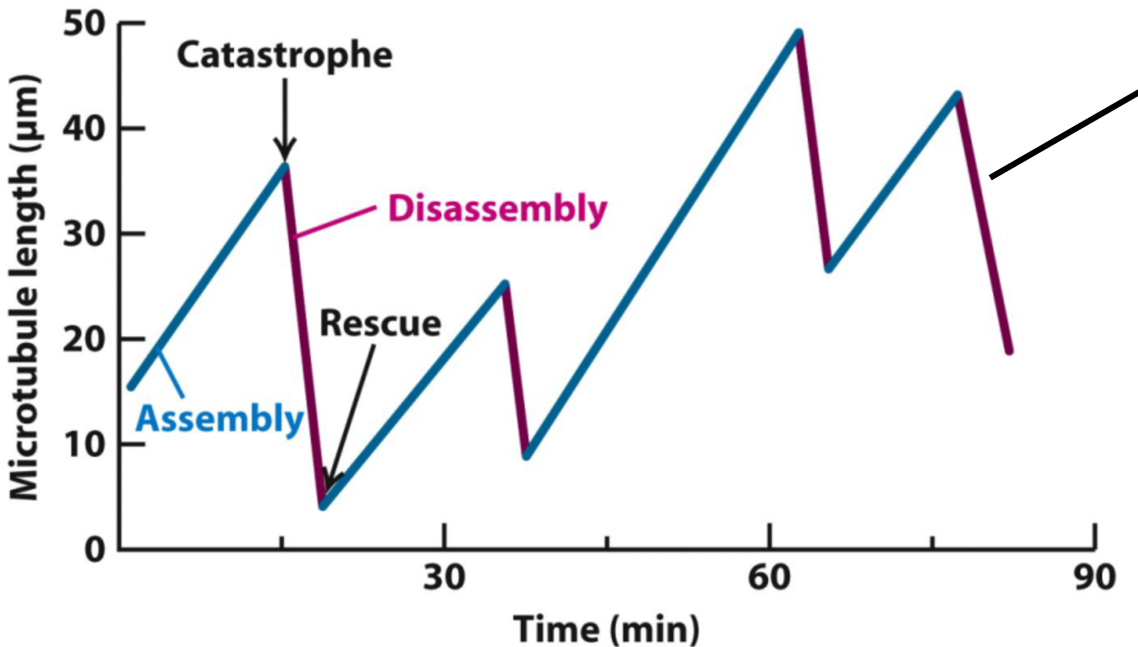
- The end to which GTP-tubulin adds faster is called the **plus-end**
- **GTP addition** to the protofilaments and **MT closure along the seam** are timely different processes

- During depolymerization the protofilaments are **peeling off** the MT
- The reason is **natural bending** of protofilaments if containing only GDP-tub
- Within the MT, however, protofilaments (containing only GDP-tub) do **laterally interact** (so unpeeling is constrained)
- This results in stored mechanical energy which **can do work** during shrinking (e.g., chromosome separation in mitosis)

MT poly- and depolymerization

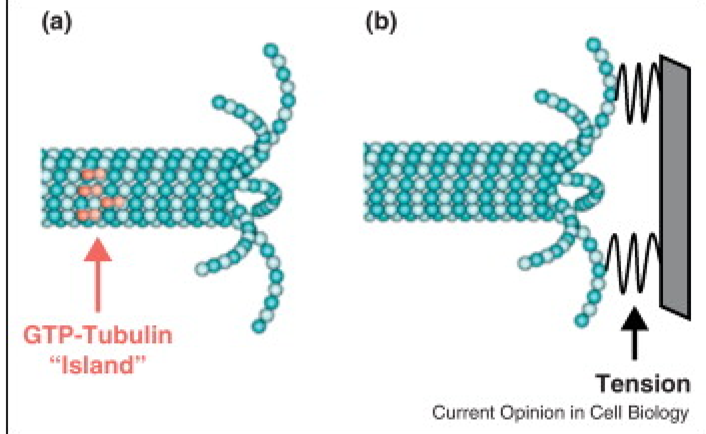
Dynamic instability is a special feature of microtubules

Both *in vitro* and *in vivo* MT oscillate between **fast ("catastrophic") shrinkage** (7 μm/min) and **slow ("rescue") growth** (1 μm/min) => **dynamic instability**



Dynamic instability of isolated MT can be observed using light microscopy (DIC)

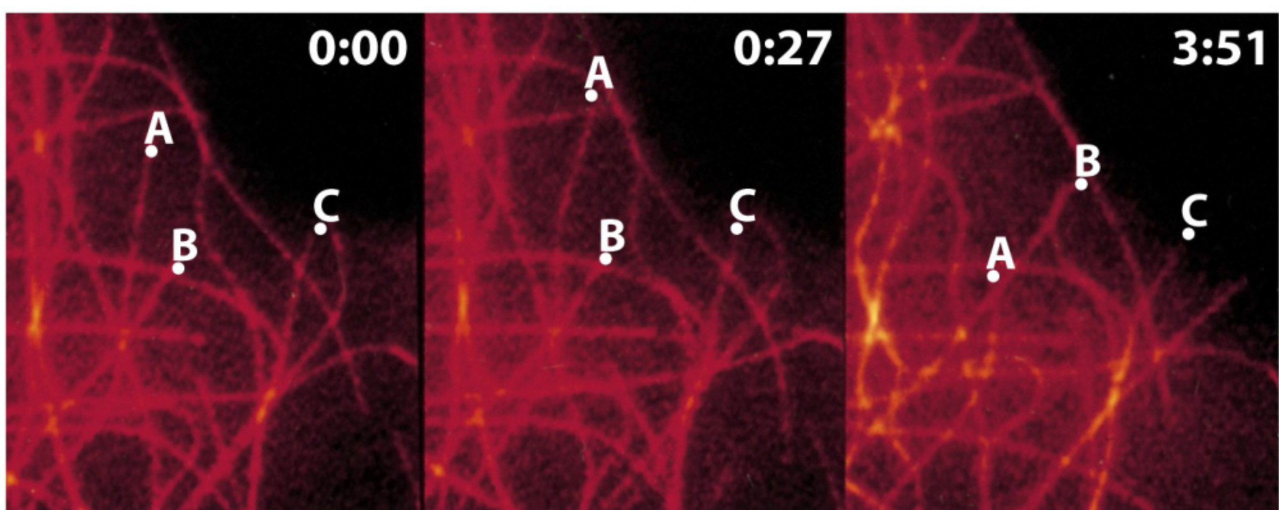
Why do some MTs not completely depolymerize?
Because they hit an **island of GTP-tubulin** (somewhere inside the MT) from which they start to re-polymerize (rescue)



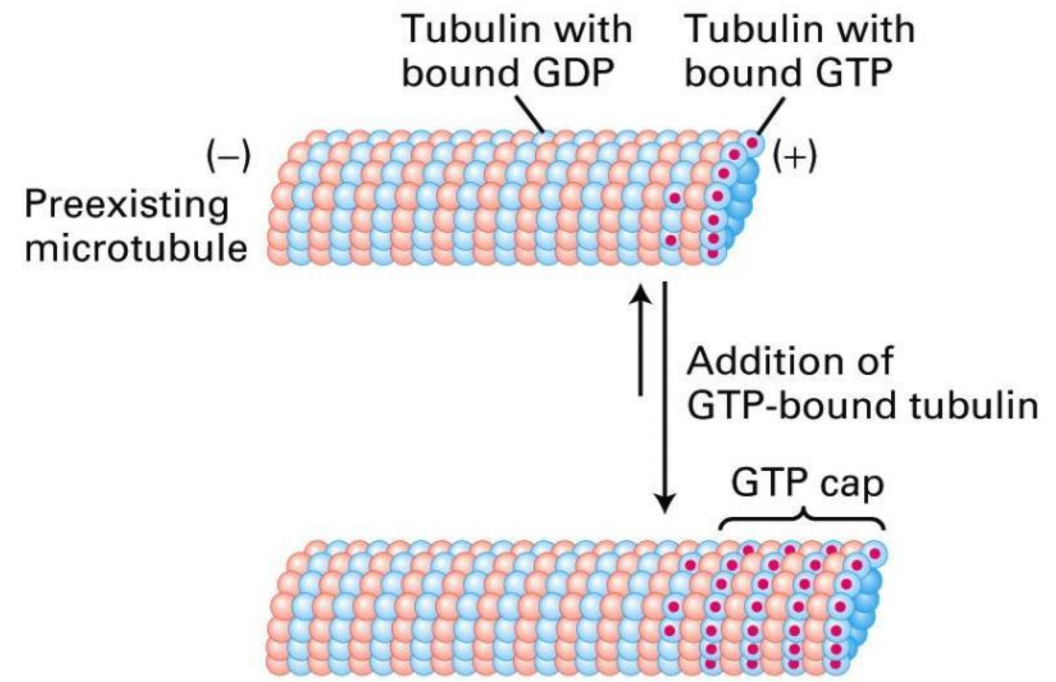
MT can do work during depolymerization (e.g., displacing chromosomes)

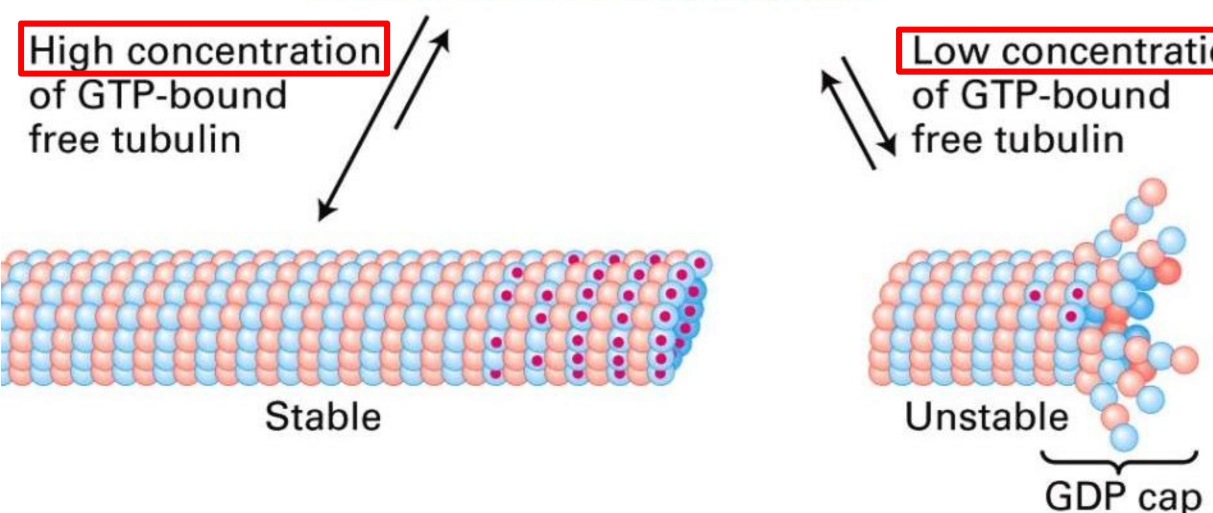
Making dynamic instability of microtubules visible in cells

- Cells **chilled down** to *depolymerize* MT => microinjection of fluorescent αβ-tubulin => **incubation at 37°C** to *repolymerize* MT
- Dynamic instability is highly limited to the plus-end because minus-end is attached to the MTOC
- Dynamic instability **occurs near the Cc**: some MT already grow fast while others start to shrink
- Growing microtubules may eventually "find" a target in the cell (organelles or other structures) that **stabilizes the plus-end** and protects the MT from catastrophe
- Thus, MTs seem to perform a constant **"search and capture"** that is important for cellular microtubule organization



Stability of MT depends on the presence of a GTP-cap



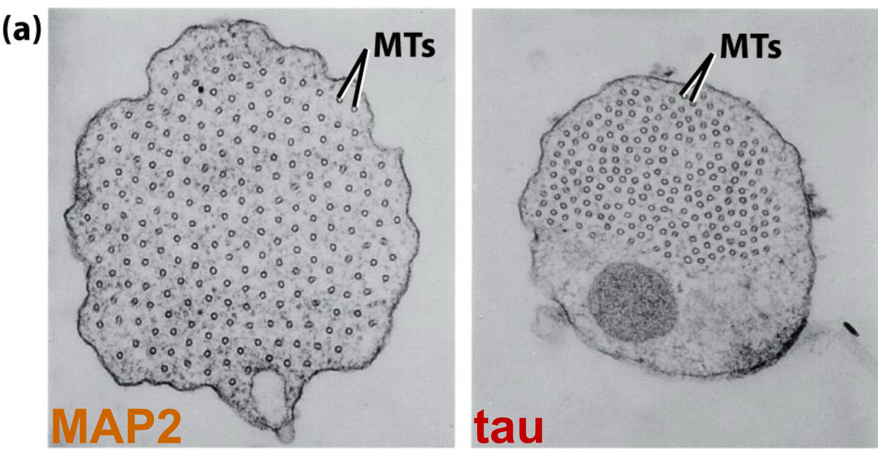


- Because GTP hydrolysis is a slow process, **GTP-cap forms**
- When all GTP-tubulin is used up, **GTP-cap shortens**
- **Fast shrinkage happens because GDP-tubulin dissociates from the MT much faster (4x) than GTP-tubulin**
- A GTP-cap is therefore important to **protect** the MT from shrinking
- Adding **GTP-tubulin rescues** the MT

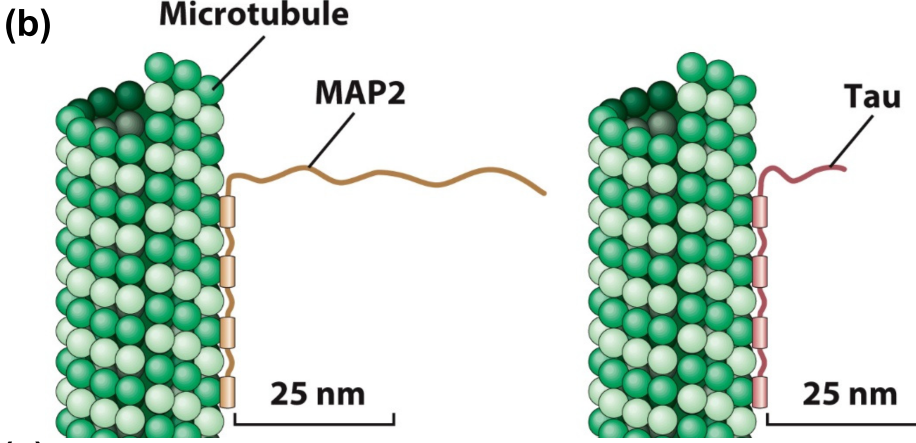
"The existence or non-existence of the GTP-cap is the major reason for dynamic instability"

Dynamic instability is controlled by MT-binding proteins

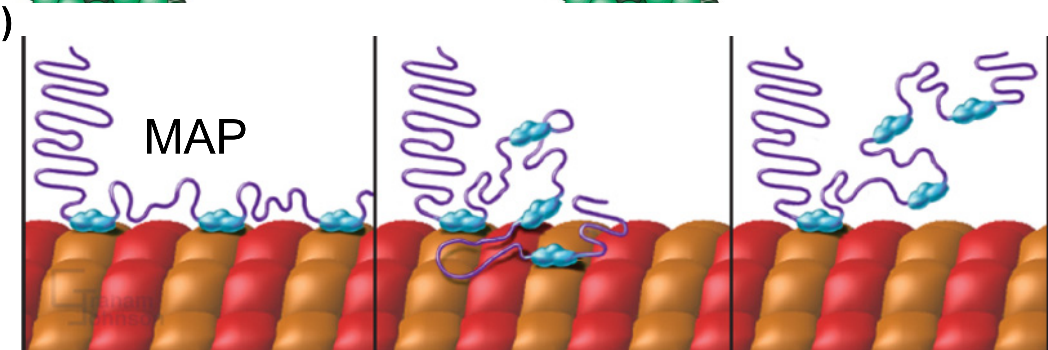
Proteins controlling MT stability are called **MAPs = microtubule associated proteins**



- Insect cells expressing either **MAP2** or **tau** grow *axonlike processes*
- Spacing between the MT depend on the expressed MAP type



MAPs have a **basic** MT-binding domain (positively charged = binds to the negatively charged MT) and a **acidic** projection domain (binds to membranes or IFs)

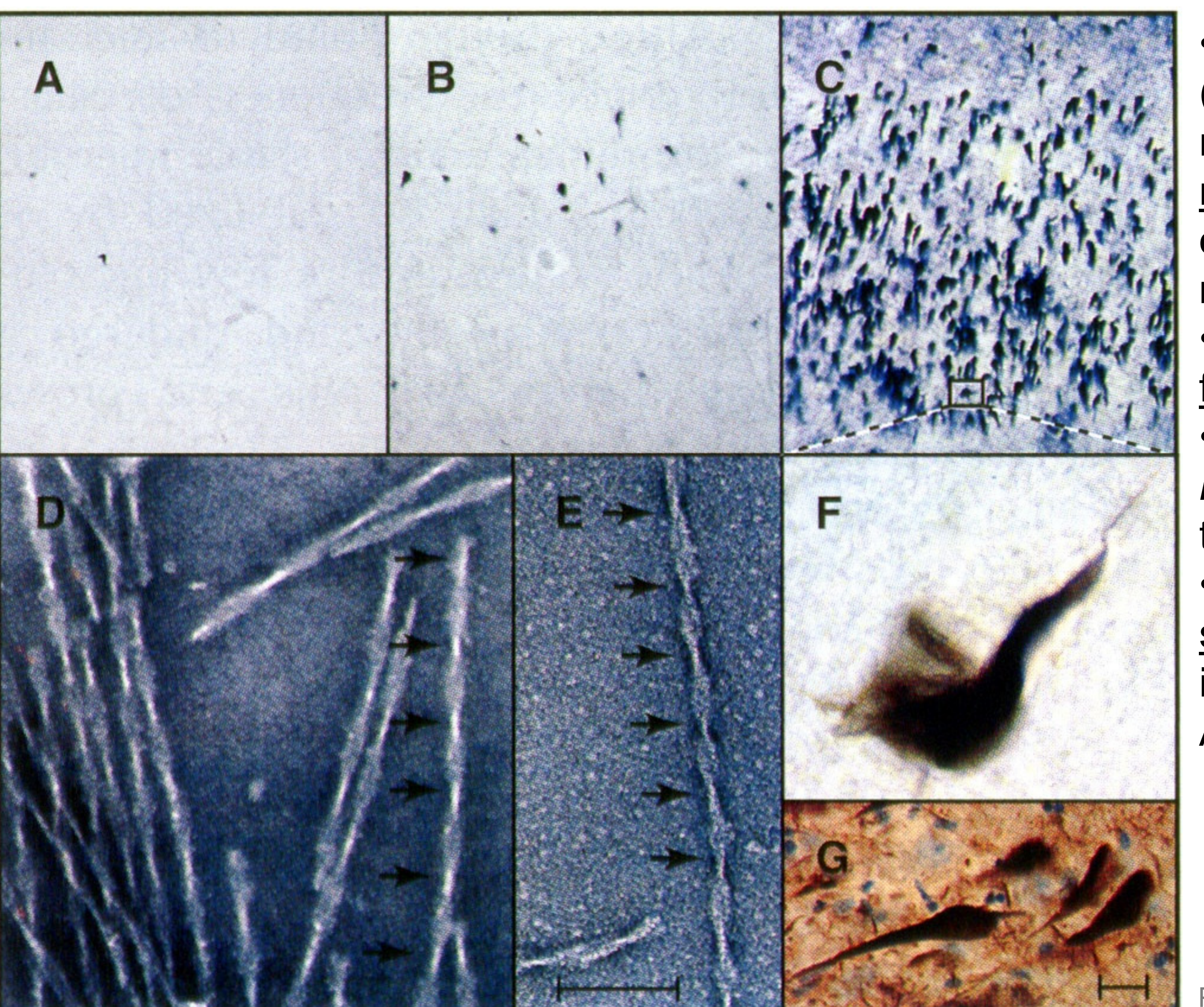


The **basic** MT binding motifs are **dynamic** and rapidly change positions on the tubulin subunits

Pollard 3rd ed

Tau is associated with Alzheimer's disease

- Tau is associated with several neurodegenerative diseases: **tauopathies**
- In **Alzheimer's**, fibrous aggregates composed of tau appear in pathogenic neurons



- A-C: three stages (I, II and V) in Alzheimer's disease show neurofibrillary tangles of **paired helical filaments** in the brain
- D: Isolated helical filaments in EM
- E: polymerized **tau** *in vitro* (looks similar to helical filaments)
- F+G: Antibody staining against **tau** in the brain of Alzheimer's patients

Pollard 3rd ed

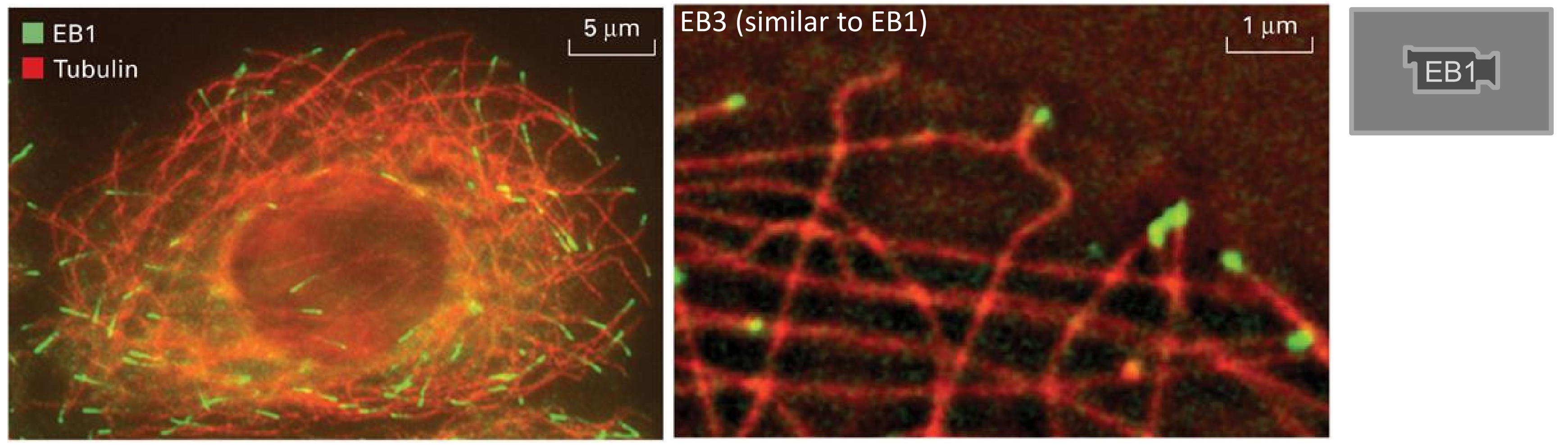
Lateral binding MAPs

- **MAP1** and **MAP2** are large, filamentous molecules mostly found in neurons
- MAP2 is exclusively found in dendrites (**can be used as a marker**)
- **MAP4** is the most widespread MAP which regulates MT stability in mitosis
- **Tau** boosts MT polymerization and stabilizes MTs
- **CLIP170** connects MTs to chromosomes during mitosis
- MAP-binding to MT is controlled by phosphorylation via **MAPK** (MAP kinases):
    - **Phosphorylation** of MAPs inhibits their binding and **promotes MT *instability***
    - **Dephosphorylation** of MAPs increases their binding and ***stabilizes* MT**
- MAP4 is phosphorylated by **CDK** (cyclin-dependent kinase)
- Tau is regulated by **MARK/Par-1** (*m*icrotubule-*a*ffinity *r*egulating *k*inase)

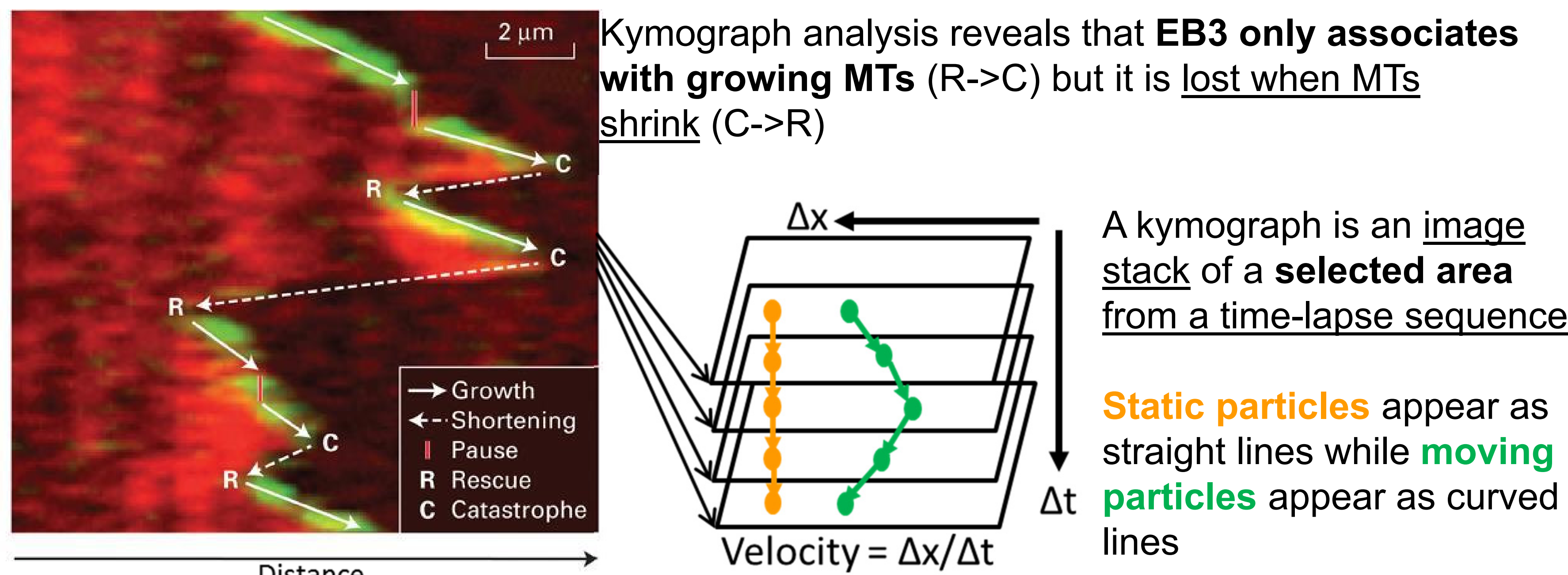
Protein	MW	Location	Function
MICROTUBULE-STABILIZING PROTEINS			
MAP1	250,000–300,000 (heavy chain)	Dendrites and axons; non-neuronal cells	Assembles and stabilizes MTs
MAP2	42,000 and 200,000	Dendrites	Assembles and cross-links MTs to one another and to intermediate filaments
MAP4	210,000	Most cell 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

MAPs that bind to the plus-end of MTs are called +TIPs

- A major +TIP (plus-end ***t***rack***i***ng ***p***rotein) is EB1 which recognizes a specific structure in the (blunt) growing end of MTs
- +TIPs not only stabilize MTs but they also link MTs to membranes, F-actin or chromosomes



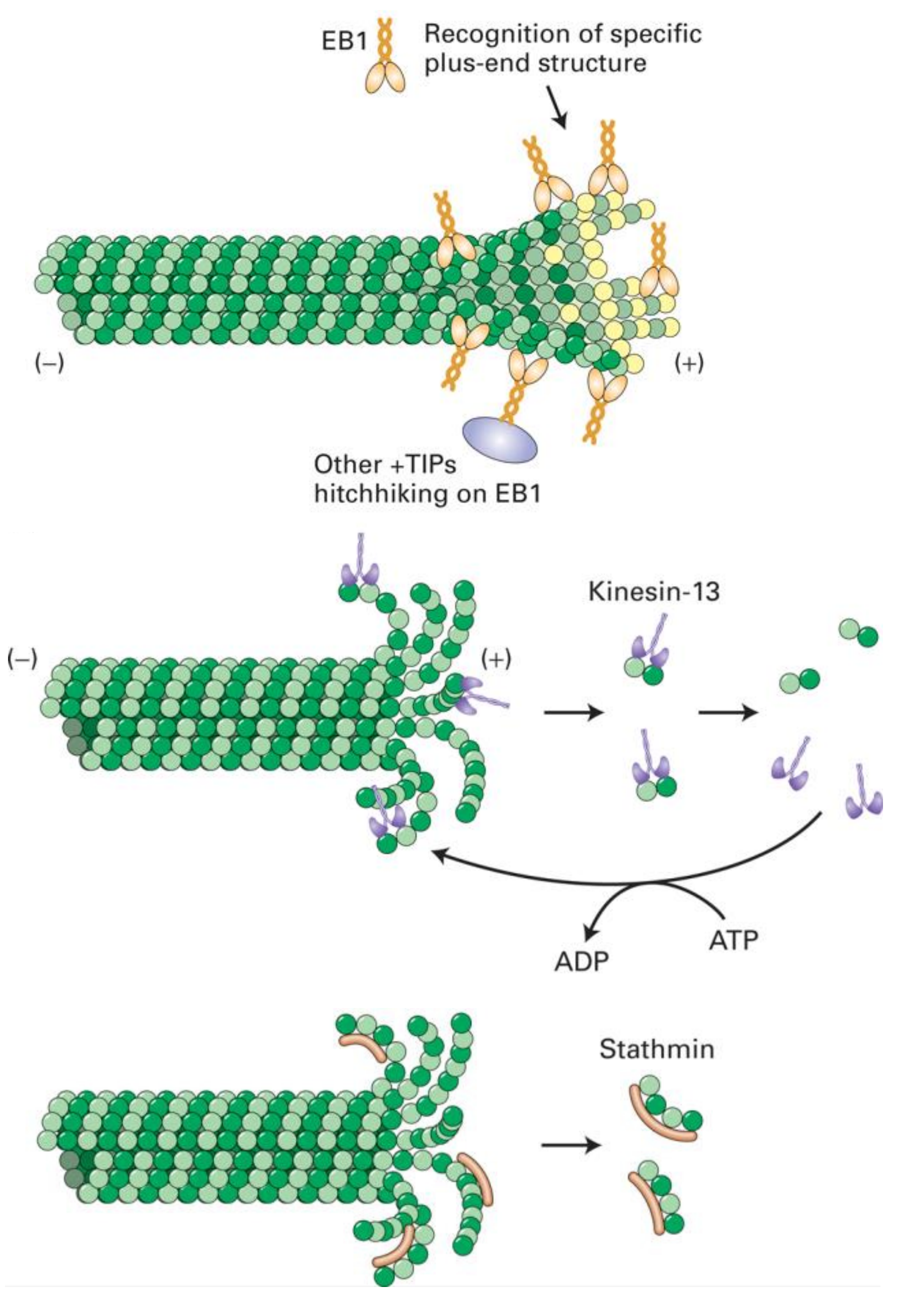
Kymograph analysis reveals that **EB3 only associates with growing MTs** (R->C) but it is lost when MTs shrink (C->R)



A kymograph is an image stack of a **selected area** from a time-lapse sequence

Static particles appear as straight lines while moving particles appear as curved lines

Microtubule-end stabilizing and destabilizing proteins



Stabilizing proteins

- EB1/EB3 are the major plus-end stabilizing proteins
- **Other +TIPs** usually have to first bind to EB1 (hitchhiking)

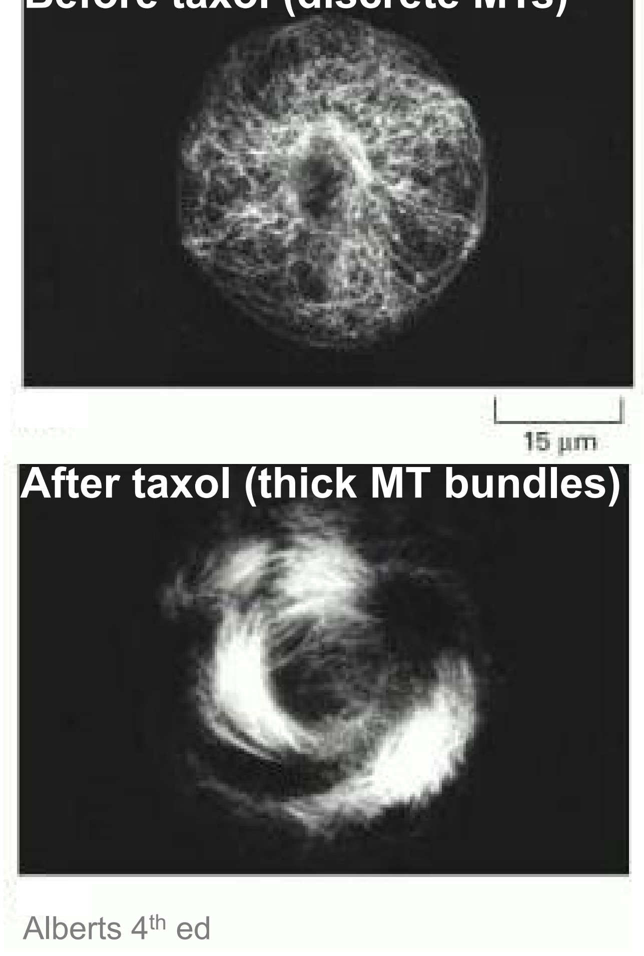
Destabilizing proteins

- **Kinesin-13** are ATPases that bind to both ends of MTs
- Kinesin-13 bind and curve the MT end into the characteristic GDP-β-tubulin conformation (thereby destabilizing it)
- They then **remove tubulin dimers** and the dissociation from the dimer is regulated by ATP hydrolysis

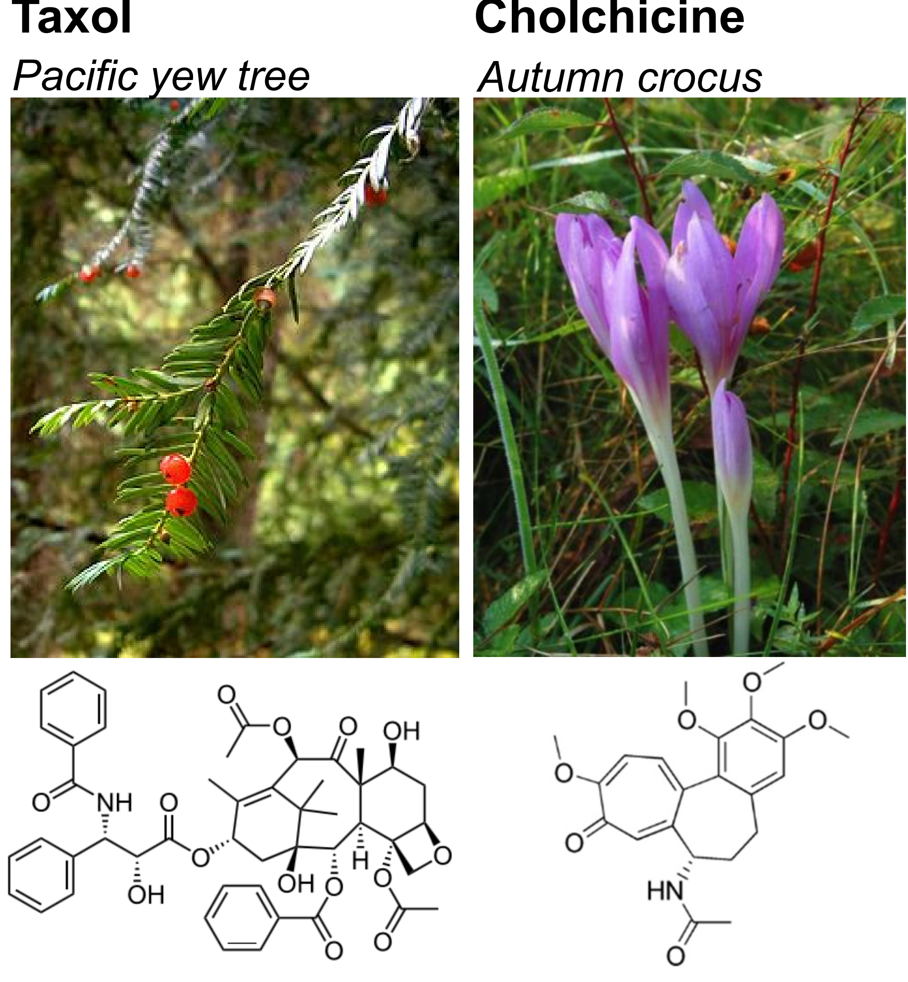
- **OP18/stathmin** is also a destabilizing protein and was first discovered in cancers (OP18, oncoprotein 18)
- It binds two tubulin dimers and when dephosphorylated it is deactivated

Microtubule stabilizing and destabilizing drugs

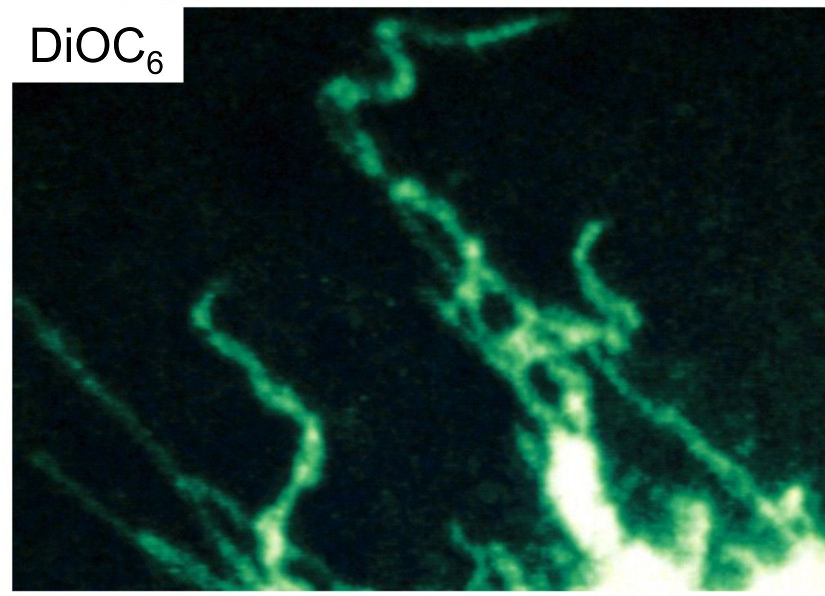
- **Taxol** prevents MT from depolymerization thus “freezes” fast growing tumors at metaphase during mitosis (mostly used to **treat breast and ovarian cancer**)
- **Colchicine** or **colcemid** facilitates MT depolymerization / effect on tumor cells is similar since mitosis is also impeded here (mitotic inhibitors) / also treatment of gout



Natural sources and chemical formulas:



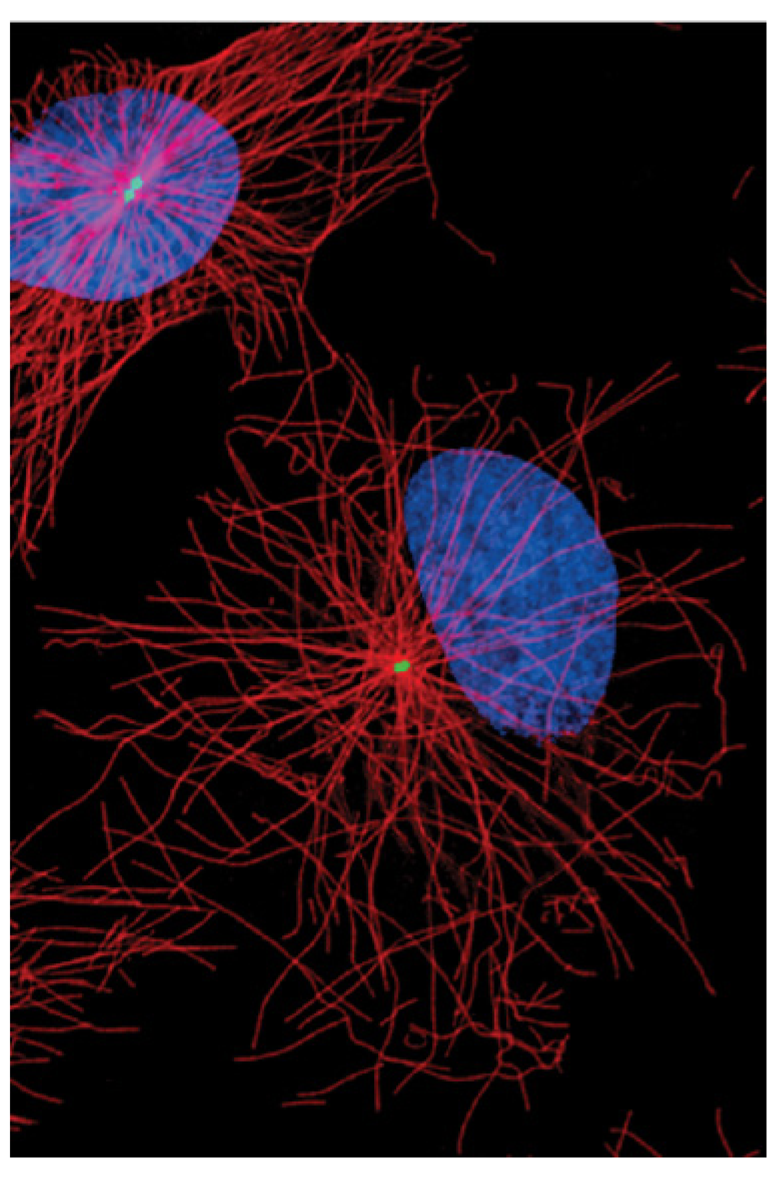
Microtubules organize position and structure of the ER and Golgi



- A broad mass of the <u>ER is directly associated with MTs</u>
- If MTs are depolymerized with <u>colchicine</u> then the ER also <u>loses</u> its organized <u>structure</u>
- Similarly, the <u>Golgi closely associates with MTs</u>
- During **mitosis,** when MT depolymerize, also the **Golgi breaks down** in several tubular-like vesicles
- <u>After mitosis</u>, when cytoplasmic MT reform, the <u>Golgi reassembles</u> to the typical tubular-like network

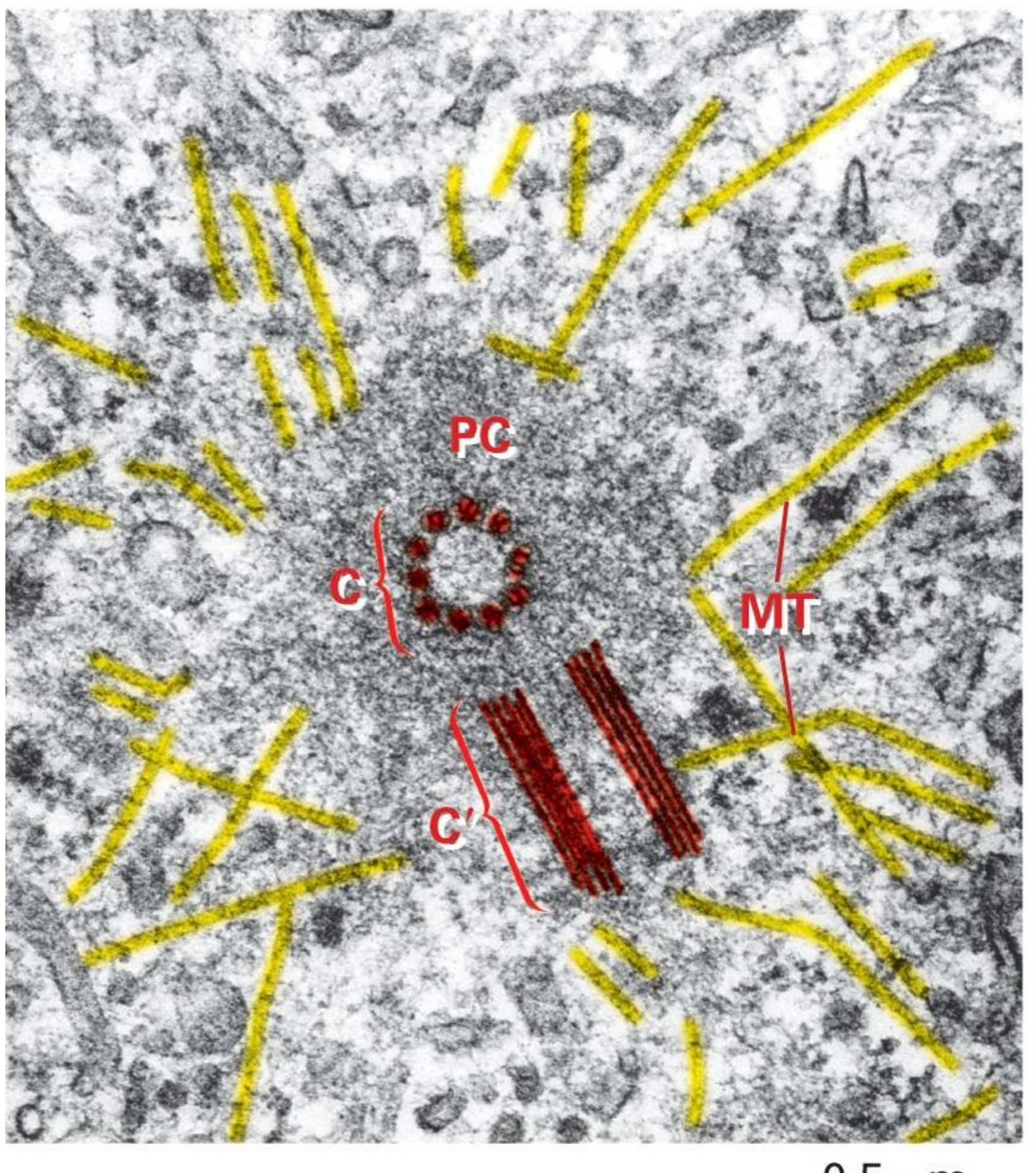
DiOC$_6$ is a fluorescent dye which **specifically stains** the **ER**

Microtubule organizing centers (MTOCs)



- MT polymerization is a rather **unfavorable reaction** and spontaneous nucleation rarely occurs in cells
- Hence, MTs **nucleate from specific structures** known as **MTOCs** (microtubule organizing centers)
- In interphase cells, the MTOC is the **centrosome** (near the nucleus) which contains a pair of **centrioles**
- MTs grow plus-end out from MTOCs towards the cell border
- This orientation is critical for vesicle transport (**kinesins** => **exocytotic vesicles**, **dynein** => **endocytotic vesicles**)
- At the cell border MTs often **bend** and undergo growth and shrinkage
- Plants do not have centrosomes or centrioles

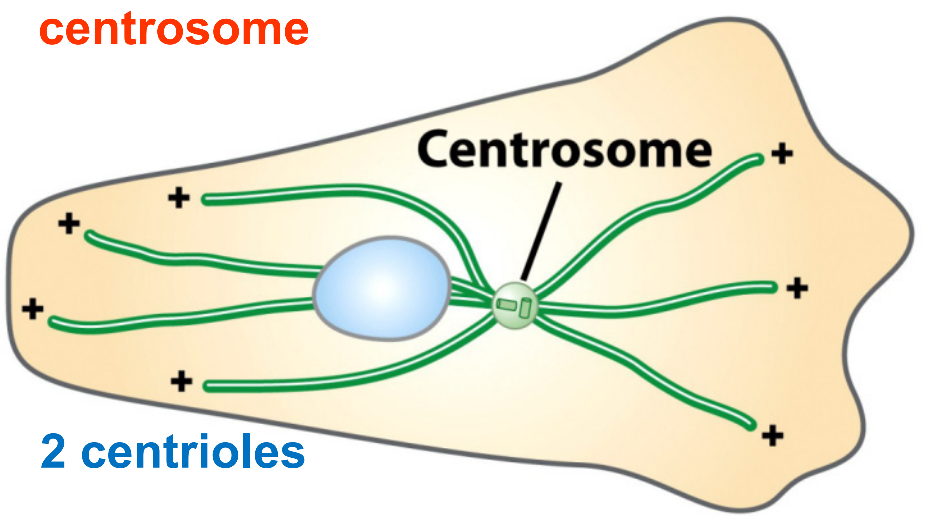
The centrosome is a complex MTOC containing various proteins



- **2 Centrioles** = part of the centrosome => each composed of **9 triplet MTs** arranged in a ring
- Centrioles do not directly bind MT
- The two centrioles are **orthogonally aligned**, thus, one centriol appears in cross section and the other longi-tudinal
- Part of the centrosome is the peri-centriolar matrix (PCM) containing **PCM1**, **pericentrin** and **γ-tubulin**
- Centrioles are thought to organize the PCM (to bring the matrix into a T-shaped and 3D type structure)

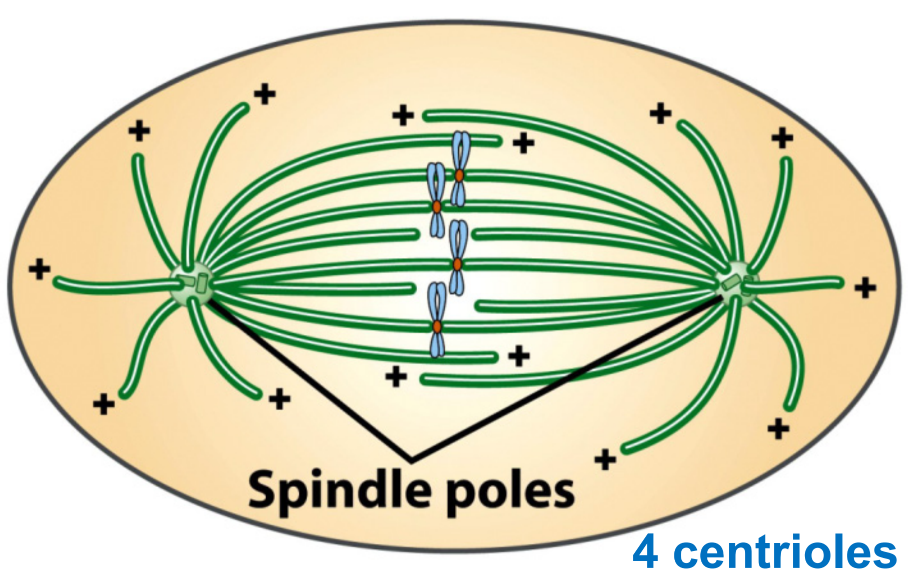
MTOCs in interphase and mitotic cells

MTOC in an <u>interphase cell</u> is called **centrosome**



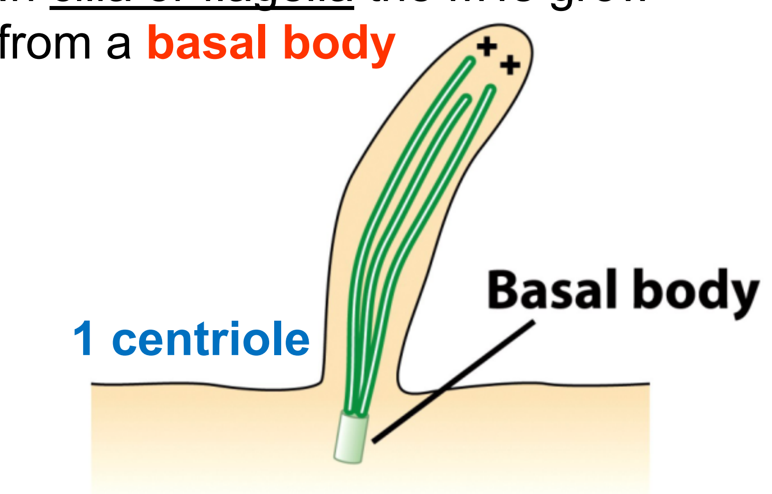
2 centrioles

The two (duplicated) MTOCs in the <u>mitotic cell</u> are called **spindle poles**



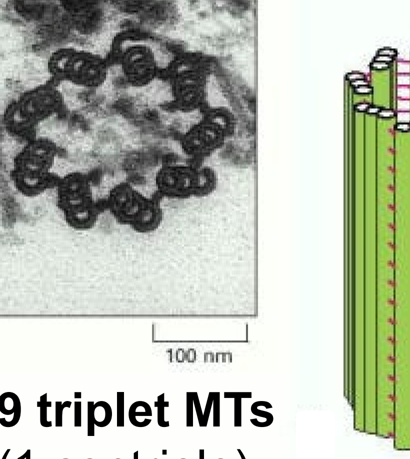
4 centrioles

In <u>cilia or flagella</u> the MTs grow from a **basal body**



1 centriole

Basal body (EM)



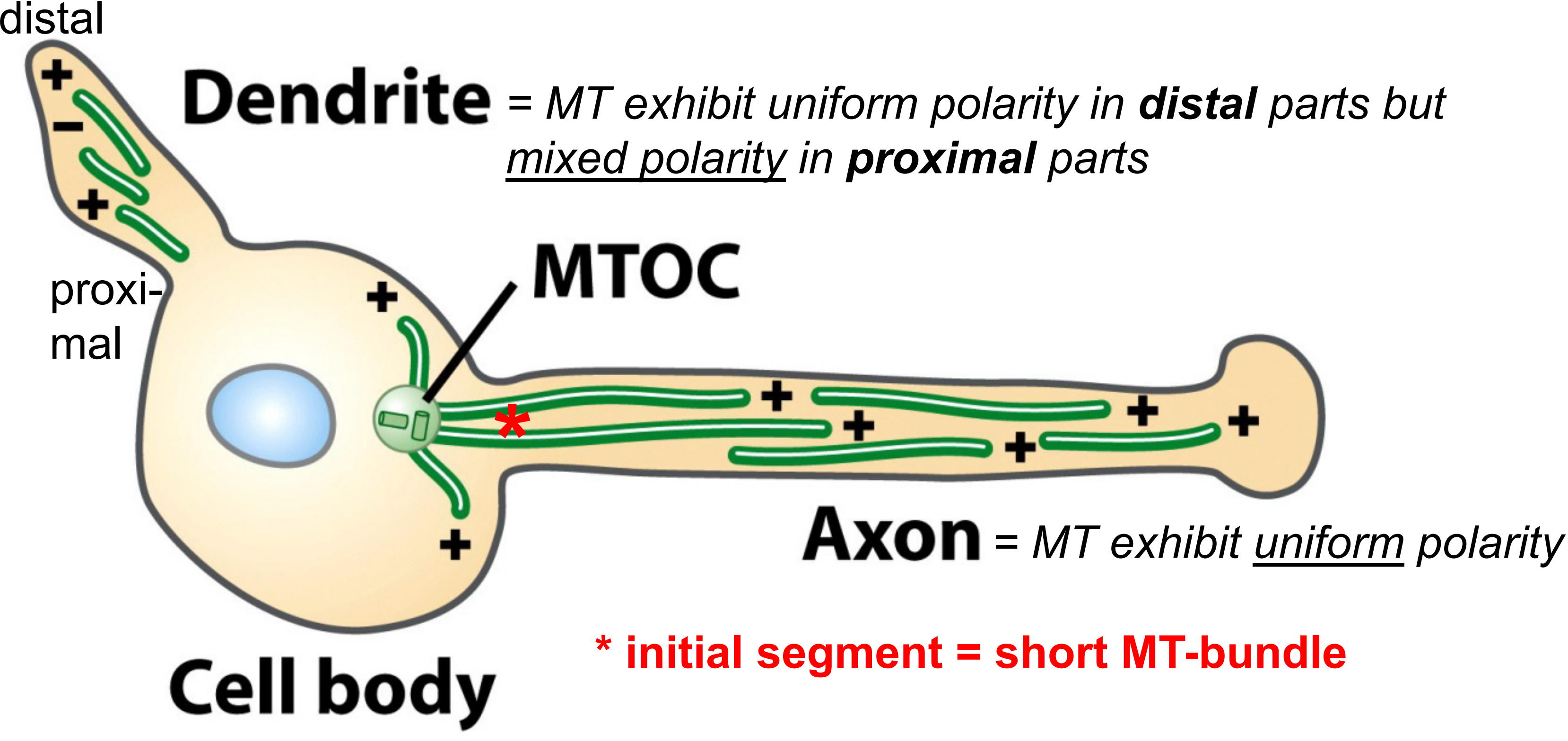
9 triplet MTs
(1 centriole)

A B C

Alberts 4th ed

Basal body is an MTOC composed of <u>*one* centriole</u>

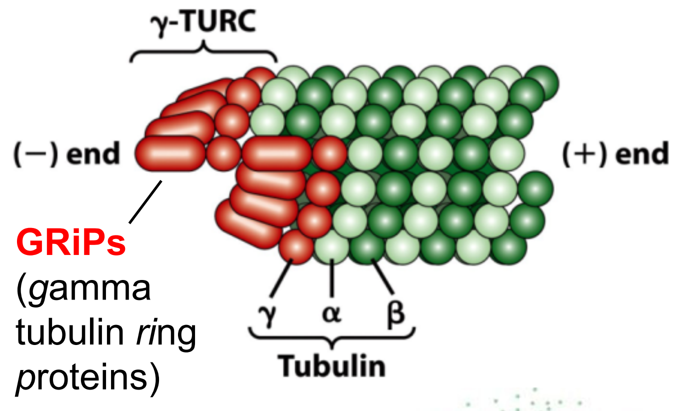
MTOCs in neurons

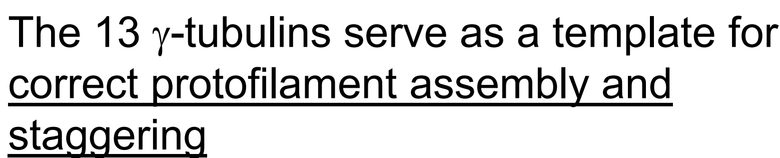


- No basal bodies at the base of dendrites or axons
- MTs grow from an MTOC in the cell body and are then released into the dendrites or axons

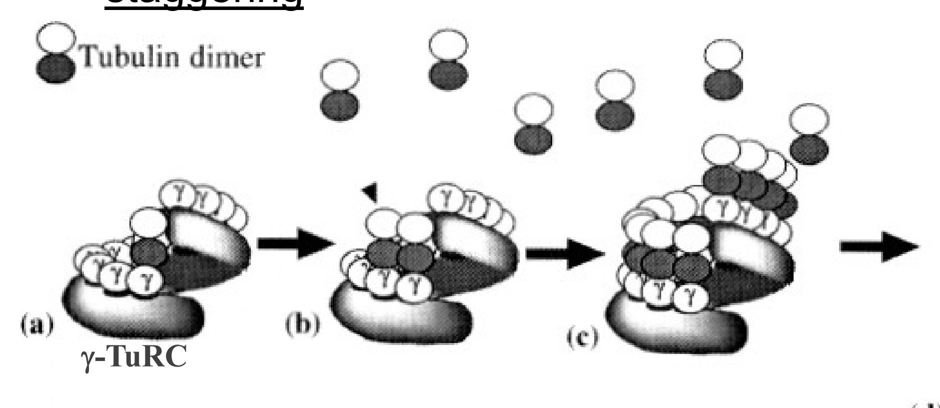
The γ-tubulin ring complex (γ-TuRC) is part of the centrosome and nucleates MT polymerization

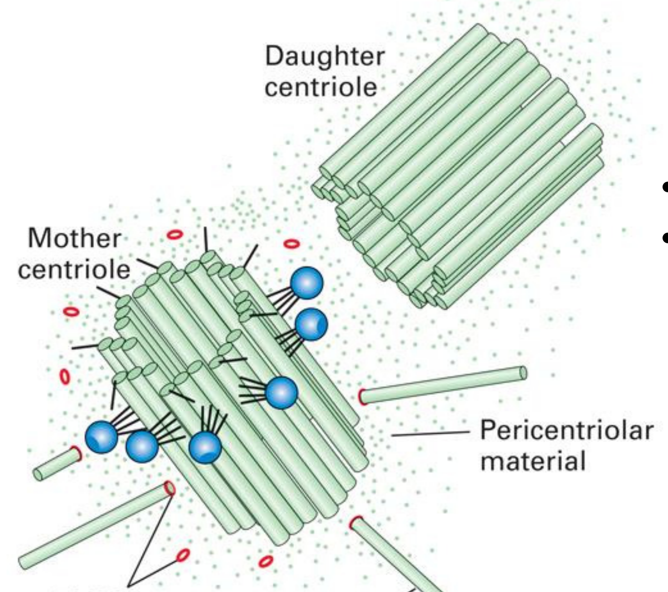
The **γ-tubulin ring complex** (γ-TuRC) is a ring-like structure that <u>facilitates tubulin polymerization below the Cc</u>





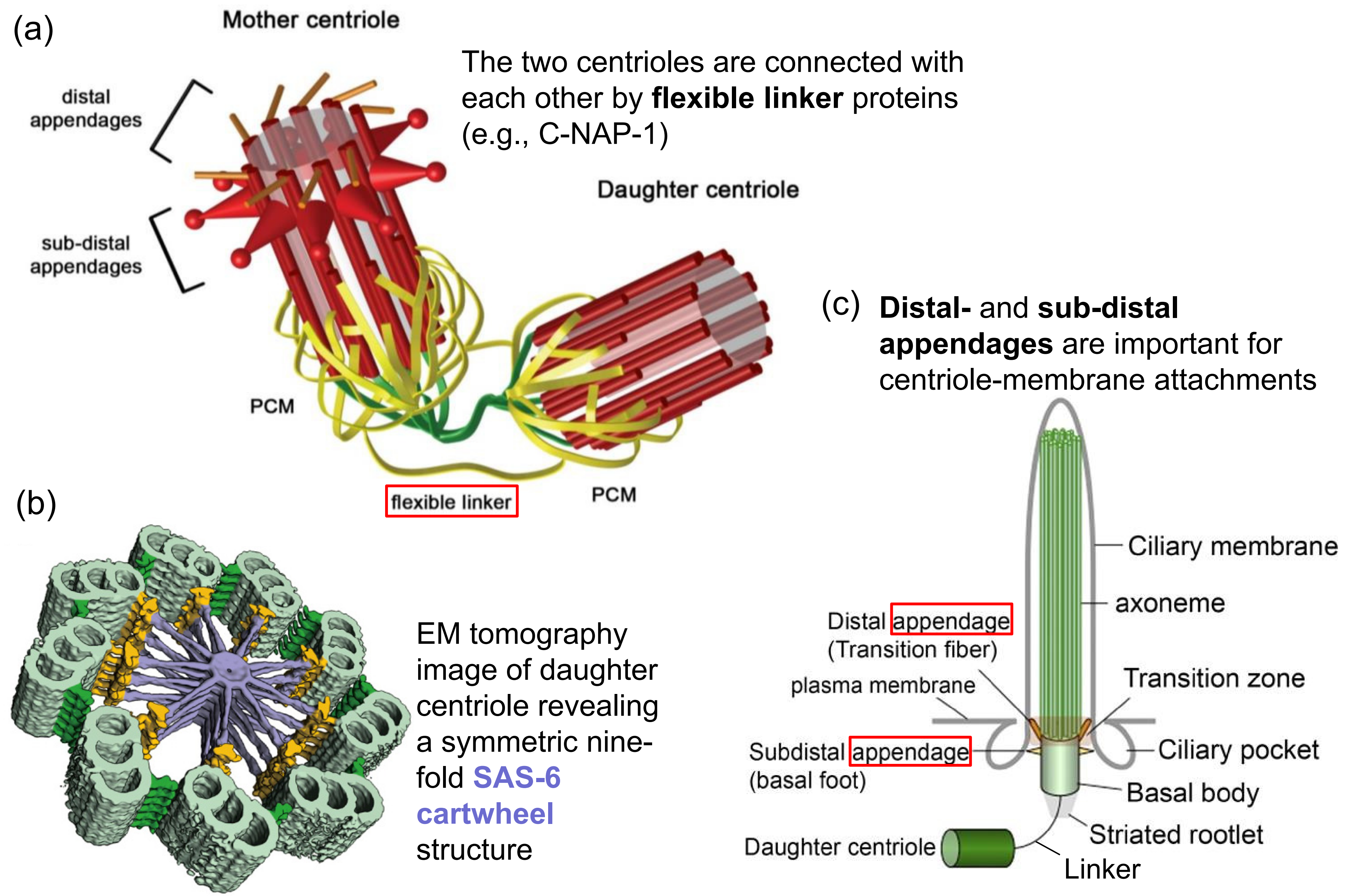
The 13 γ-tubulins serve as a template for <u>correct protofilament assembly and staggering</u>





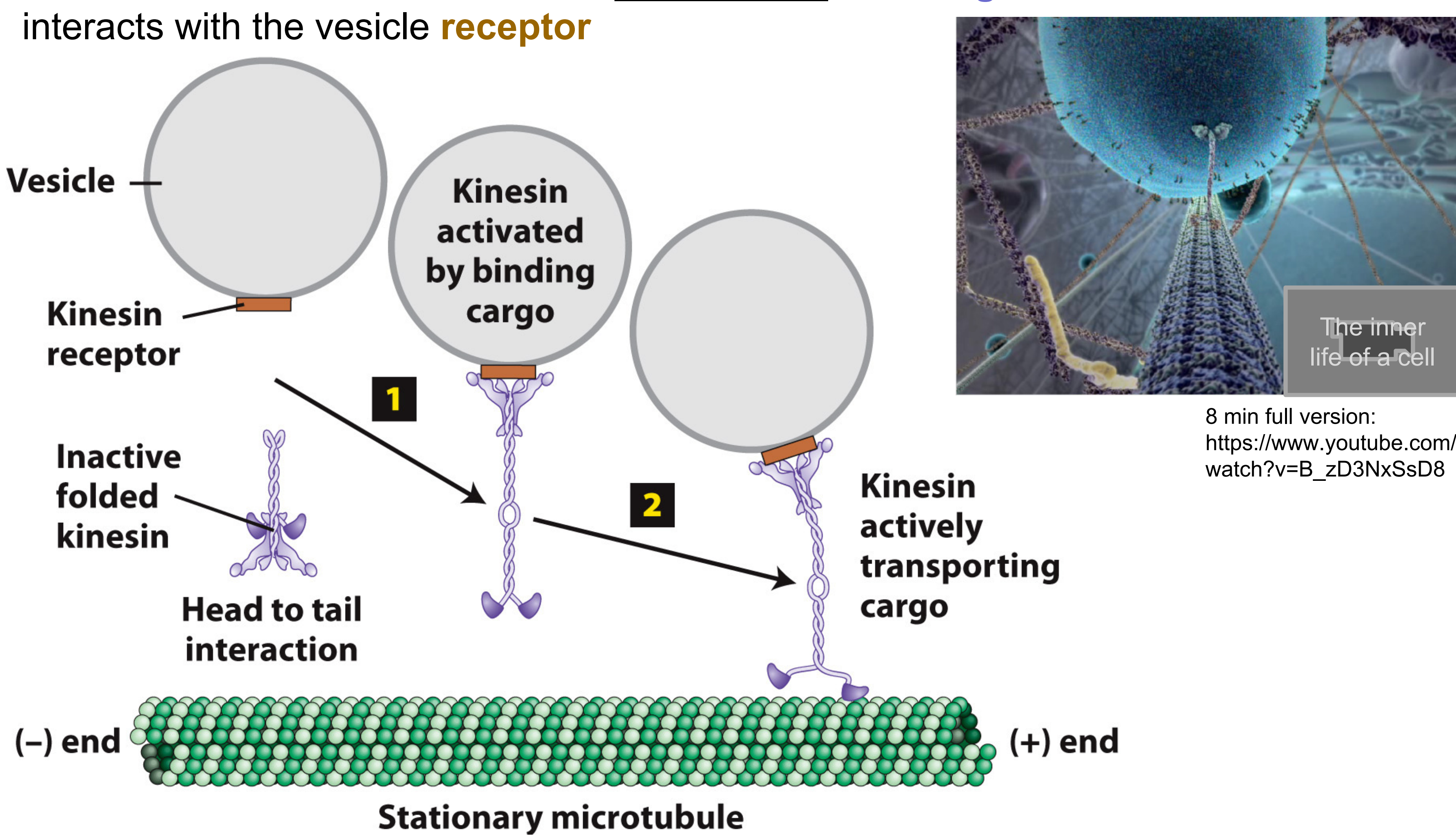
- Lots of γ-TuRC can be <u>found in the PCM</u>
- In contrast to the daughter centrioles, the mother centrioles has **appendages** attached (serving as membrane anchoring sites)

Centrioles are linked to each other and appendages connect basal body to ciliary membrane



Model of kinesin-based vesicle transport

- Kinesins are usually deactivated in cells via intramolecular folding
- Intramolecular folding is released when kinesin binds to a cargo (e..g, vesicle)
- **Globular *motor* domain** binds to microtubules and the **globular *tail* domain** interacts with the vesicle **receptor**



8 min full version: https://www.youtube.com/watch?v=B_zD3NxSsD8

The variety of kinesin structure and functions

Kinesin-1 (conventional)
Kinesin-2 (heterotrimeric)
Organelle transport
(–)
(+)
*

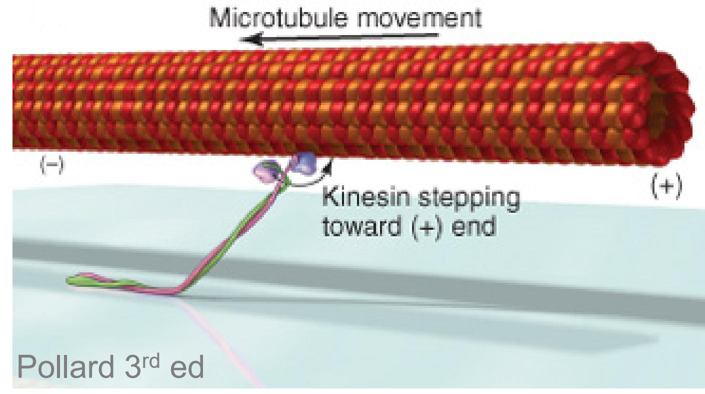
**
Kinesin-5 (bipolar)
(–)
(+)
Sliding
(+)
(–)

Kinesin-13
End disassembly
(–)
(+)

(total 14 classes;
only selected shown)

Two major functional groups

- Cytosolic kinesins:
  - **Kinesin-1** (conventional) transports various organelles (lysosomes, mitochondria, RNA ganules)
  - **Kinesin-2** (heterotrimeric) is a dendritic motor
  - **Kinesin-3** (monomer inactive; dimer active) transports synaptic vesicles
- Mitotic kinesins:
  - **Kinesin-4**: links chromosome *arms* to polar MTs
  - **Kinesin-5**: (bipolar) spindle pole separation via MT sliding
  - **Kinesin-7**: links chromosome *centromeres* to kinetochore MTs
  - **Kinesin-13**: (no motor activity) depolymerizes kinetochore MTs

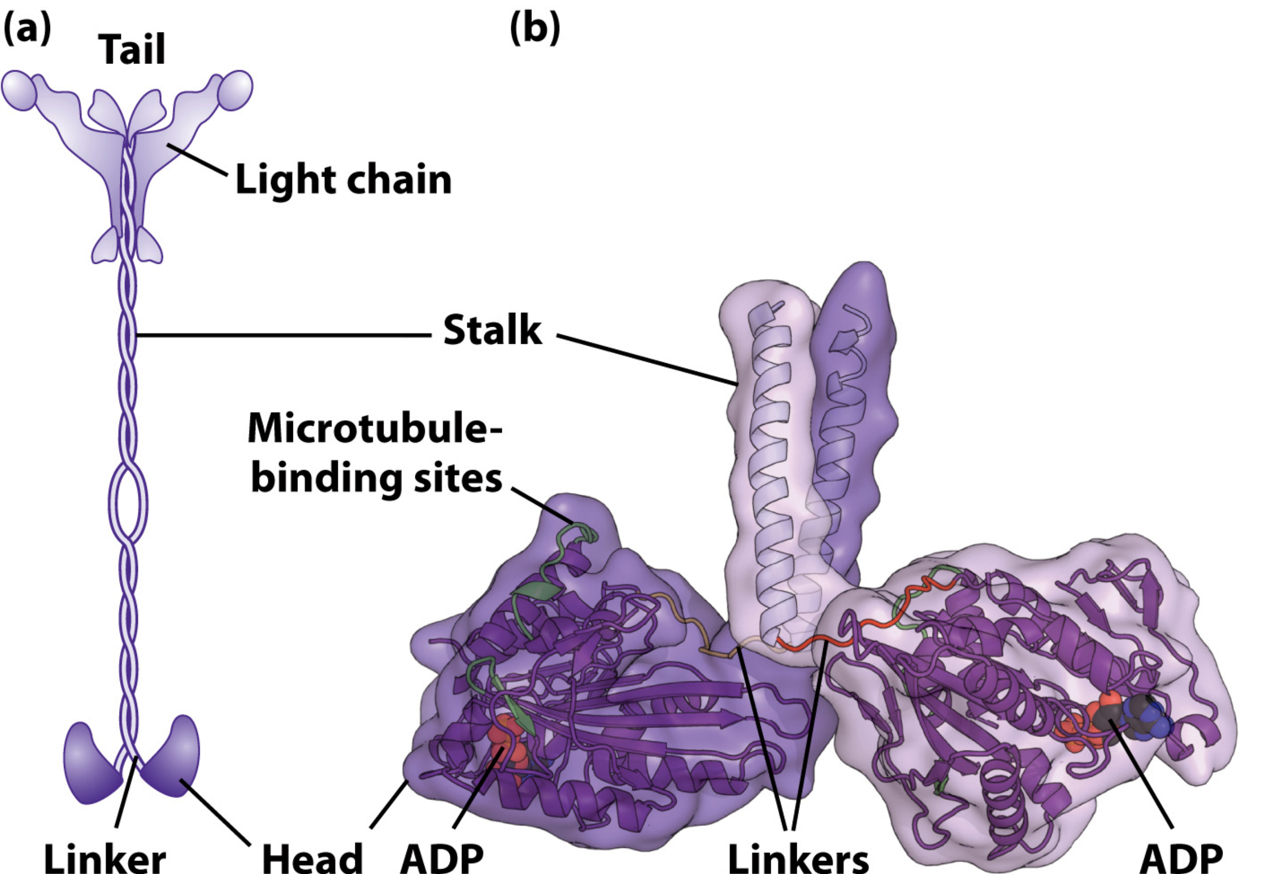


* **MT fixed** = organelle moving into plus-direction

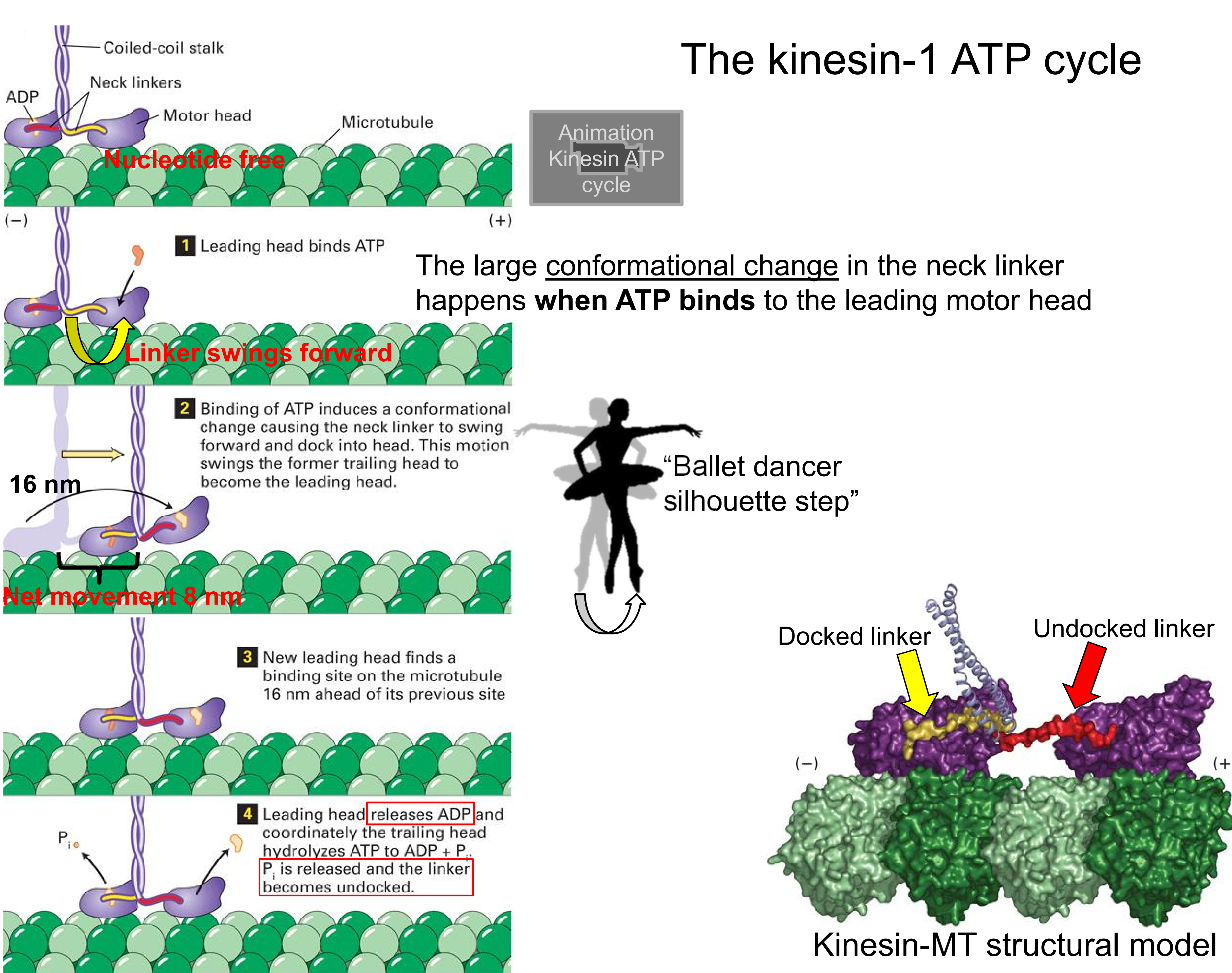
** **MT free** = kinesin push MT into minus-direction

Structure and function of Kinesin-1

- **Kinesin-1** (or "conventional kinesin") is a dimer of 380 kDa composed of: **two heavy chains** (110-135 kDa) and **one** associated **light chain** (60-70 kDa)
- The **head domain** binds to the microtubule and converts chemical energy (from ATP hydrolysis) into mechanical energy (to move along the MT)
- The **tail domain** binds to the cargo via adaptor proteins
- Each head is connected via a **neck-linker** to an α-helical stalk (coiled-coil)
- **MT interaction** (green helix) is regulated by the **nucleotide** (red) at the opposite site



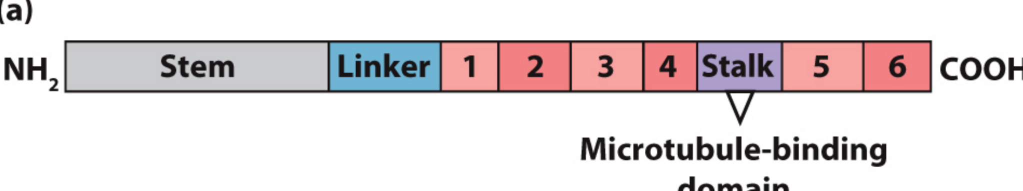
The ATP-binding pocket has the same structure as myosin's => Evolution must have twice generated the same structure

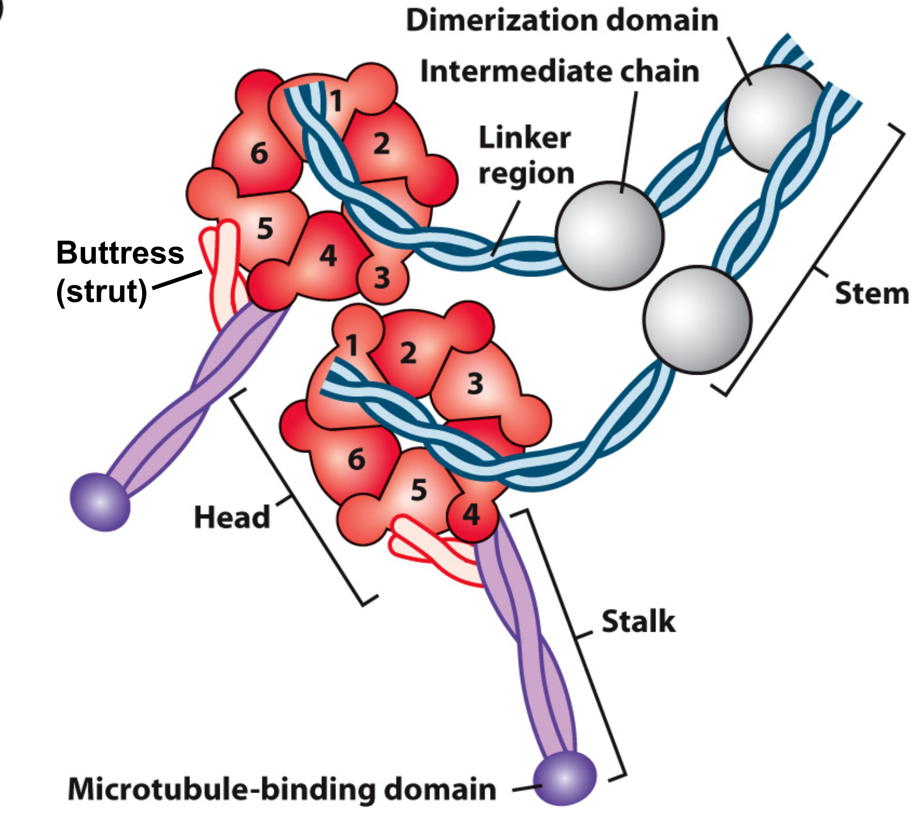


The kinesin-1 ATP cycle
Coiled-coil stalk
Neck linkers
ADP
Motor head
Microtubule
Nucleotide free
(−)
(+)
Animation
Kinesin ATP
cycle
1 Leading head binds ATP
Linker swings forward
The large conformational change in the neck linker
happens when ATP binds to the leading motor head
2 Binding of ATP induces a conformational
change causing the neck linker to swing
forward and dock into head. This motion
swings the former trailing head to
become the leading head.
16 nm
Net movement 8 nm
"Ballet dancer
silhouette step"
3 New leading head finds a
binding site on the microtubule
16 nm ahead of its previous site
Docked linker
Undocked linker
(−)
(+)
4 Leading head releases ADP and
coordinately the trailing head
hydrolyzes ATP to ADP + Pi.
Pi is released and the linker
becomes undocked.
Pi
Kinesin-MT structural model

Dyneins are large minus-end directed motors

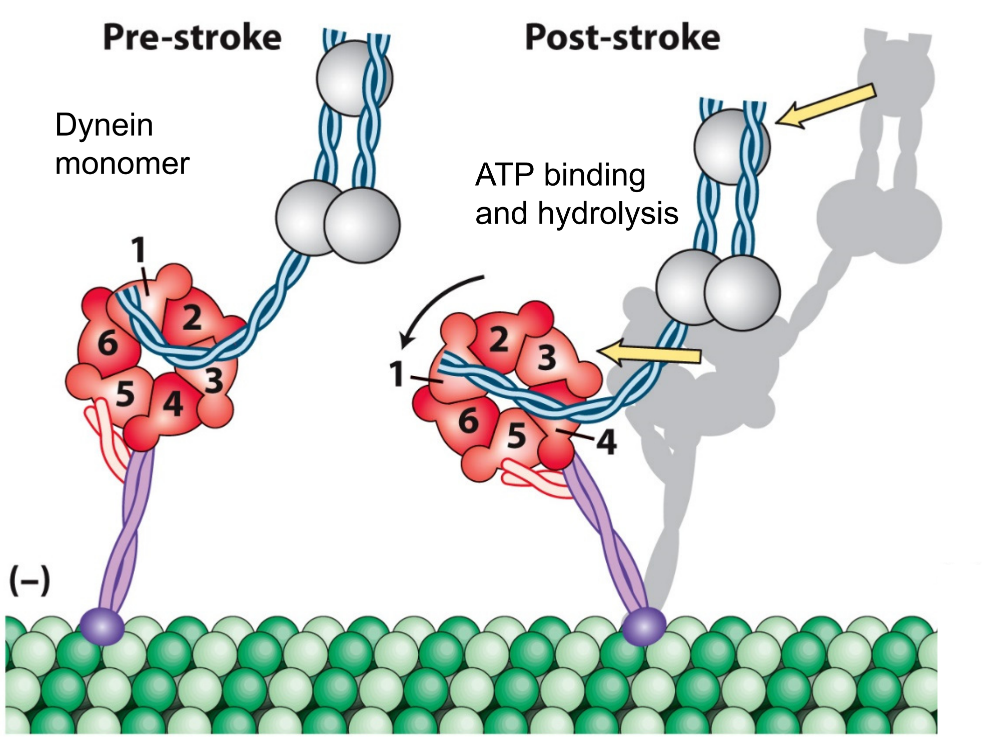
- **Dyneins** move cargo retrogradely in axons and Golgi vesicles in non-neuronal cells
- Dyneins are **large dimers** (>1 MDa) with each heavy chain carrying six **AAA ATPase** repeats (***A***TPases ***a***ssociated with cellular ***a***ctivities) as well as a **stem** and a **stalk**
- The **stalk** with the **MT binding domain** lies between the 4th and 5th AAA repeat
- Two functional classes exist: **cytosolic dynein** and **axonemal dynein** (cilia, flagella)





In the relaxed stated (pre-stroke), when ADP + P_i is bound, the **linker** associates with AAA1 and AAA3

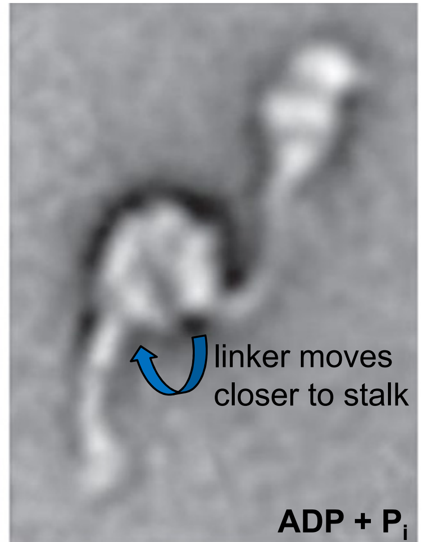
Dynein power stroke is mediated by a linker-to-AAA association



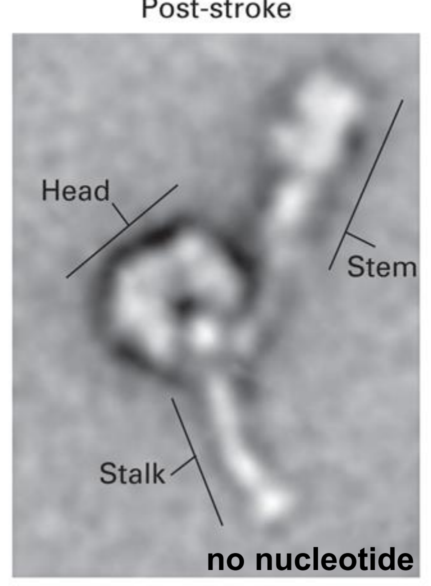
- During the power stroke the **linker** changes its **AAA1/AAA3** interaction **to** an **AAA1/AAA44** interaction
- The head is then tilted towards to the minus-end of the microtubule (in relation to the position of the stalk)
- Similar, the **stem** (with its cargo) would be then **brought closer to the stalk** (so the cargo would be moved towards the minus-end)

Dynein walks on MT like a drunken sailor

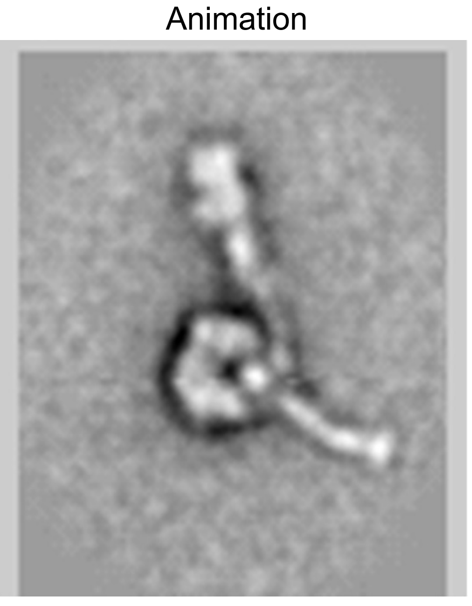
Pre-stroke



Post-stroke



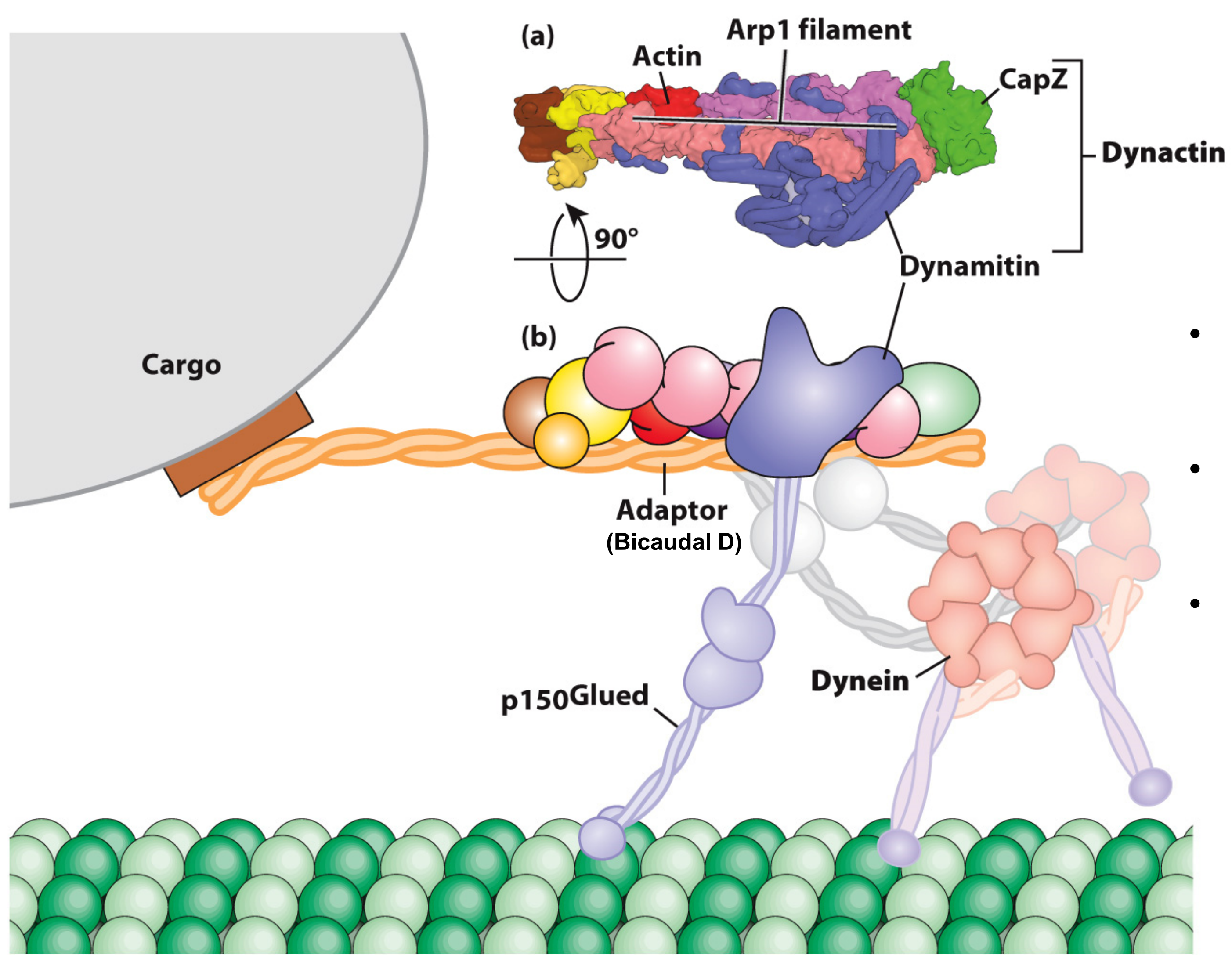
Animation



Averaged, multiple TEM images of dynein monomer (most right = stacked images)

Dynein exist in a complex with another huge protein

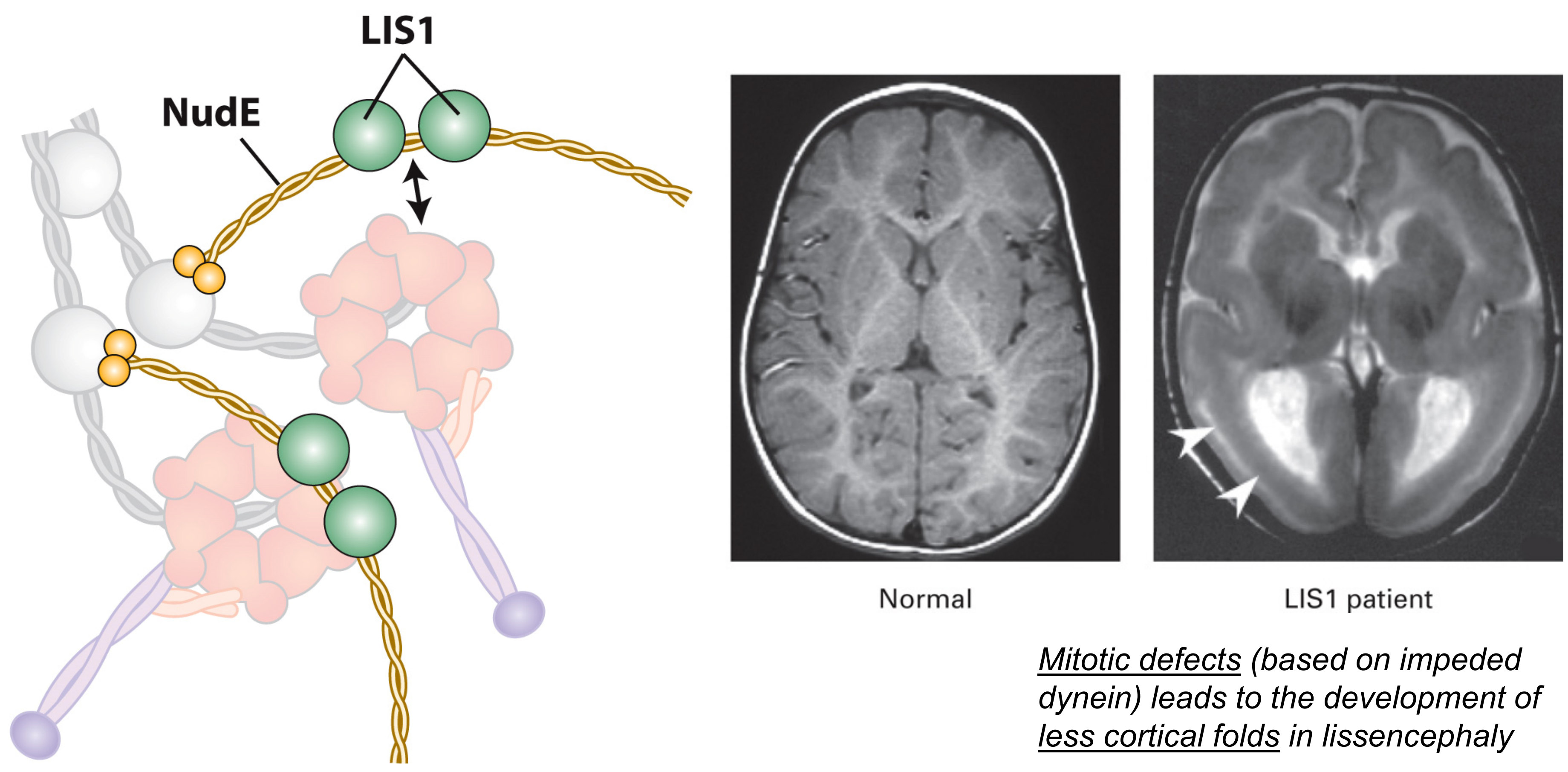
- Dynein can transport cargo only in conjunction with the adaptor protein **dynactin**
- Dynactin consists of 11 subunits including **p150^{Glued}**, **Arp1 polymer** and **dynamitin**
- **p150^{Glued}** has a MT binding site as well as a dynein binding site
- **Dynamitin** holds Arp1 and p150^{Glued} together
- The **Arp1 polymer** is **capped** with plus-end (CapZ) and minus-end capping proteins



- **Dynactin** is not actively moving but "skates" along the MT
- It stabilizes dynein, thus making the motor more processive
- **Dynein** is able to associate with EB1 when *inactive*. In that way it moves with growing MTs to the cell cortex. Arrived there it becomes active and moves towards the minus-end.

Dynein is regulated by LIS which is involved in brain diseases

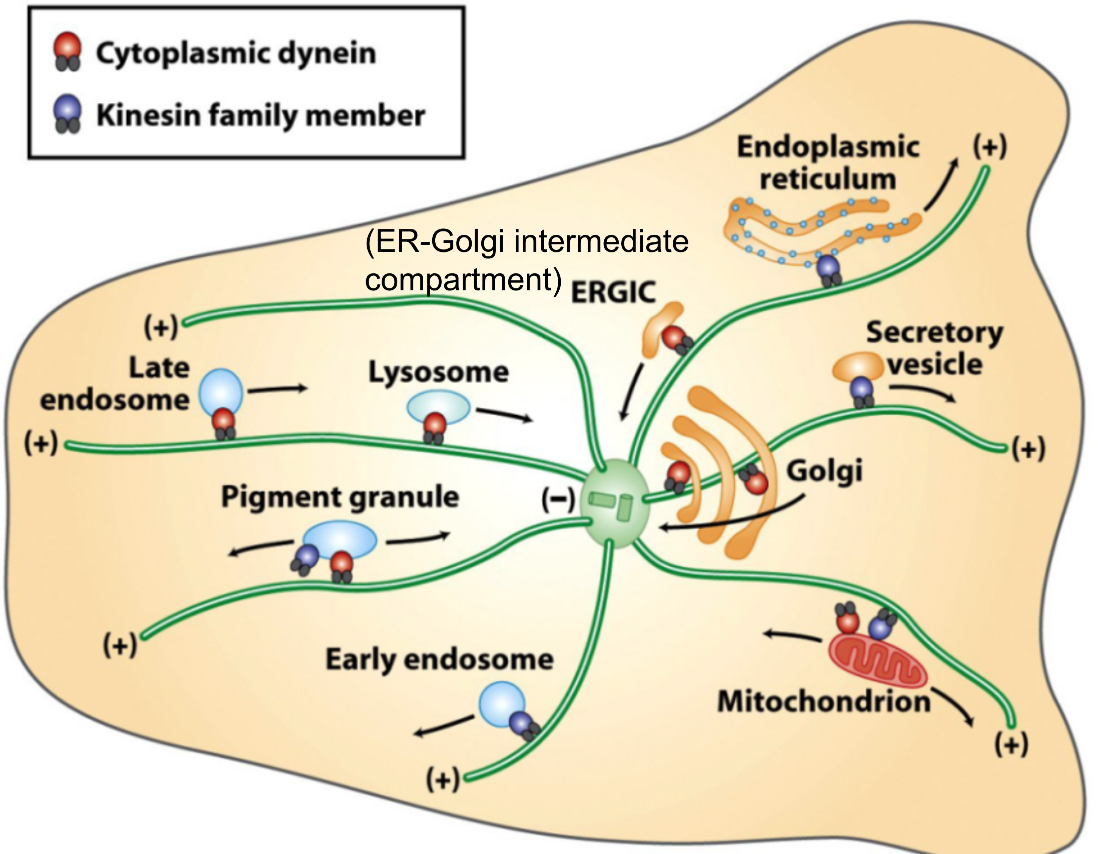
- NudE is a protein that associates with **dynein's IC** and **LC**
- Associated with NudE is **LIS** which can interact with the AAA resulting in **prolonged power strokes** and **increased dynein processivity**
- Defects in LIS cause **lissencephaly** (mental retardation) which leads to "smooth brain" structures



Mitotic defects (based on impeded dynein) leads to the development of less cortical folds in lissencephaly

Kinesin and dynein specialize and cooperate in cargo transport

The fixed orientation of the MTs from the MTOC leads to specialized transport mechanisms powered by specific type of motors



Dynein motor:

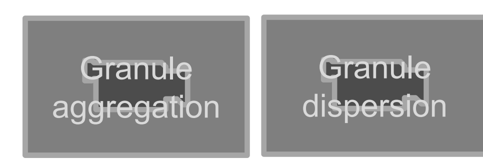
- Golgi *trans-cis* transport
- Lysosome transport

Kinesin motor:

- Secretory vesicles
- ER fragments

Dynein/kinesin **cooperative** transport:

- Mitochondria
- Pigment granules

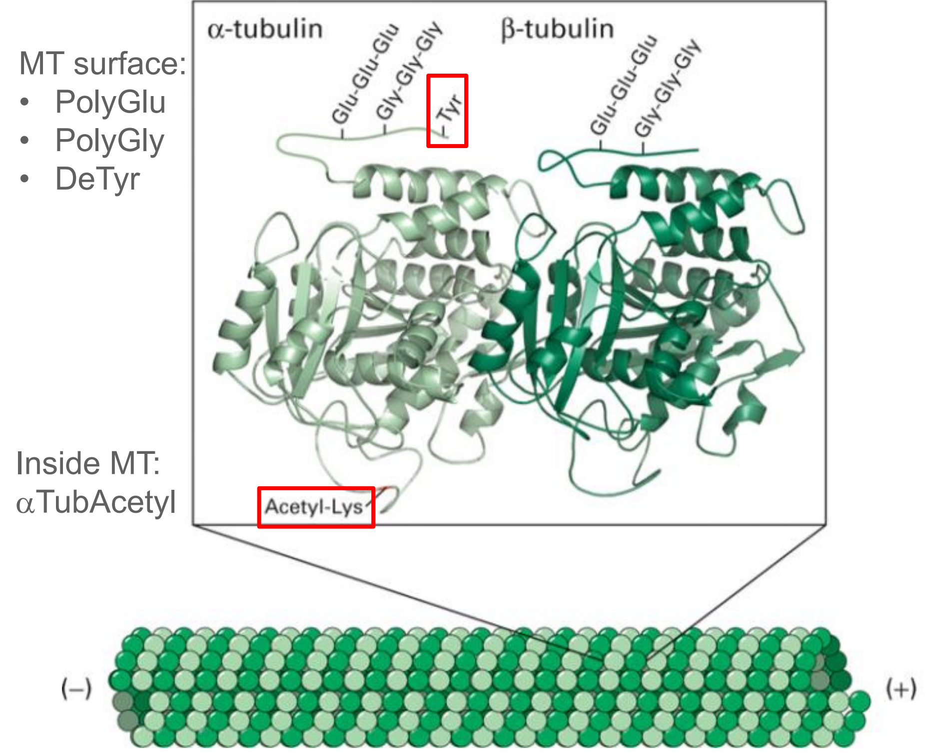


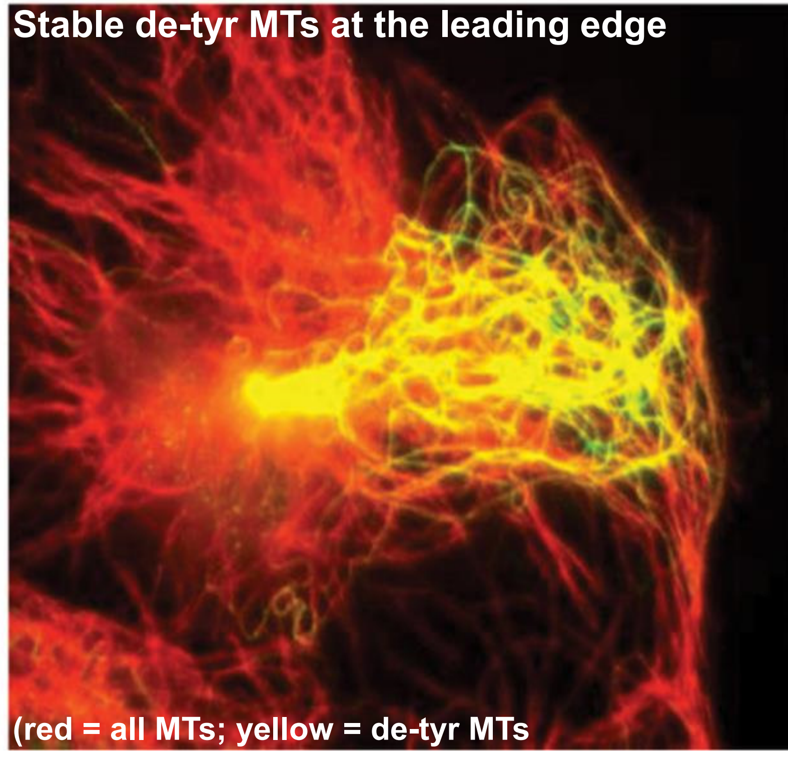
Fish skin perfused with hormone

- Animals (e.g. fish or frog) frequently change the skin color and brightness (camouflage, social interactions)
- This is accomplished by specialized skin cells named **melanophores**.
- They contain **pigment granules** that are fast transported in a **collaborative effort** by dynein and kinesin-2 (**high cAMP** = dispersed via kinesin = dark skin; **low cAMP** = centered via dynein = bright skin)

Post-translational modifications of tubulin control motor association

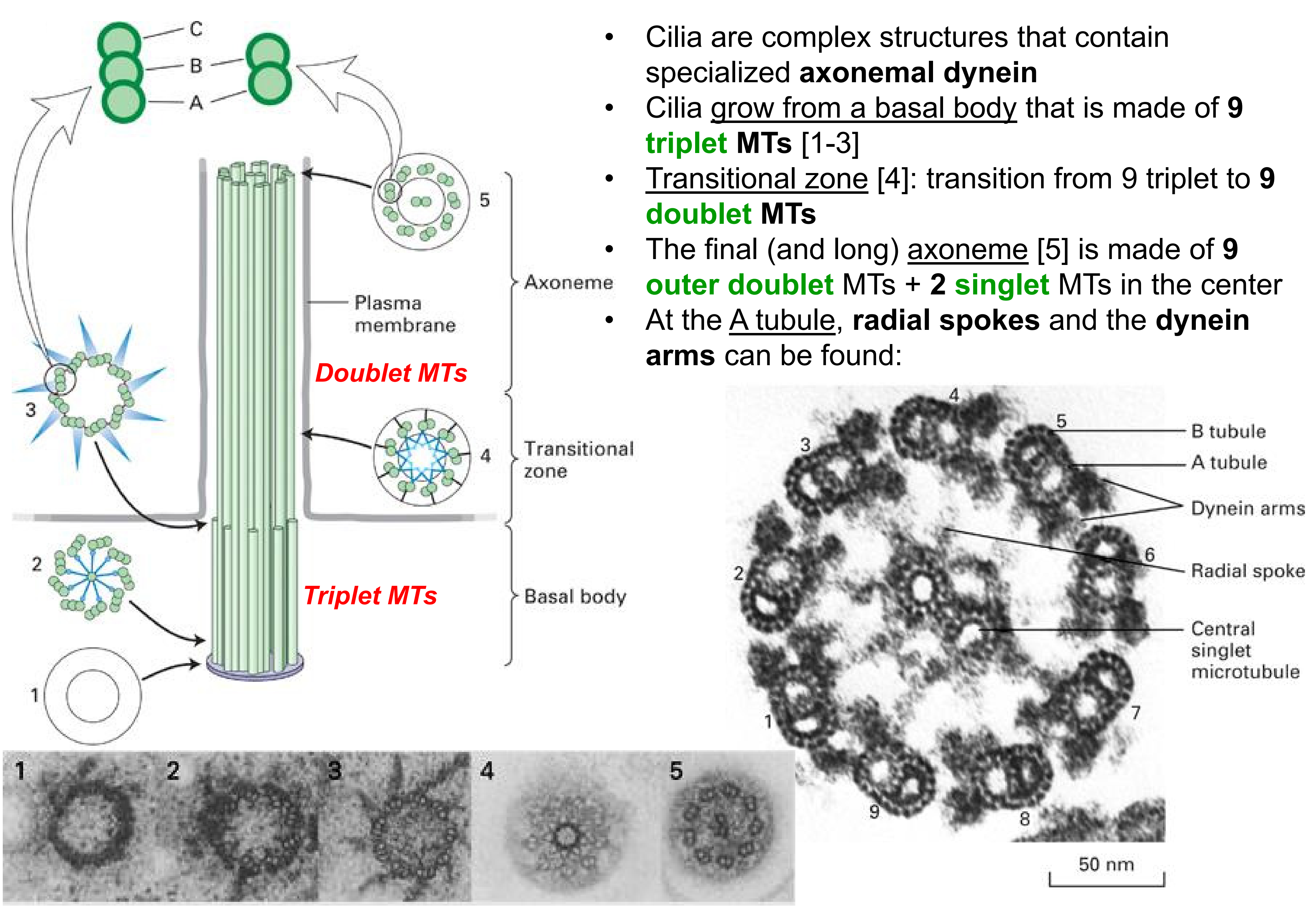
- **Post-translational modifications** regulate stability and function of microtubules
- Examples for posttranslational modifications are (always *after* polymerization):
  - Only **α-tubulin**: lysine **acetylation**, **detyrosynation**
  - Both **α- and β-tubulin**: **polyglutamylation**, **polyglycylation**
- Usually the effects are to stabilize microtubules:
  - **Acetylation** = long-lived and stable MTs (centrioles, basal bodies, cilia)
  - **Detyrosynation** = makes MTs more stable and more resistant to depolymerization by kinesin-13; these MTs are also found at the **leading edge of migrating cells**
  - **Polyglutamylation** and **polyglycylation** both make MTs more stable
- **Acetylation** and **detyrosynation** both stabilize neuronal MTs in **axons** and positively affecting the binding of kinesin-1, thus enhancing axonal transport



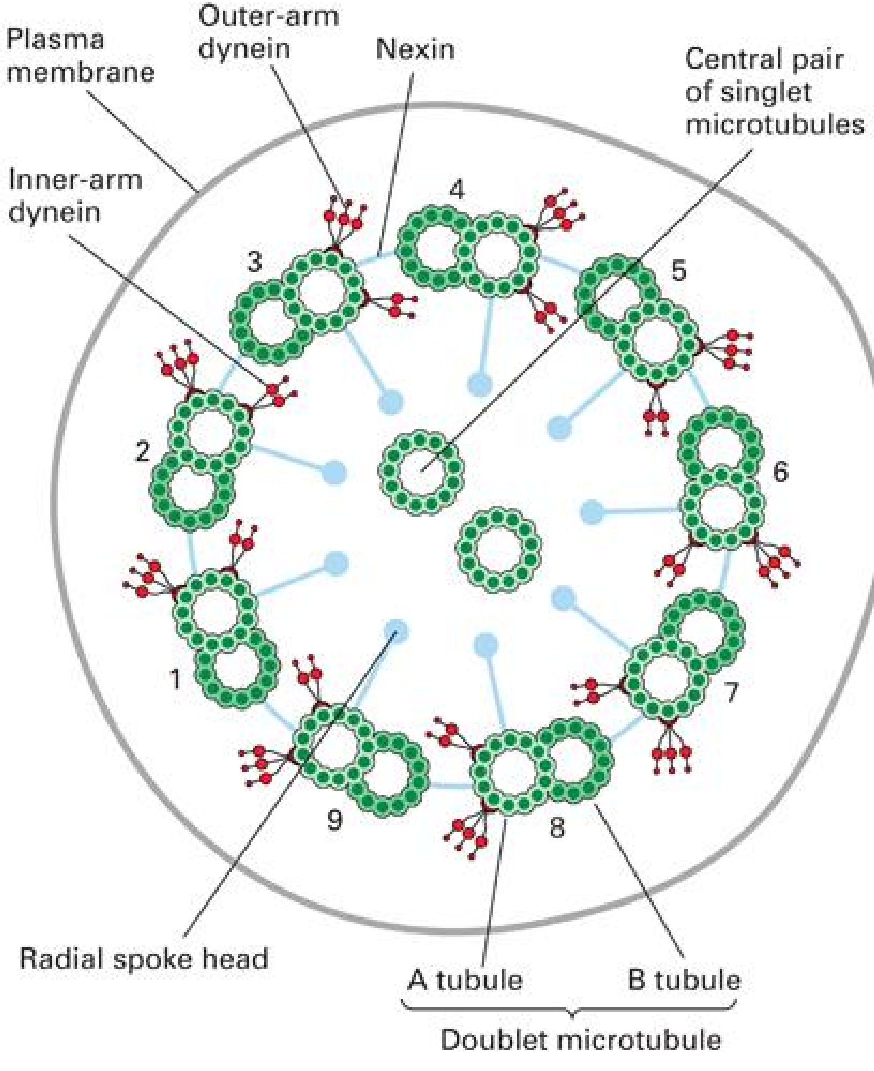


Cilia and flagella are microtubule-filled cellular extensions

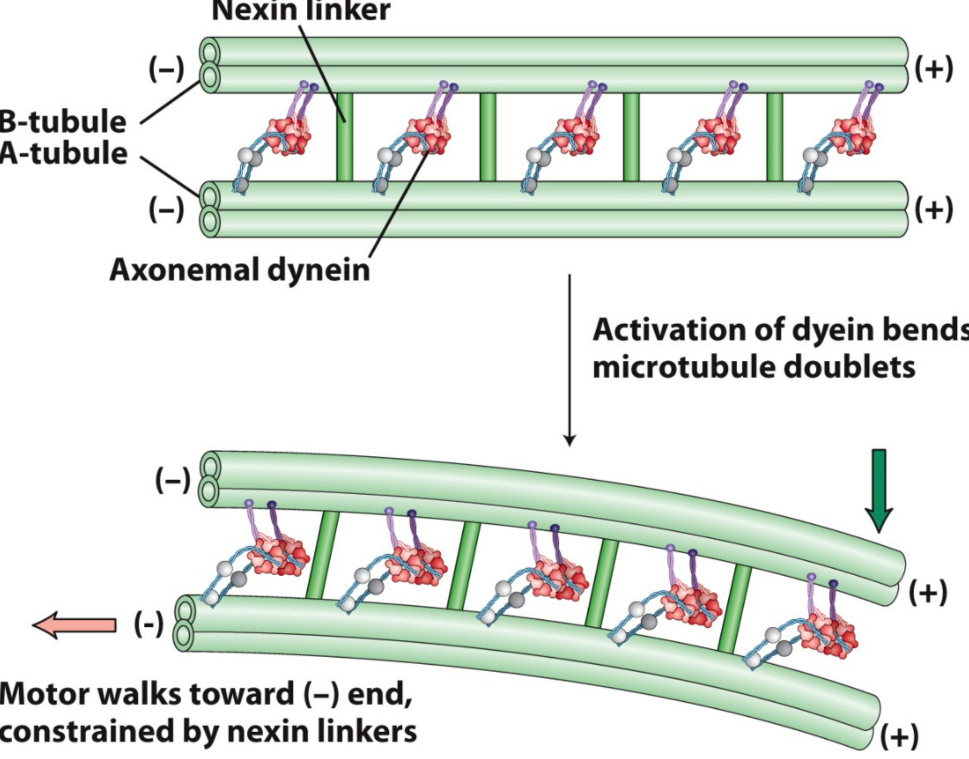
- Cilia are complex structures that contain specialized **axonemal dynein**
- Cilia grow from a basal body that is made of **9 triplet MTs** [1-3]
- Transitional zone [4]: transition from 9 triplet to **9 doublet MTs**
- The final (and long) axoneme [5] is made of **9 outer doublet** MTs + **2 singlet** MTs in the center
- At the A tubule, **radial spokes** and the **dynein arms** can be found:



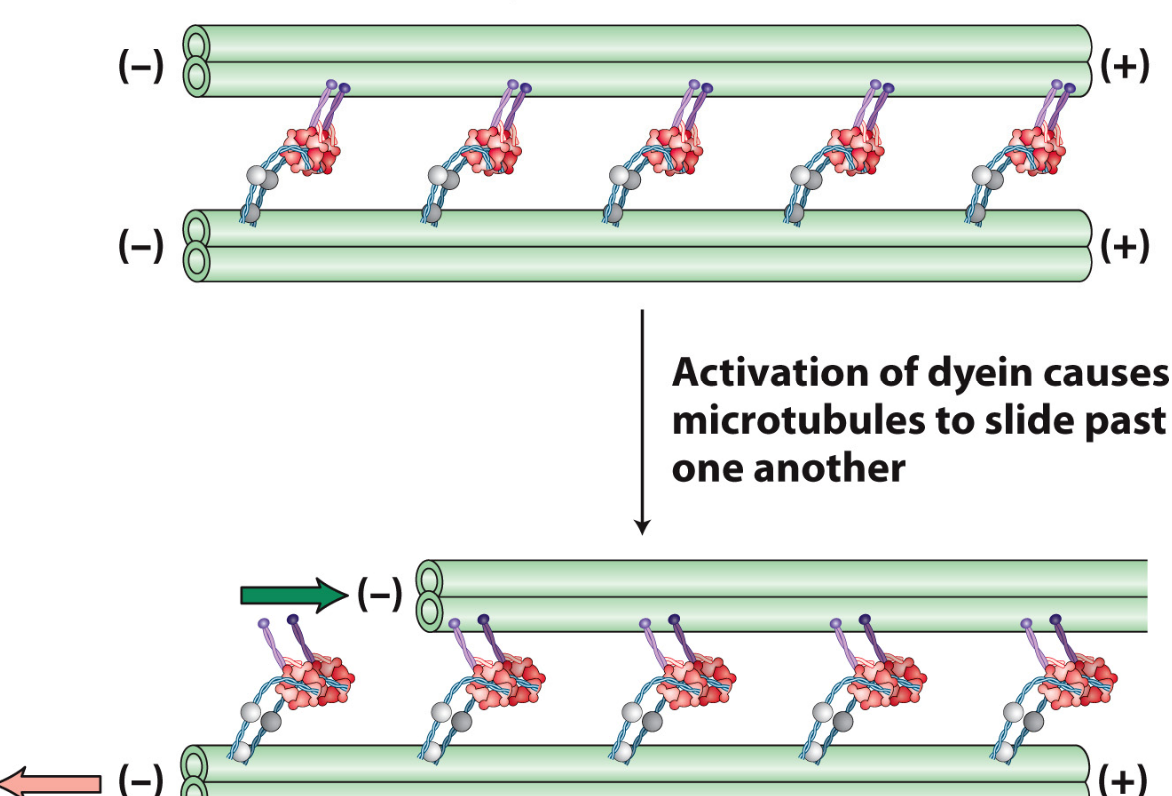
Cross section of the axoneme



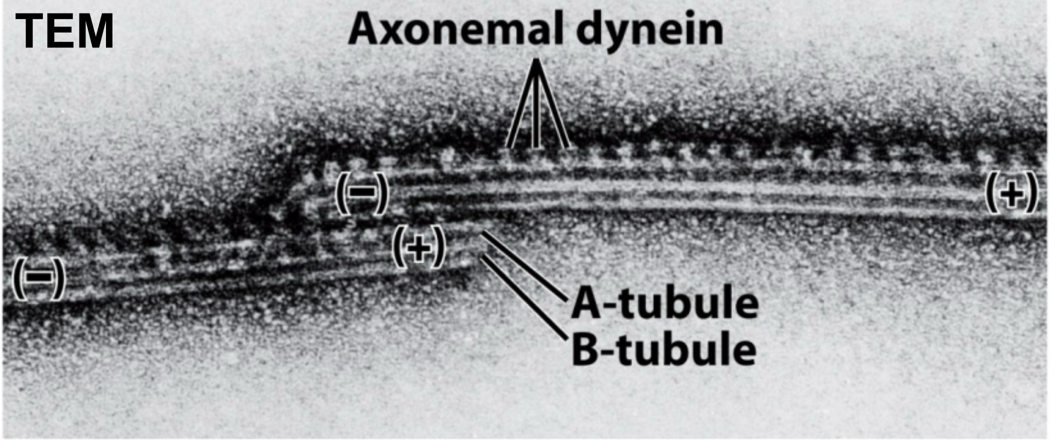
- The 9 outer doublet MTs are held together by **nexin**
- Also the inner MTs are held together by cross-linking proteins (not shown)
- **Bending** of cilia occurs when dynein arms at the A tubule walk down on a neighbor the B tubule
- **Polyglutamylation of the B tubule** positively affects its interaction with inner-arm dynein



A classic experiment revealed that bending occurs in a ATP dependent manner

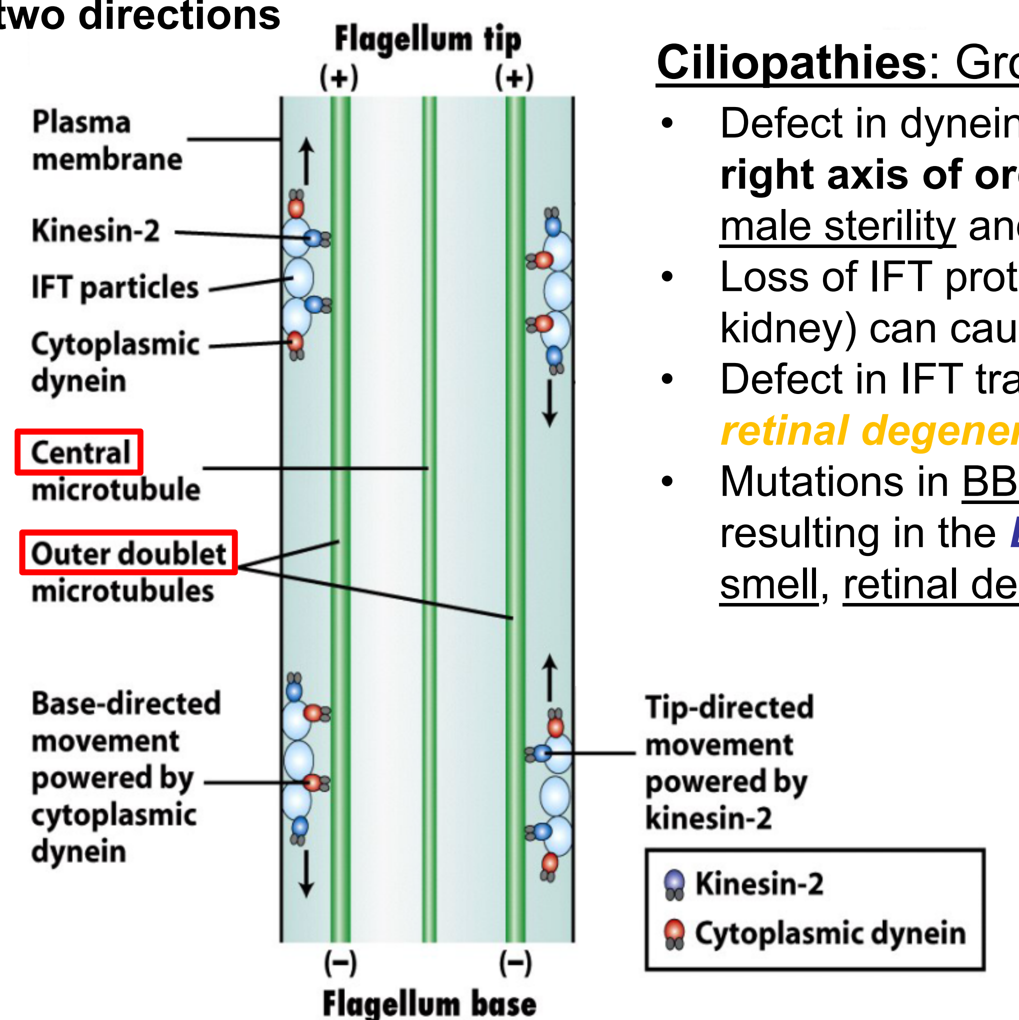


1) Open the plasma membrane with mild **detergents**
2) Proteolysis of cross-linking proteins (nexin cleaved by protease)
3) Add ATP: doublet MTs largely slide past each other (visible in EM)



Cooperative motor activity in the intraflagellar transport (IFT)

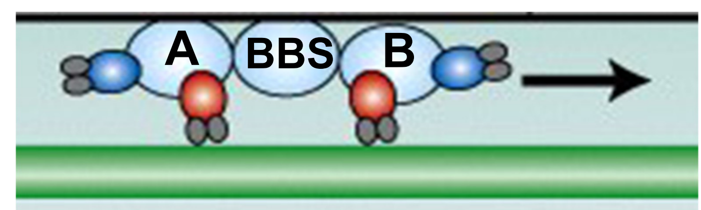
• Besides **axonemal dynein** that powers cilia bending, a set of motor proteins composed of **kinesin-2** and **cytoplasmic dynein** coordinate movement of particles within the cilia
• New material is constantly transported to the tip of the flagellum to promote MT **growth** and **turnover** (≈2.5 μm/s anterograde, ≈4 μm/s retrograde)
• Both **kinesin-2** and cytoplasmic **dynein** are attached to the particles fast shuttling cargo into **two directions**



Ciliopathies: Group of diseases related to cilia defects

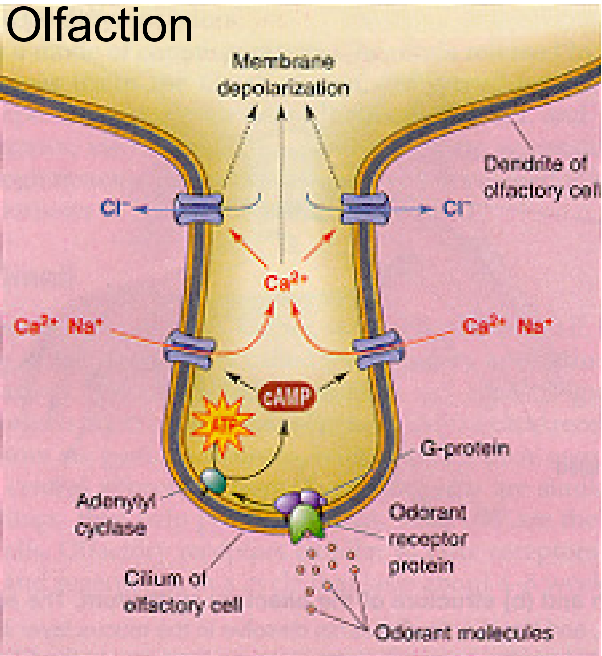
- Defect in dynein's outer arms in cilia: **Reversal of left-right axis of organs** (***Kartagener's triad***) resulting in male sterility and bronchial problems
- Loss of IFT proteins in primary cilia (detect fluid flow in kidney) can cause **PKD** = ***polycystic kidney disease***
- Defect in IFT transport of **photoreceptor cilia** can cause ***retinal degeneration***
- Mutations in BBS proteins cause defects in primary cilia resulting in the ***Bardet-Biedl syndrom***: loss of ability to smell, retinal degeneration as well as obesity

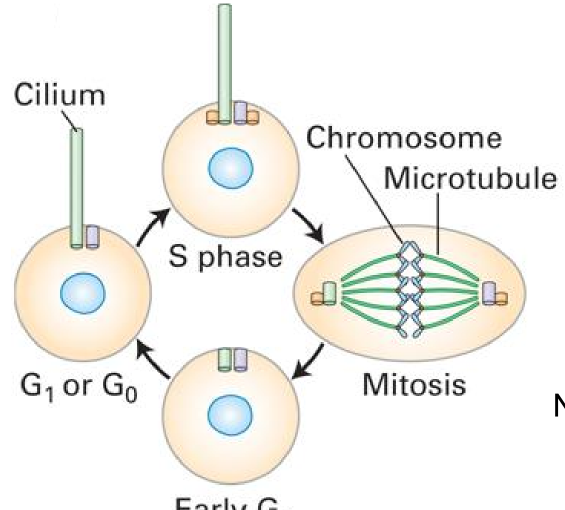
IFT particles are composed of **particle A** and **particle B** which are held together by **BBS proteins**



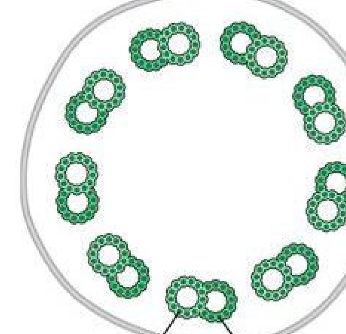
Primary cilium are non-motile cilia involved in sensing the environment

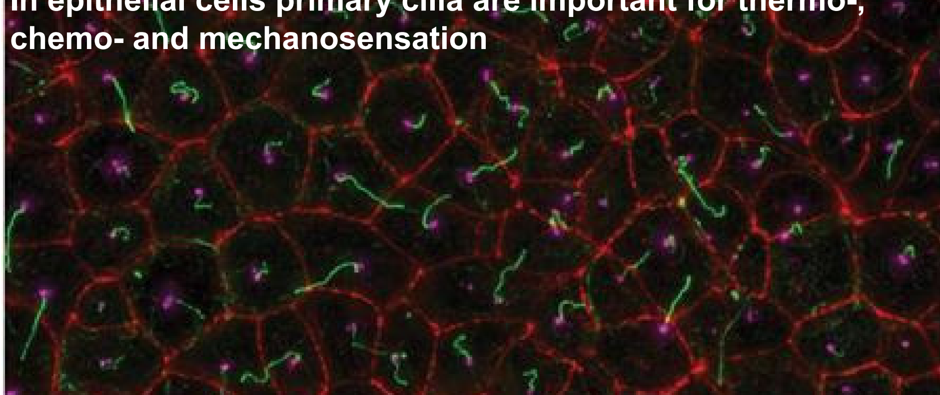
- In cilia of **dendritic sensory neurons** important **signaling molecules** and **receptors** need to be transported back and forth (recycling):
  - **Odorant** receptors in olfactory neurons
  - Retinal **opsin** in photoreceptors (2000 molecules/min!)
- Microtubules in primary cilia are resistant to colchicine and are highly lysine acetylated
- Primary cilia **cannot bend** because they lack the central MT pair and axonemal dynein





- A **primary cilium** is always accompanied by a daughter centriole (which duplicates during S phase)
- **Spindle pole centrioles** serve as a basis for primary cilia grow at G_1/G_0

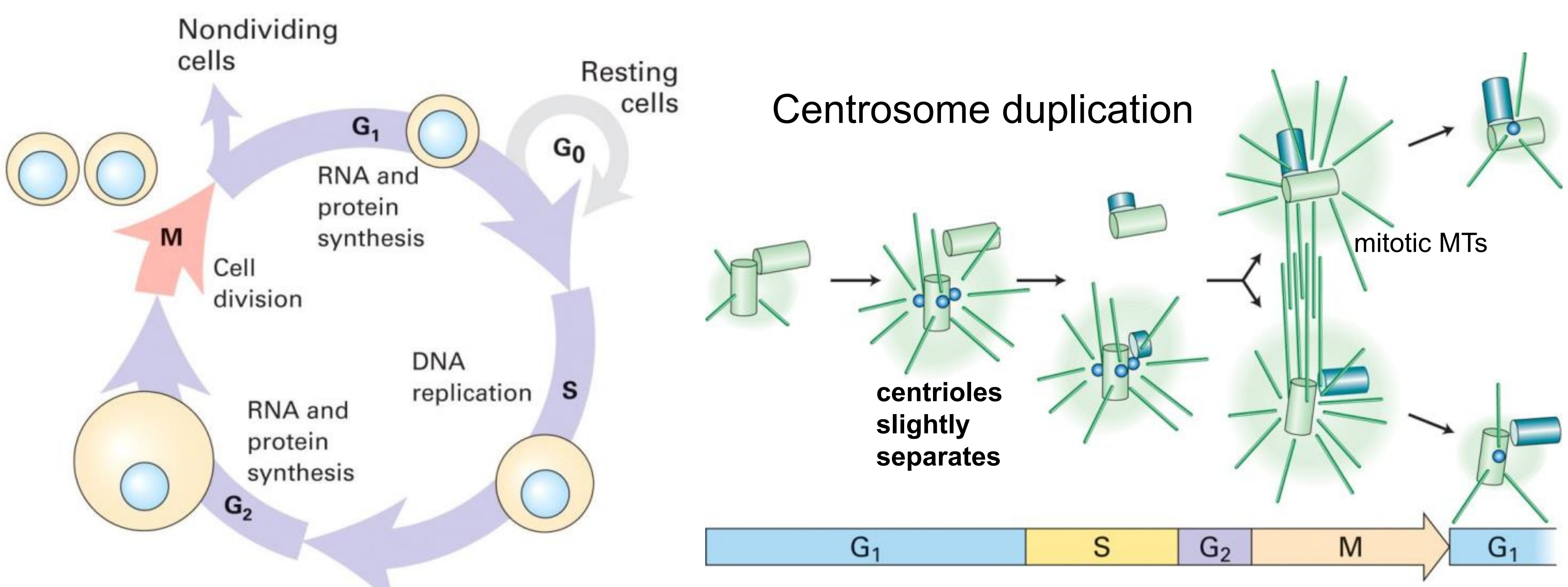




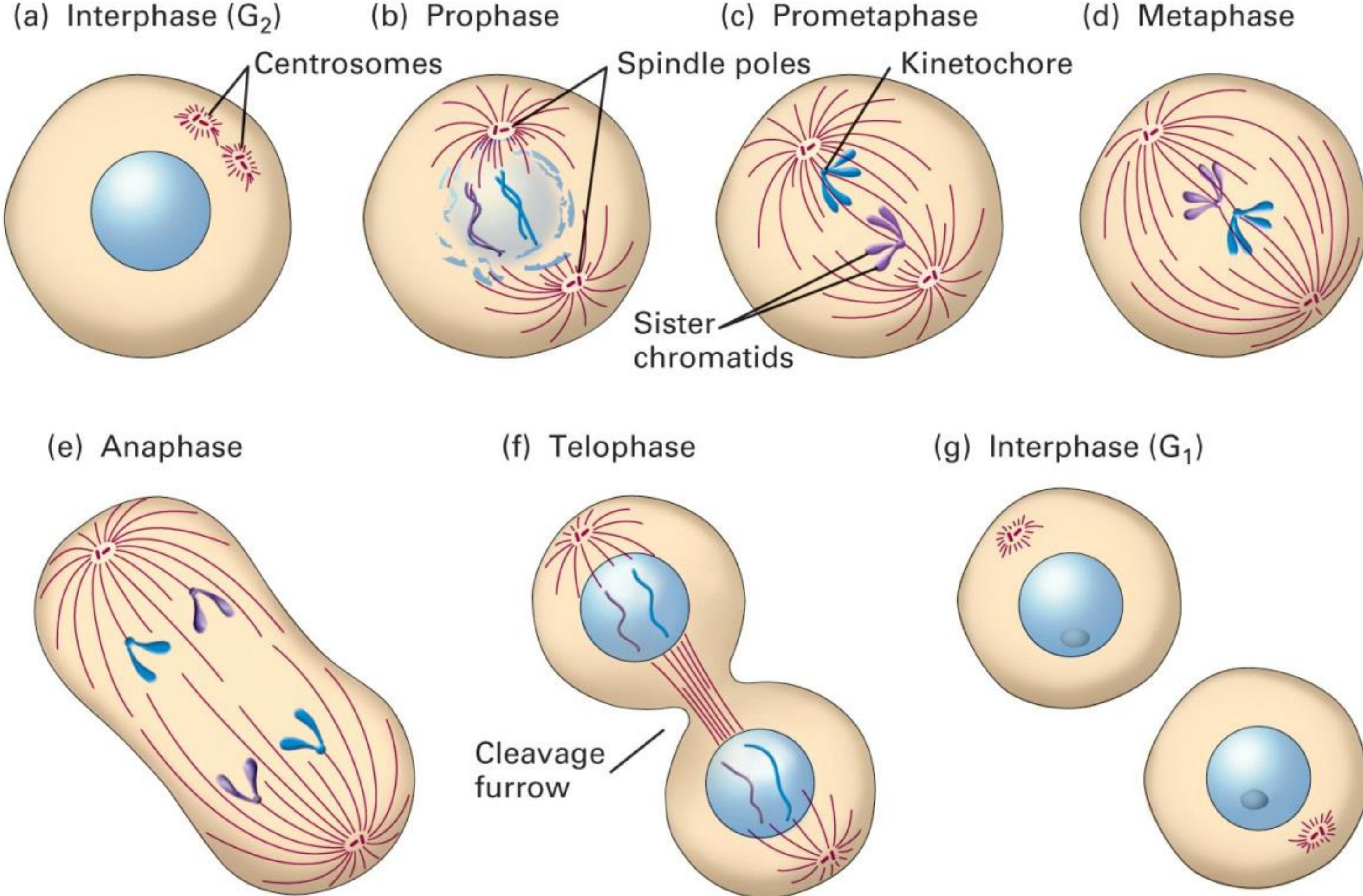
Mitosis

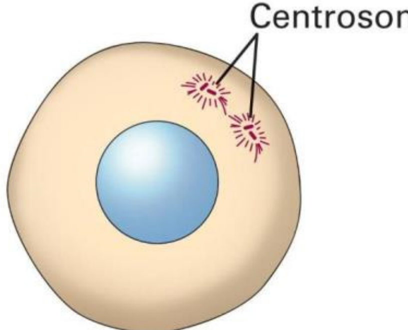
Molecular basis of mitosis

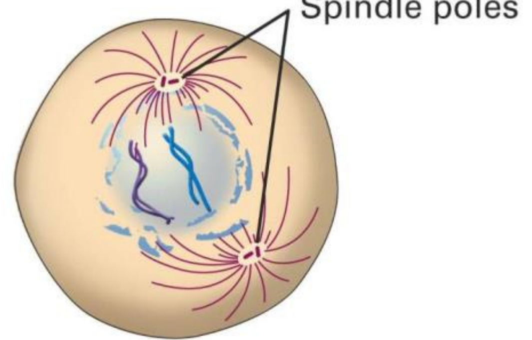
- During the **cell life cycle** duplicated chromosomes (from the S phase) are segregated into the two daughter cells, a process called **mitosis**
- Mitosis takes about 1 hour: cytosolic MT depolymerize and then build up the **spindle apparatus** that captures and aligns the chromosomes
- A critical first step is the **duplication of the MTOCs** (aka spindle poles or centrosomes):
  - **G phase**: the centrioles slightly migrate from each other
  - **S phase**: a daughter centriole buds off the mother centriole
  - **M phase**: two pairs of centrioles complete and migrate to the cell poles; **polar**- and **aster-type MTs** are now visible
- In **cancer cells** MTOC duplication is often erroneous resulting in **multiple centrosomes** per cell leading to aneuploidy (unequal numbers of chromosomes)

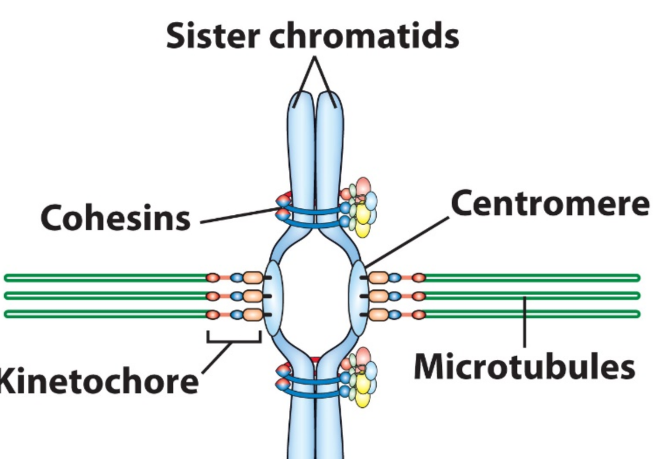


The 6 steps of mitosis









Interphase (G_2):

- **Duplication of centrosomes**
- **Four copies of** each chromosomal **DNA** (**4*n***) (from previous S phase): 2 copies of chromosomes and each chromosomes has one (sister) **chromatid**

Prophase events are largely coordinated by the activity of kinase cyclin-CDK:

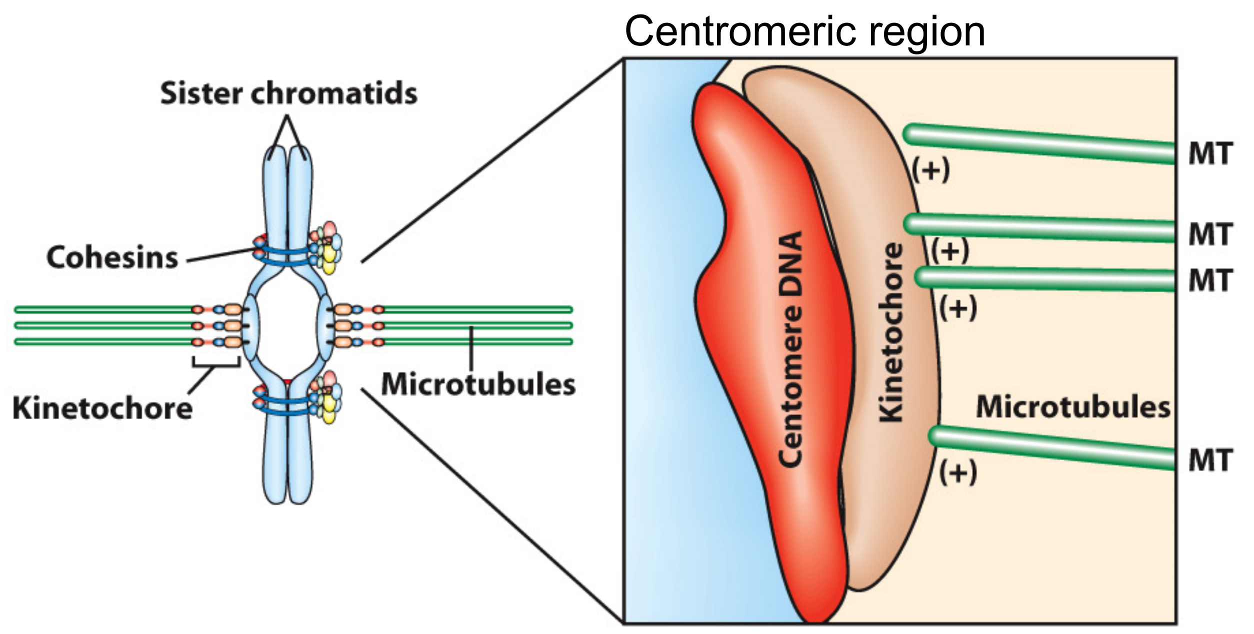
- Interphase MT **depolymerize** and mitotic MTs **reform** onto the new centromeres
- **+ TIP** activity increases to promote MT growth
- Centrosome **migrates** to the cell poles and **aster MTs** visible (that are pushed apart by bipolar **kinesin-5**)
- Chromosome condensation: two identical filaments (sister **chromatids**) appear
- Chromatids are held together by the **centromere**, a structure composed of **cohesin protein complex**
- **Kinetochores** start to assemble (MT-DNA interface)
- **Nuclear envelope fragments**

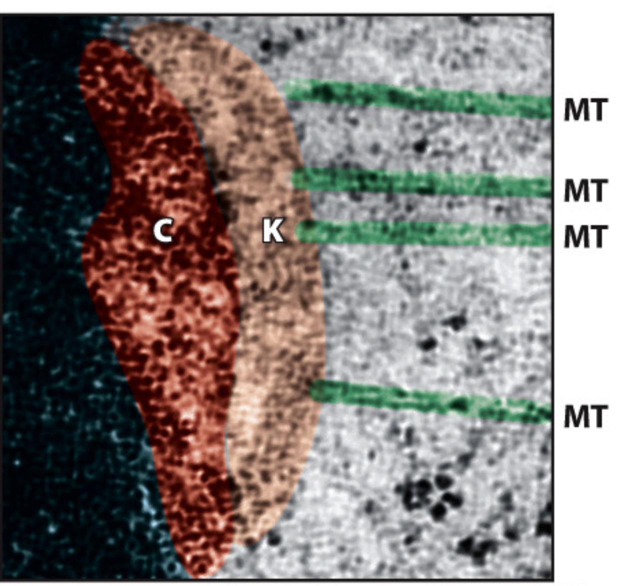
The breakdown of interphase MTs during prophase results in a change of cell shape (less polarity and more round) as well as in a breakdown of large membranous structures as ER and Golgi

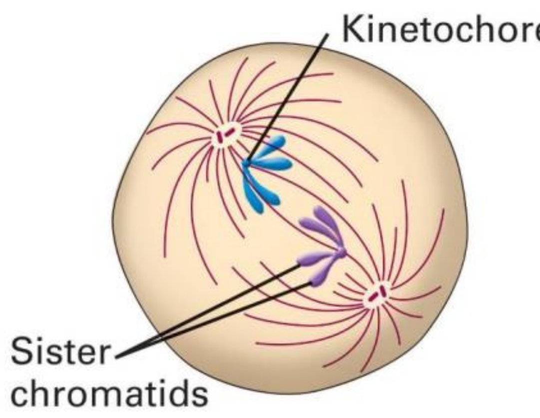
Detail prophase: structure of the centromere

- Region where kinetochores assemble at each sister chromatid is called the **centromere** (near the center of each chromosome)
- It contains **highly repetitive** and **non-coding** centromeric **DNA**
- **Kinetochores** consists of several protein complexes as well as the centromeric DNA followed by the **inner**- and **outer kinetochore layers**
- The outer kinetochore layer has several MTs attached

Centromeric region

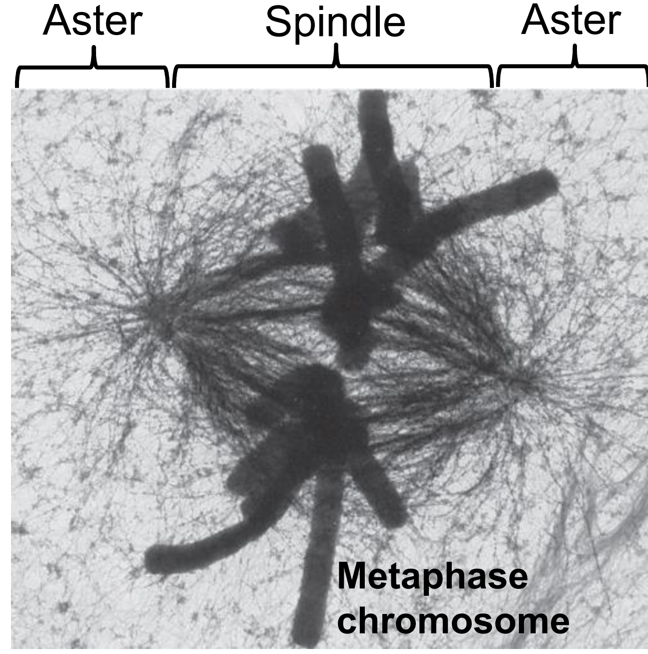


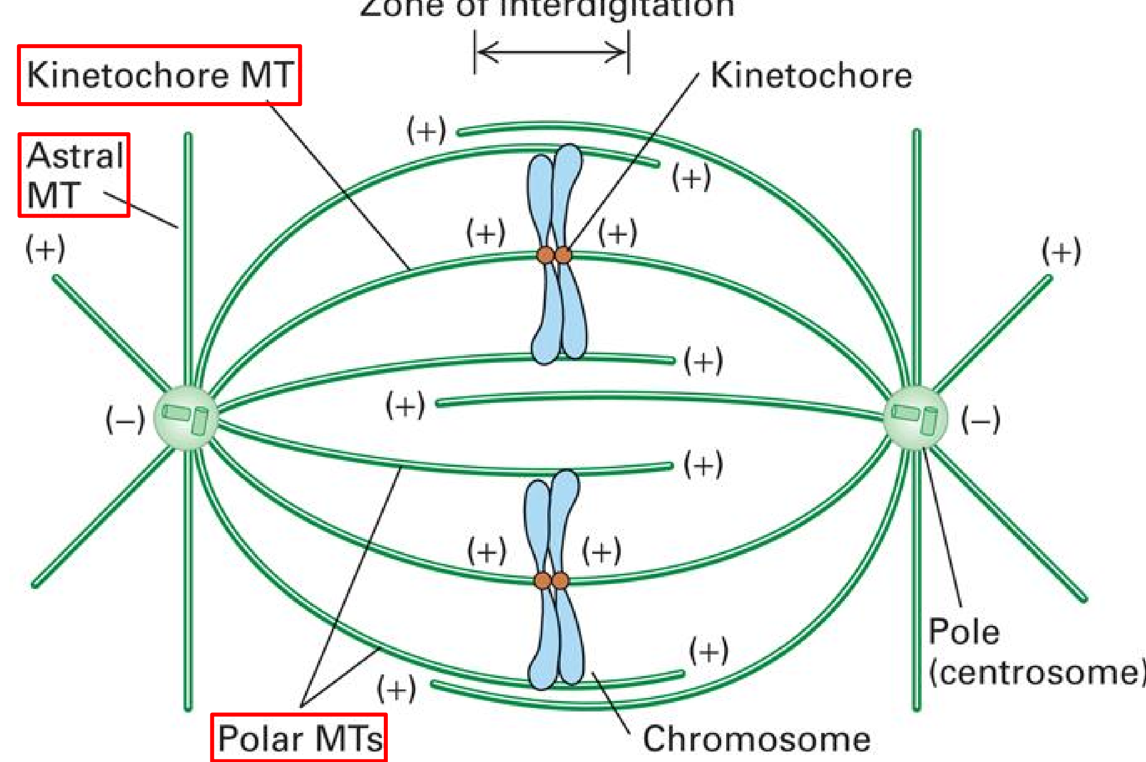




- **Kinetochore microtubules** assemble from the spindle poles and "search and capture" chromosomes (attach to kinetochores)
- Chromosomes become eventually attached to both spindle poles: bi-oriented chromosomes
- **Chromosome congression** processed until all become aligned in the equatorial plate
- 3 functional distinct sets of mitotic microtubules:
  - **Aster MT**: attached to the cortex; position the spindle
  - **Kinetochore MT**: spindle MTs; "catch" chromosomes
  - **Polar MT**: overlap with opposite polar MT; do not connect to kinetochores; move centromeres and cell poles apart

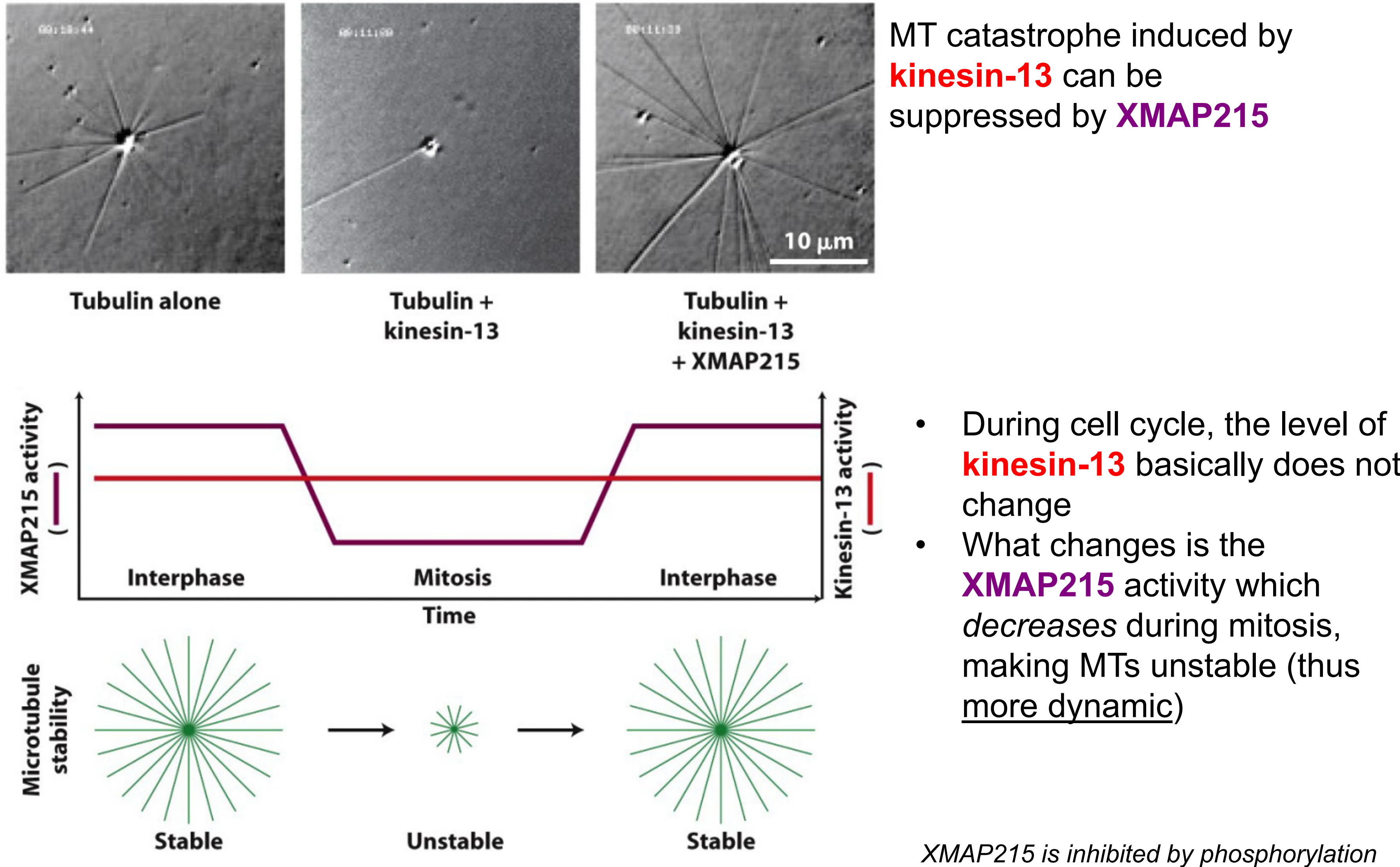
TEM of spindle apparatus





Detail prometaphase: Regulation of MT dynamics

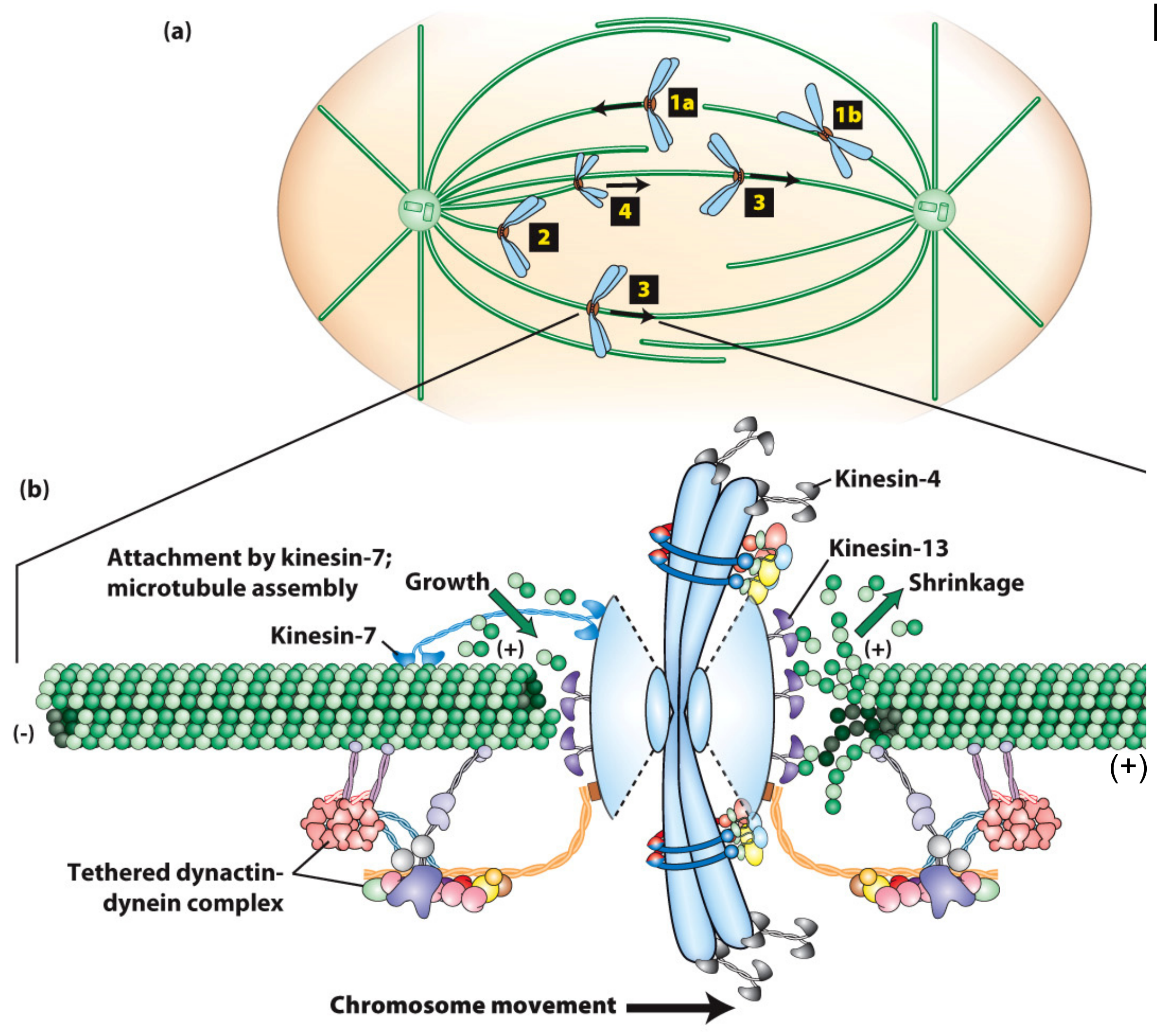
Mitotic MTs are very dynamic and this behavior is regulated by *destabilizing* **kinesin-13** and *stabilizing* (*Xenopus* microtubule-associated protein) **XMAP215**



MT catastrophe induced by **kinesin-13** can be suppressed by **XMAP215**

- During cell cycle, the level of **kinesin-13** basically does not change
- What changes is the **XMAP215** activity which *decreases* during mitosis, making MTs unstable (thus more dynamic)

XMAP215 is inhibited by phosphorylation



More details prometaphase

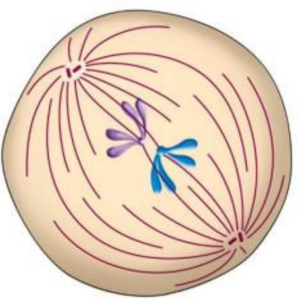
[1a/b]: "<u>Search and capture</u>" leads to kinetochore-MT interactions (can be also laterally, **1b**). This process is promoted by the G protein Ran.

[2]: **Dynein/dynactin** moves the <u>chromosome to the spindle pole</u>; the **other free end of the chromosome becomes more exposed**

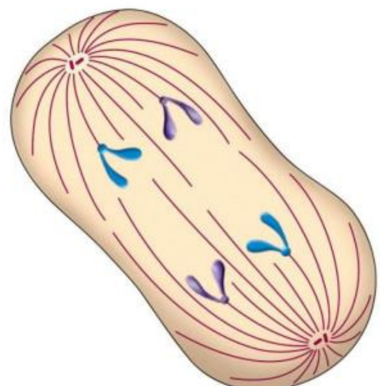
[3]: Exposed chromosome-end eventually captured by opposing MT; <u>chromosome becomes bi-oriented</u>

[4]: Some chromosomes from step **[2]** also use **kinesin-7** (attached to their free kinetochore) to interact with ***other*** <u>kinetochore MTs</u> to move more towards the center of the spindle

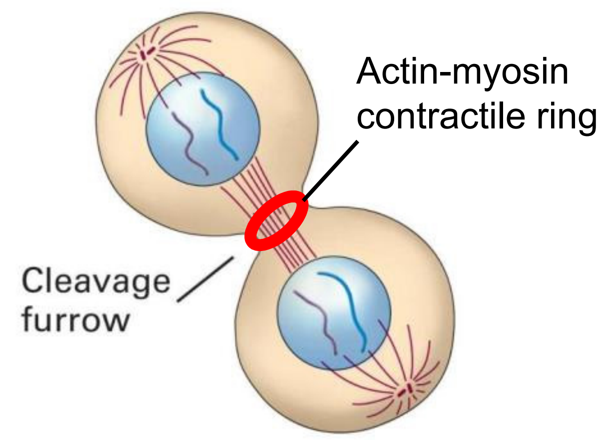
Detail of **[3]**: The movement of <u>bi-oriented</u> chromosomes to the center (equatorial plate) is called <u>congression</u> and powered by **polymerization on one side** (with **kinesin-7** fixing the MT) and **depolymerization on the other side** (powered by **kinesin-13**) with additionally **dynein** <u>movement to the minus-pole</u>; further, **kinesin-4** moves the chromosome arms to the plus-end of ***<u>polar</u>*** **MTs**



- All chromosomes now aligned in the equatorial plane (metaphase plate)



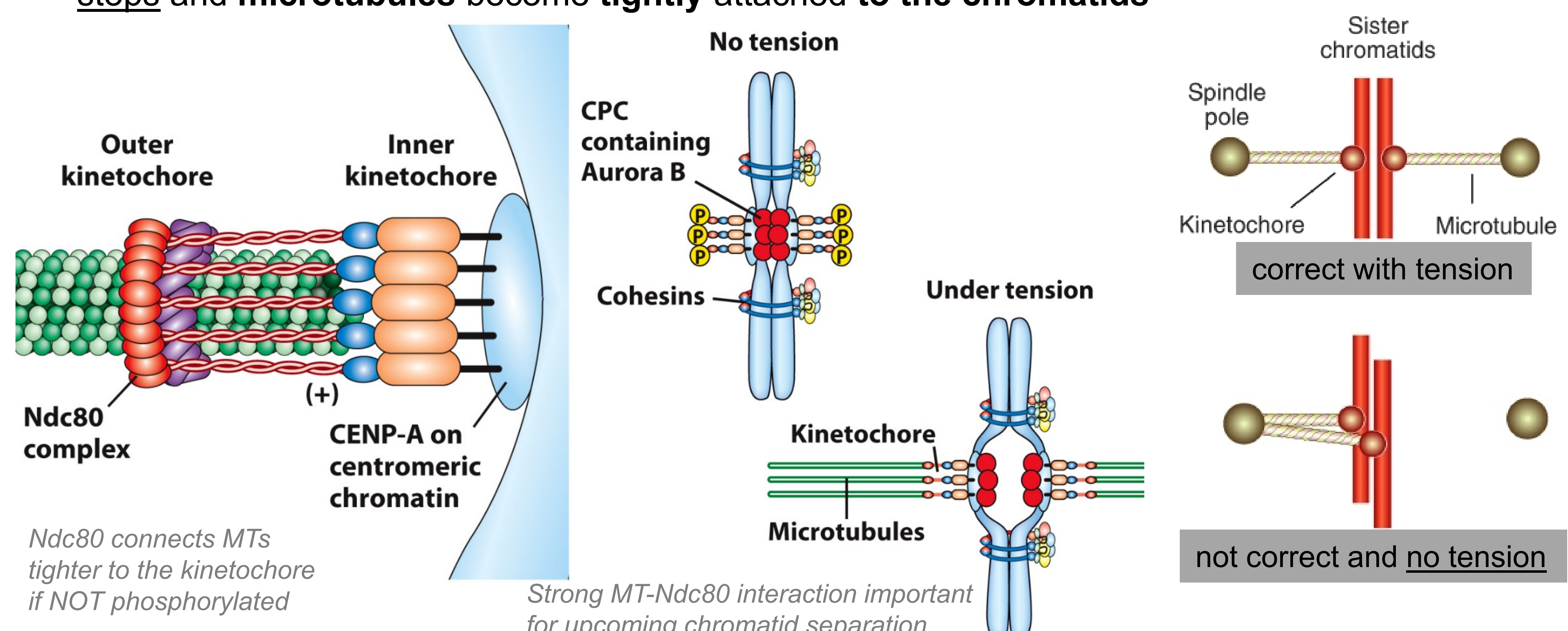
- **APC/C** (anaphase-promoting complex/cyclosome) **activated** that leads to the **destruction** of the **cohesin complexes**
- Now the two sister chromatids can be pulled to the poles via kinetochore MTs (**anaphase A**)
- At the same time cellular poles moves further apart (**anaphase B**)



- **Nuclear membranes** reform
- Chromosomes **decondense** and become less obvious
- **Mitotic MTs** depolymerize and cell starts to divide powered by the **contractile actin-myosin ring** (cytokinesis)
- Signal cascade that activates formation of **actin-myosin ring**: **CPC** (chromosomal passenger complex) → **centralspindlin** **RhoA** → **formin** → **F-actin** (→ = activates/recruits)

Detail Metaphase: Arranging chromosomes in the equatorial plate

- Perfect bi-orientation of chromosomes in the metaphase plate is **very important** for the following anaphase (chromatid separation)
- Example for **bad orientation**: two kinetochore-MTs from the same spindle pole attach to both kinetochores of one chromosome = chromatids won't be split apart (**aneuploidy** happens)
- One feature of **correctly bi-oriented chromosomes** is that they are under stronger tension as opposed to non-correct oriented chromosomes (indeed, in the cell, when chromosomes are not under high tension, they will be continuously reoriented)
- How does the cell detect tension? If NO tension, both chromatids associate with **Aurora B kinase** (part of CPC, *c*hromosomal *p*assenger *c*omplex) which **phosphorylates Ndc80**
- Only if chromatids are not in contact with Aurora B (under tension) phosphorylation of Ndc80 stops and **microtubules** become **tightly** attached **to the chromatids**



Ndc80 connects MTs tighter to the kinetochore if NOT phosphorylated

Strong MT-Ndc80 interaction important for upcoming chromatid separation

Kinesin-13
Shrinkage
(+)
(-)
Shrinkage
(+)
(-)
A1
A2
B1
B2
(+)
(–)
Sliding by kinesin-5
(-)
(+)
(–)
Pulling by anchored dynein
(+)

During this movement polar MTs keep growing

Details anaphase

Anaphase A

- **Dynein** already released from the centromere and <u>moved to the spindle pole</u> (prometaphase)
- **APC/C activation** leads to proteolysis of **cohesin** so the sister chromatids are <u>abruptly</u> separated (tension released!)

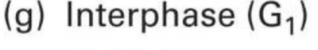
[A1/A2]: **Kinesin-13** depolymerizes **kinetochore MTs** at (+) *and* (–) end (this "shrinking force" needs to <u>counteract</u> with **kinesin-4** still moving on polar MTs)

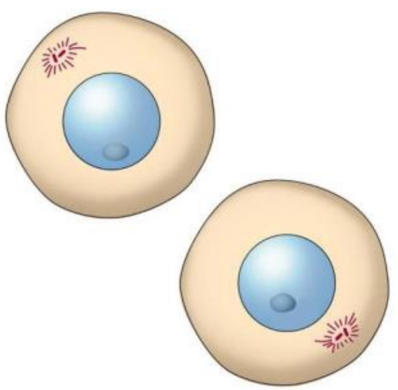
Anaphase B

[B1]: Bipolar **kinesin-5** powers **polar MTs** <u>to slide past each other</u> that <u>elongates the spindle</u>

[B2]: **Dynein** (fixed on the cortex) pulls on **astral MTs** that also <u>moves the spindle poles further apart</u>

http://www.youtube.com/watch?v=WFCvkkDSflU

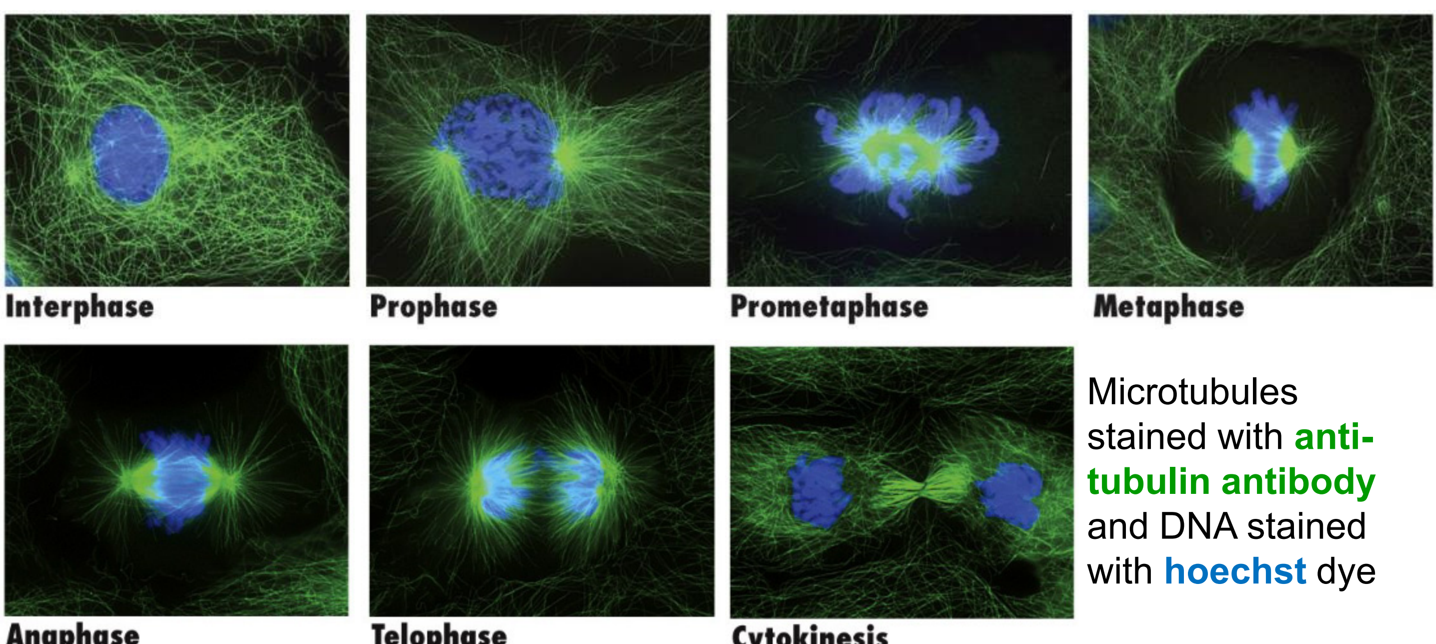




- After cytokinesis, each cell has a **double set of chromosomes**, however each chromosome consists of only one chromatid (***2n***)
- In the following S phase chromosomes duplicate (***2n*** => ***4n***)

Mitosis in lung cells

- Though mitosis is a very exact and tightly regulated process, **errors** during mitosis **happen**: *missing chromosomes* or *extra chromosomes* = **aneuploidy**
- Abnormal development and other pathologies may occur: e.g., **Down syndrome** (also called **Trisomy 21** = 3 x chromosomes 21) or **Turner syndrome** (only one X chromosome)



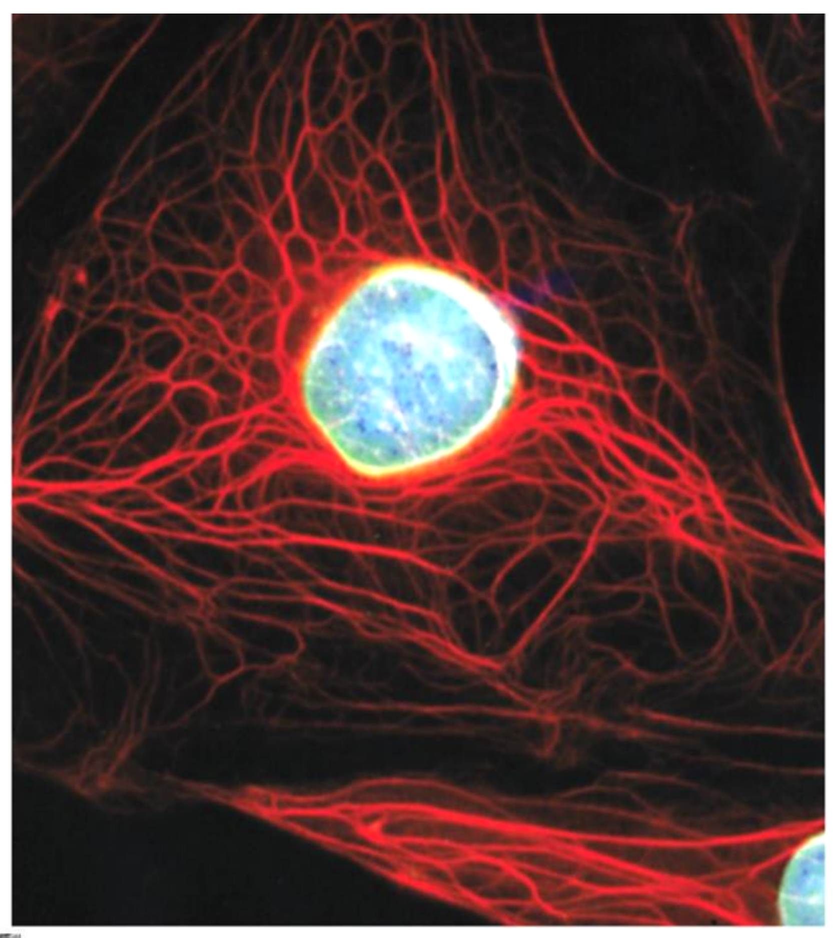
Microtubules stained with **anti-tubulin antibody** and DNA stained with **hoechst** dye

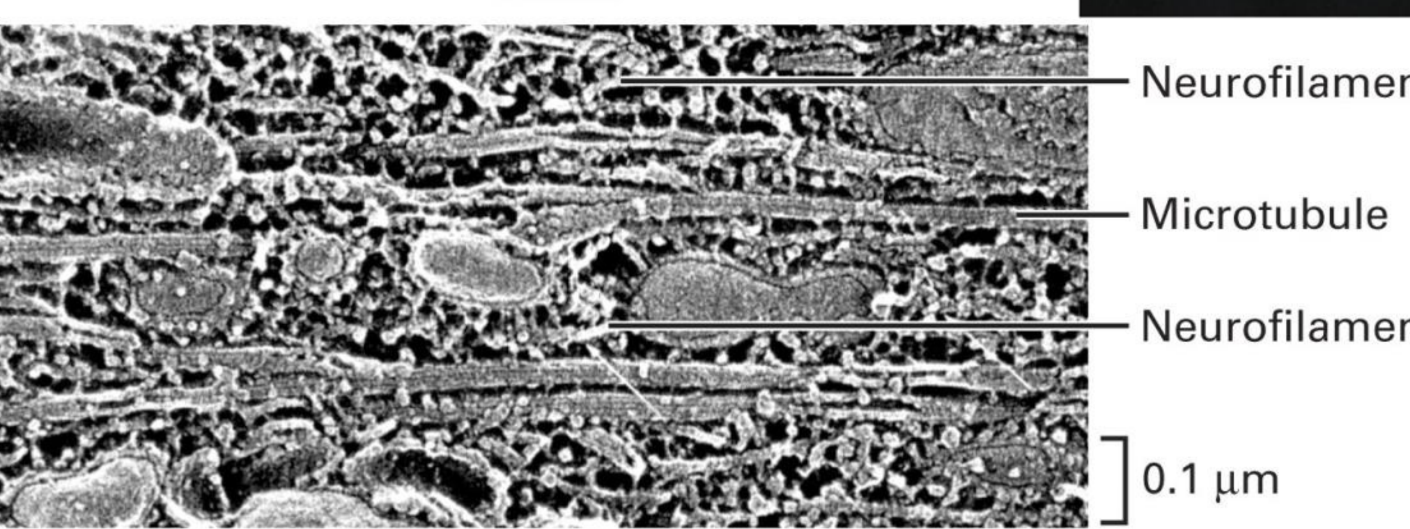
Drosophila embryos

Synchronized mitosis in *Drosophila* embryos

INTERMEDIATE FILAMENTS

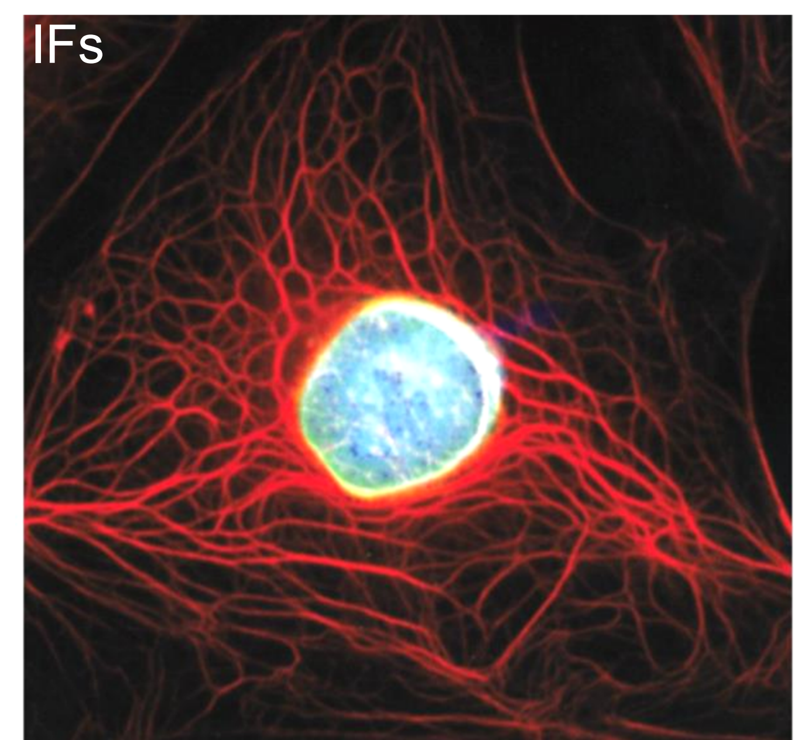
- Not found in plants and fungi
- Associate with plasma- and nucleus membrane to stabilize the position of the nucleus
- In epithelial cells, IFs provide contact with neighboring cells or the extracellular matrix
- About **40 clinical disorders** are know that are directly related to IFs
- In **neurons** IFs are called **neurofilaments**
- **NFs** are much more abundant than actin and stabilize the long and fragile axons

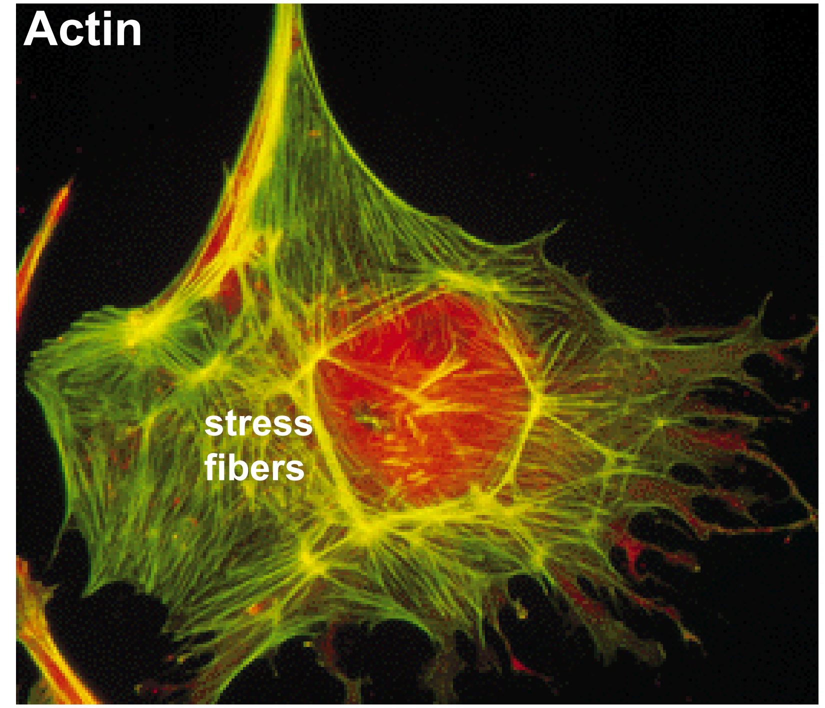


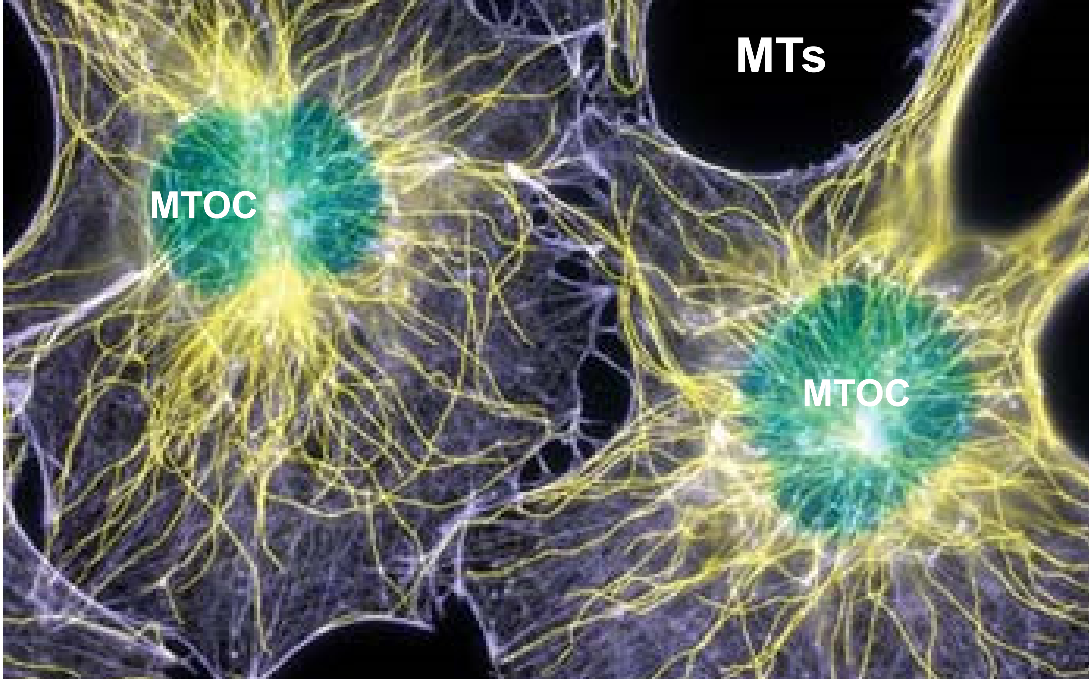


IF distribution in cells

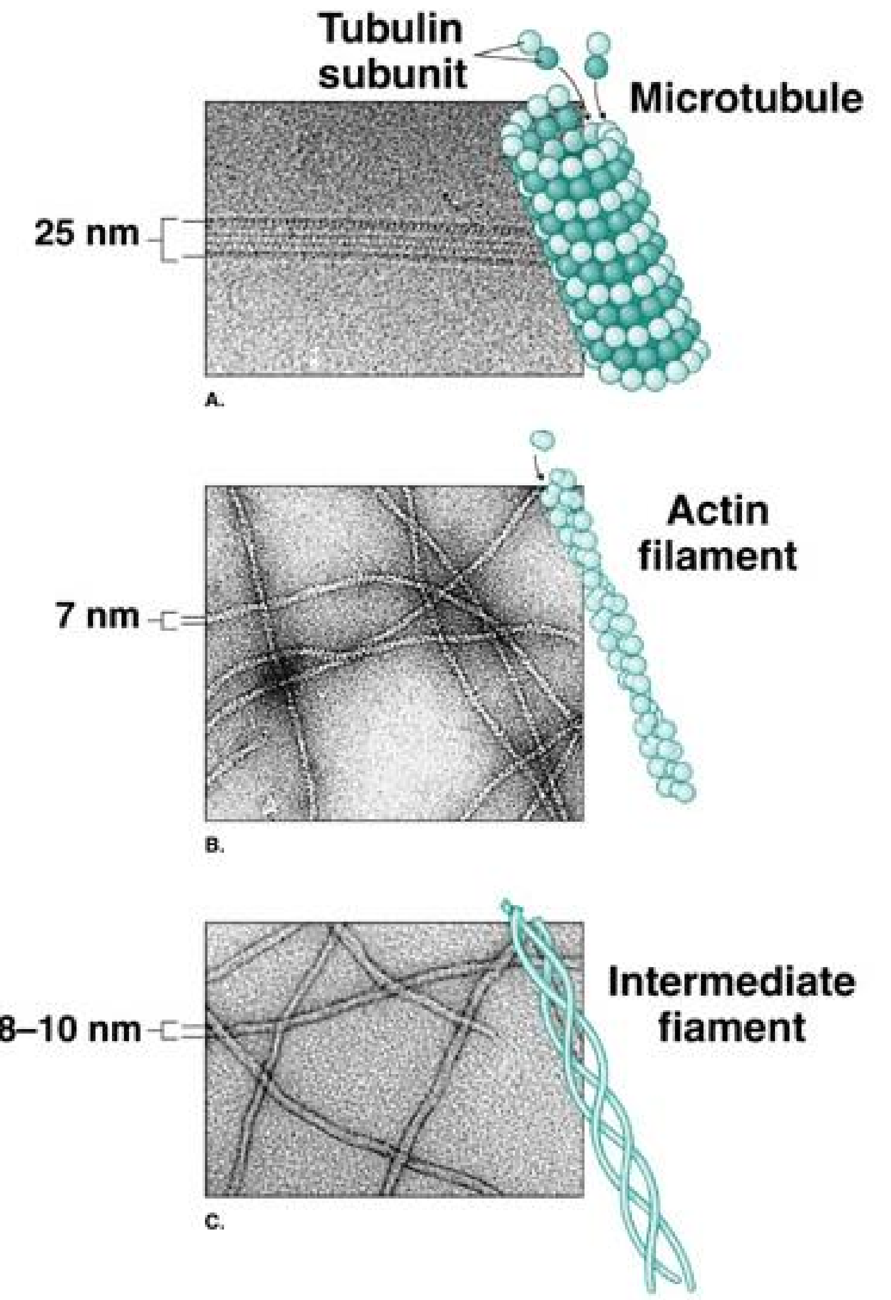
- IFs do not nucleate from central structures (as the MTOC); they do not make thick bundles as known for F-actin
- They **rather wrap around the nucleus** and form a stable and elastic network within the cell
- They do not have motors attached probably because they do not exhibit specific polarities





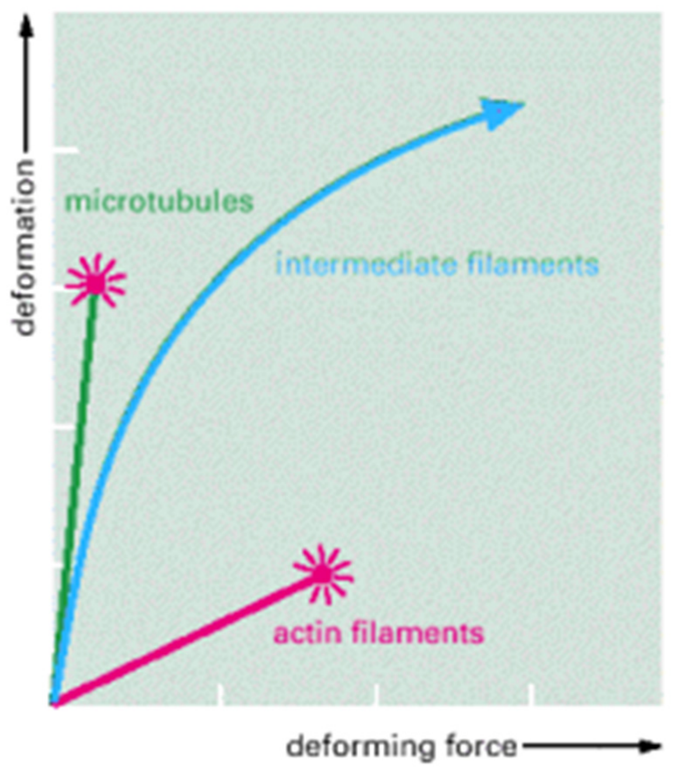


Intermediate filaments: size is *intermediate* to that of actin and microtubules



IFs differ from actin and MTs in several biochemical and mechanical properties:

- No binding of ATP or GTP
- No polarity
- No molecular motors attached
- No polymerization from globular monomers
- No depolymerization upon high salt conc.
- Much more resistant to deformation under high stress compared to actin and MTs



IFs are divided into 5 major classes based on their sequences

IFs isoforms vary greatly in their sequence and molecular weight

The Major Classes of Intermediate Filaments in Mammals

CLASS	PROTEIN	DISTRIBUTION	PROPOSED FUNCTION
I	Acidic keratins	Epithelial cells	Tissue strength and integrity
II	Basic keratins	Epithelial cells	
III	Desmin, GFAP, vimentin	Muscle, glial cells, mesenchymal cells	Sarcomere organization, integrity
IV	Neurofilaments (NFL, NFM, and NFH)	Neurons	Axon organization
V	Lamins	Nucleus	Nuclear structure and organization

Keratins interact with desmosomes and hemi-desmosomes to integrate cells into tissues

Desmosomes

Epithelial cell

Dense bodies

Smooth muscle

Z disk

Z disk

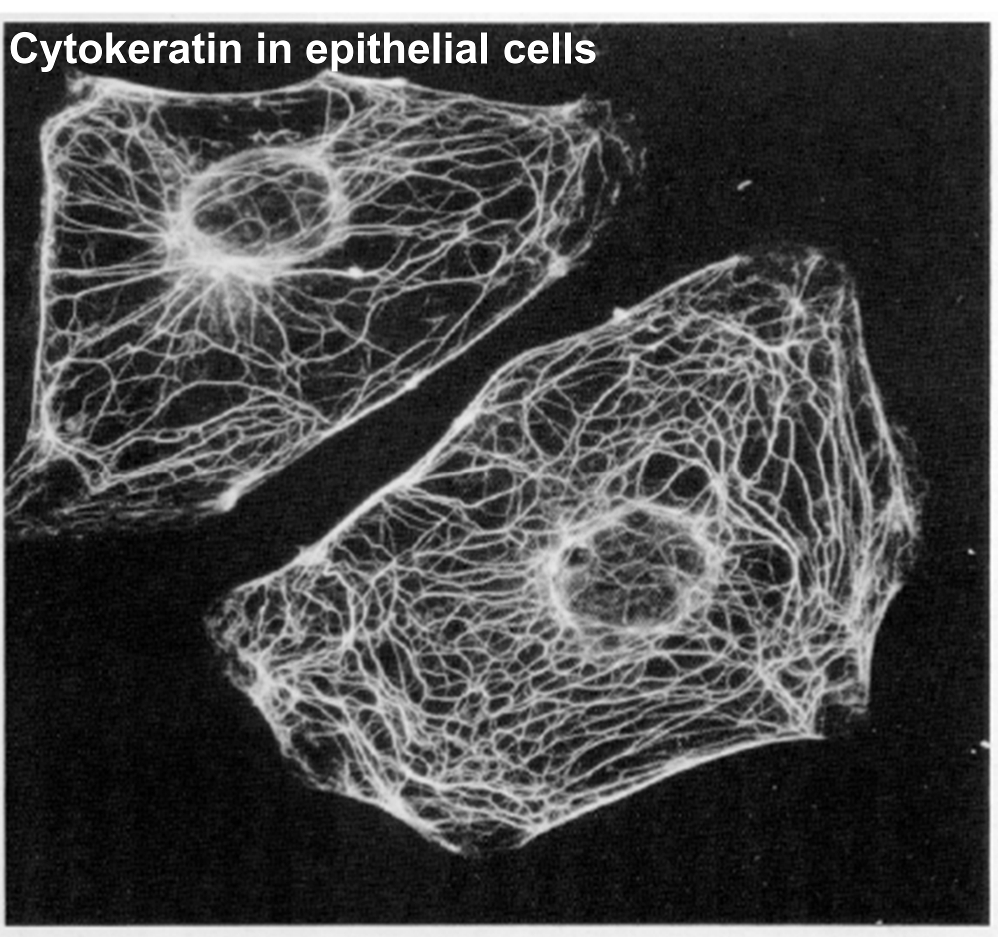
Skeletal muscle

Axon

Nucleus

Keratins (class I and II IFs)

- **Acidic** (class I) and **basic** (class II) **keratins** are expressed in epithelial cells
- 1 basic + 1 acidic polypeptide assemble to form a **obligate heteropolymer**
- 30 isoforms are known: **15 acidic** and **15 basic** keratins
- 10 of the 30 isoforms are found in **hard** epithelial tissue: **nails**, **hair**, **wool** etc.
- 20 of the 30 isoforms are found in **softer** epithelial tissues (**cytokeratins**) lining the surfaces of blood vessels and other internal cavities for structural support



How to make hair curly or straight?

- Keratins are rich in cysteine residues that can be **oxidized** to form disulfide bridges (to strengthen the keratins)
- At the hair saloon the disulfide bridges are **reduced**, the hair is reshaped and later the disulfide bonds **oxidized** again

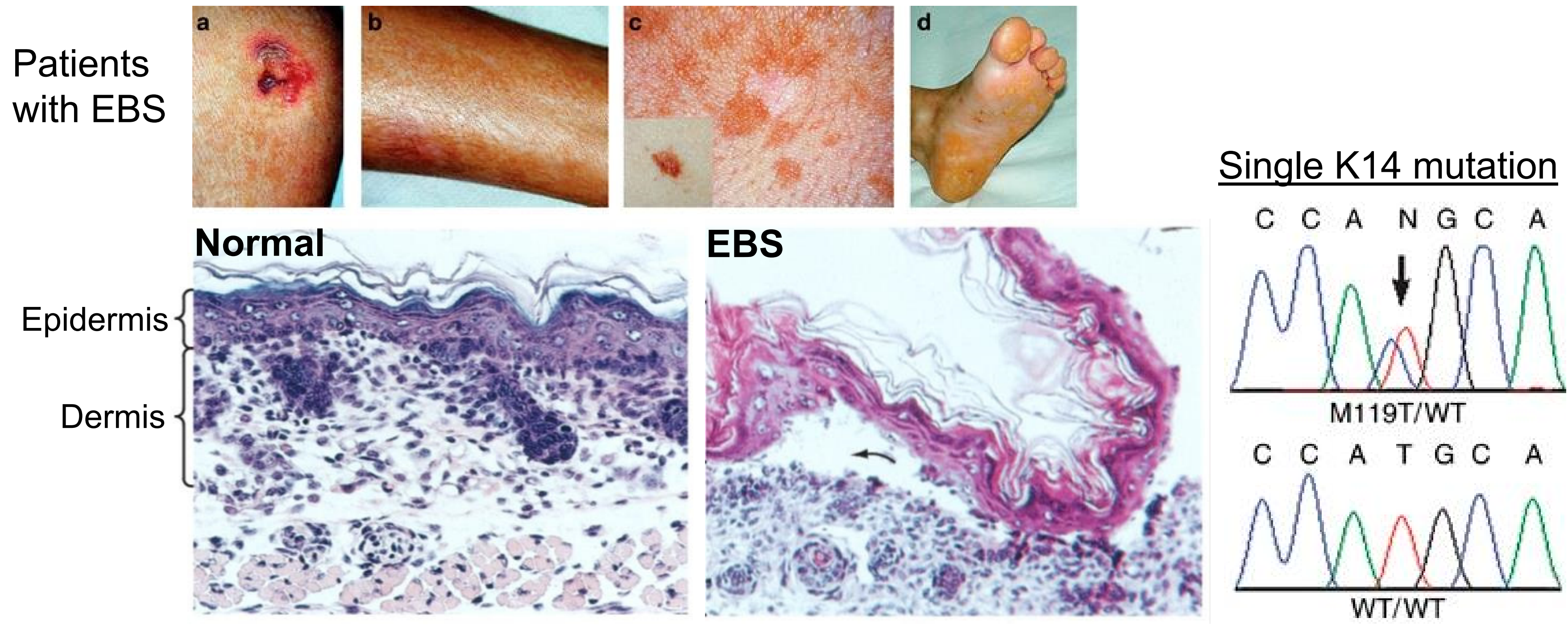
Intermediate filaments and disease

IFs in cancer drug treatment

- **Tissue specific tumors** need to be treated with drugs that precisely target these tissues
- Tumors are often **metastatic**, thus, to treat the tumor correctly the origin must be known
- Antibodies against IFs specific for different tissues (epidermal, mesenchymal etc.) can be used on tumor tissues to identify their origin

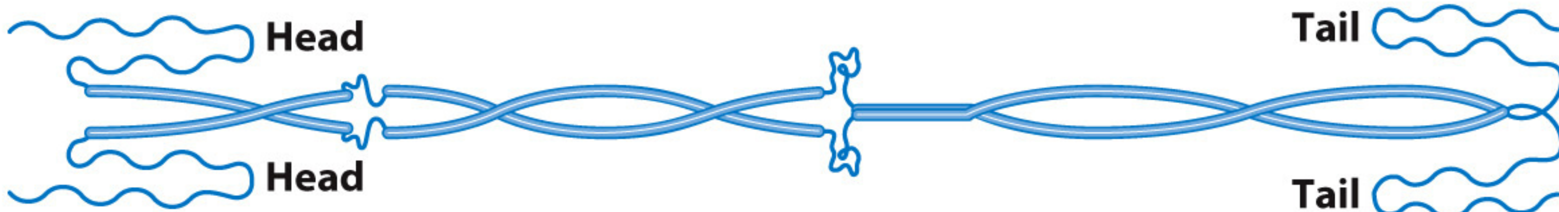
Keratin and neurofilament-based diseases

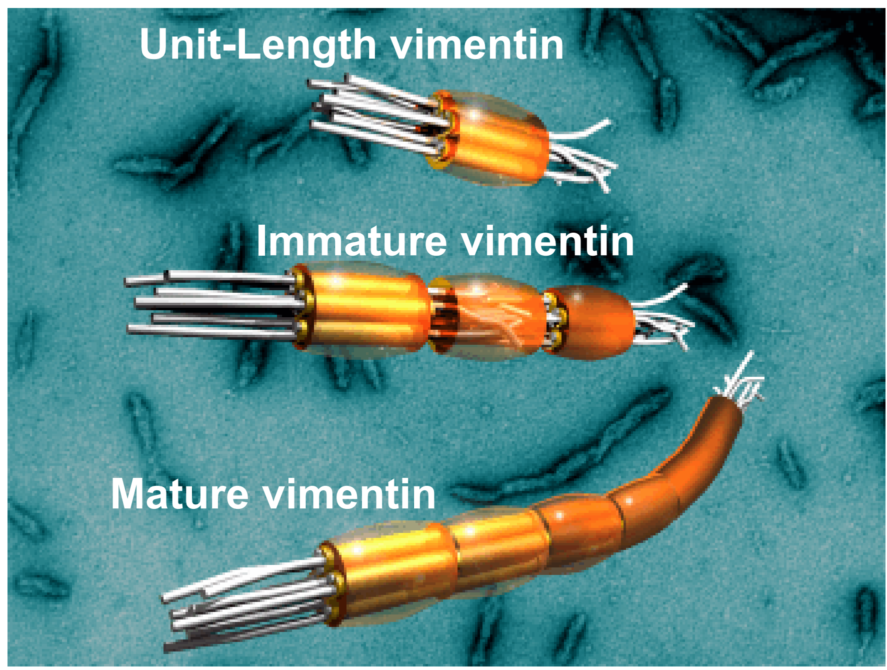
- Keratins K4 and K14 are important for connecting the hard **epidermins** with the soft **inner dermis**
- K14 mutations lead to **skin blistering** (**EBS,** *epidermolysis bullosa simplex*)
- Overexpression of **NF-L** in mice leads to **amyotrophic lateral sclerosis** (ALS)
- Mutations in **NF-L** disrupt axonal transport of neurofilaments (a hallmark of **Parkinson**)



Class III IFs

- In contrast to keratins, **class III IFs** can form both homo- and heteropolymers
- The most widely expressed class III IF is **vimentin**
- Vimentin helps to position organelles (nucleus), stabilizes the cell membrane and associate with microtubules
- **Desmin** is exclusively found in **muscle cells** (to stabilize the sarcomere)
- **GFAPs** (glial fibrillary acidic proteins) are the intermediate filaments of glial cells which **surround neurons** and **astrocytes**

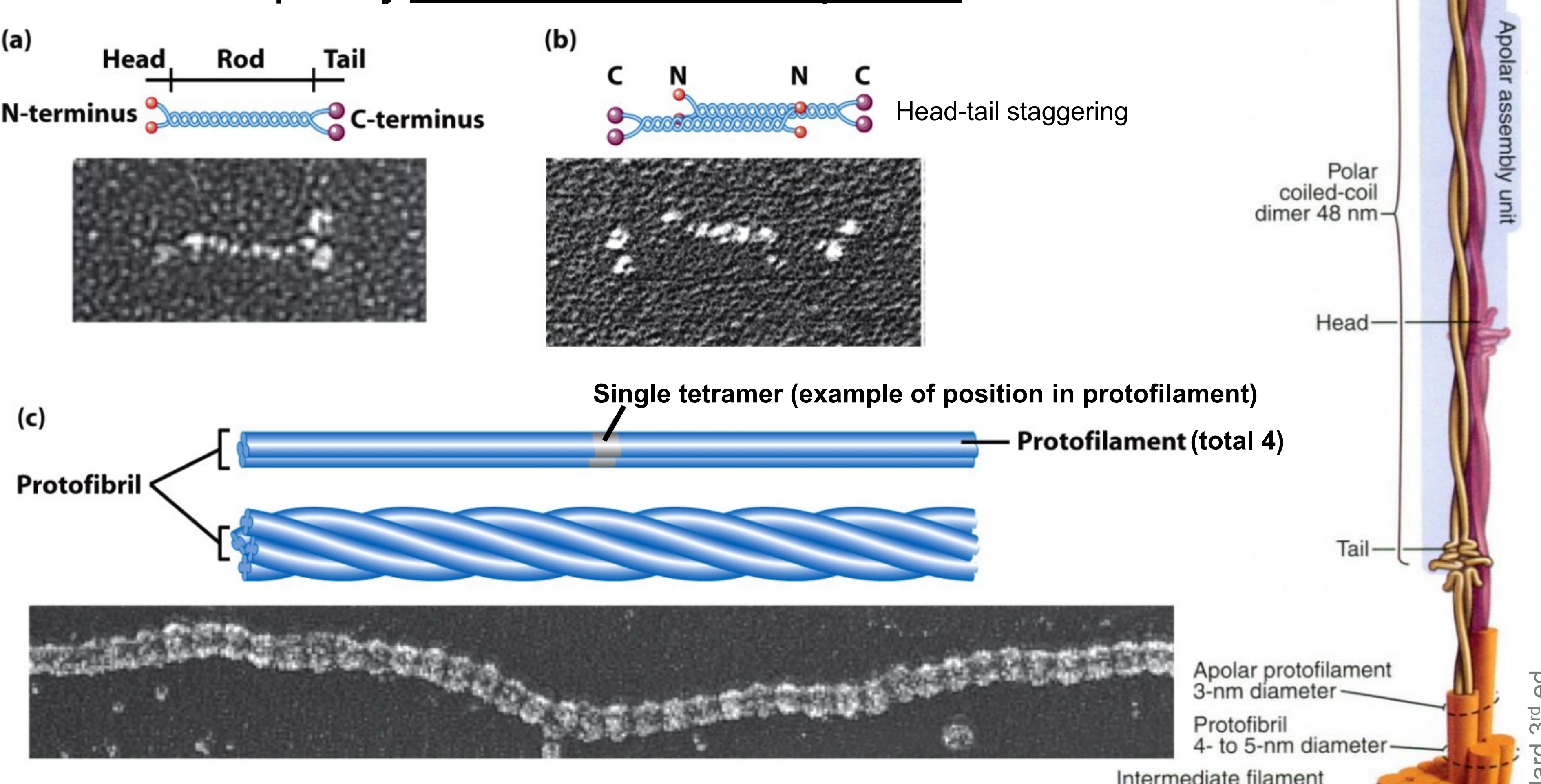




Predicted aggregation propensities of vimentin

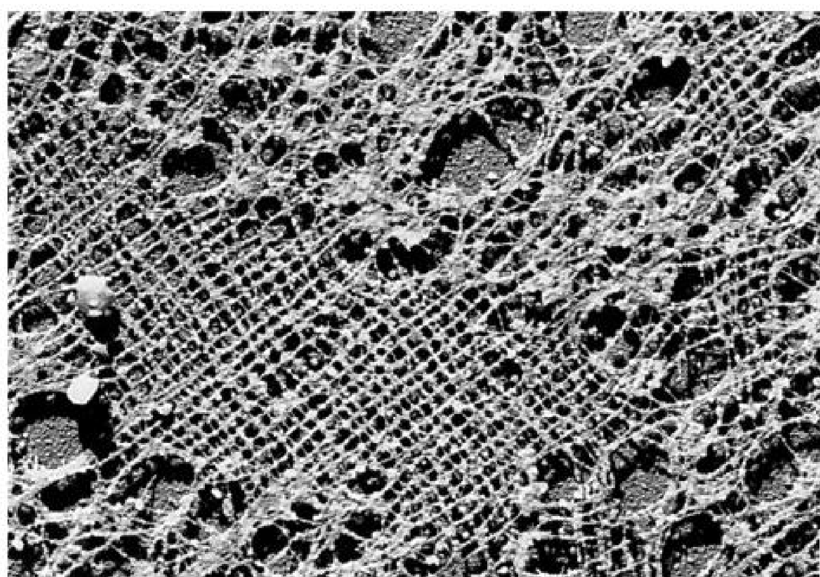
Intermediate filaments are constructed like a multi-stranded rope

- IFs assemble from α-helical monomers into **coiled-coil dimers**
- Two dimers assemble head-tail into half-staggered **tetramers**
- Tetramers form the proto**filament** by end-to-end and side-by-side assembly
- 4 protofilaments form a proto**fibril** and 4 protofibrils form the **final IF** (containing 16 protofilaments)
- IFs have **no polarity** because tetramer is not asymmetric

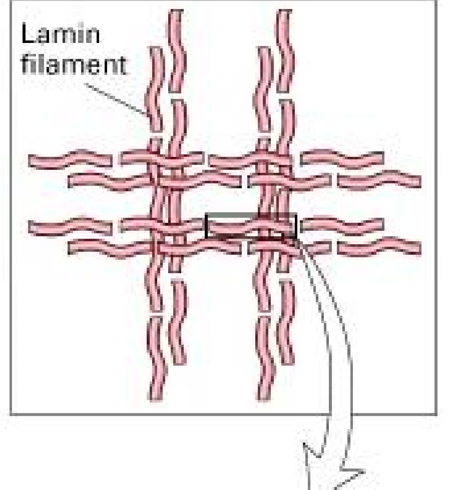


Lamins

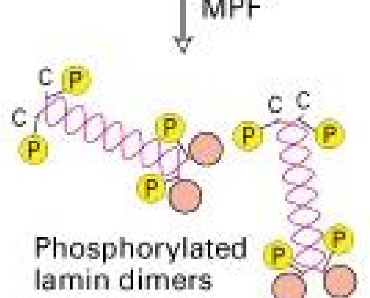
- Exclusively present ***in* the nucleus**. The **evolutionary precursor** of all IFs.
- Lamins form a stable network between the nuclear envelope and the chromatin in the nucleus. They also **organize chromatin structure**.
- 1 gene encodes **lamin A** and **C** (alternative splice products). 2 genes encodes for **lamin B**.



Lamin network on top of the inner nuclear envelope



MPF



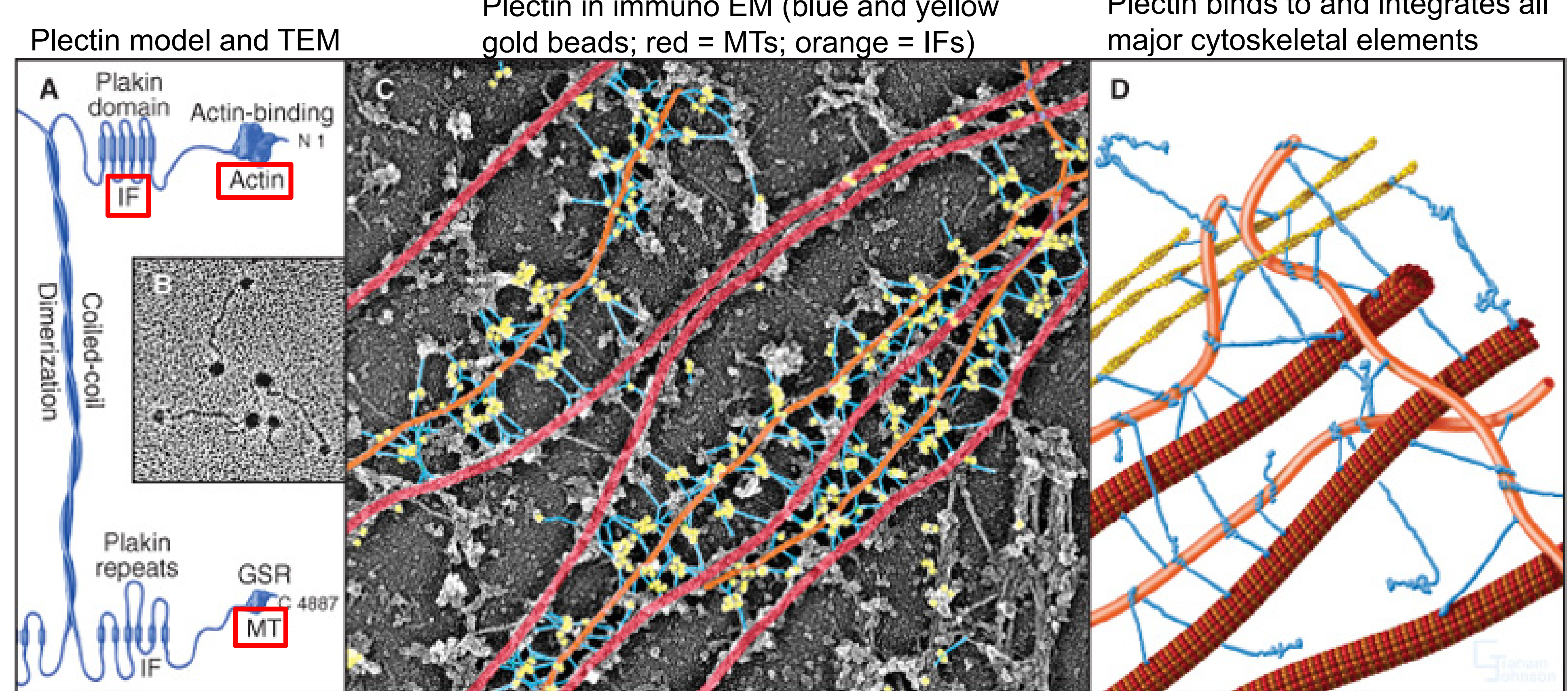
- **Post-translational isoprenylation** of **lamin B** guarantees interaction of lamin with nuclear plasma membrane
- Lamin/membrane interaction mediated by lamin-associated protein **LAP1/2** and **emerin**

Lamin-based diseases caused by mutations in lamin A gene: **muscular dystrophy** (muscle weakness), **cardiomyopathy** (heart muscle disease), **progeria** (accelerated ageing)

- During **prophase** of mitosis lamins are **hyperphospho-rylated** (regulated by MPF, maturation promoting factor) and the **network breaks down**

Intermediate filament-*associated* proteins (IFAPs)

- Though no IF capping, sequestering or severing proteins are known, IFAPs are a class of proteins known to **cross-link and bundle IFs** (they connect to all 3 major cytoskeletal elements)
- Still IFAPs *do not* control IF polymerization or IF breakdown
- IFAPs attach the IF cytoskeleton to the plasma membrane (at cell junctions) and nucleus
- Plectin is an IFAP of the plakin family with N-terminal actin and C-terminal MT binding sites (as well as plakin domains that bind to IFs)
- Plectin has the ability to integrate all three major cytoskeletal elements



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IFAPs and diseases

- **BPAG1n** is an IFAP which connects neurofilaments to F-actin in neurons
- **Filaggrin** is an IFAP that cross links keratin in epidermal cells

PROTEINS ASSOCIATED WITH INTERMEDIATE FILAMENTS

Name	Genes	Molecule	Distribution	Diseases
BPAG1	1	Alternate splicing forms BPAG1e and BPAG1n		Blistering skin and neuropathy in mice
BPAG1e		230 kD; membrane-anchored; binds keratin filaments to hemidesmosomes	Stratified epithelia	
BPAG1n		280 kD, including actin-binding domain; cross-links neurofilaments and actin filaments	Neurons	Axonal degeneration of sensory nerves
Filaggrin	1	37 kD; 10 filaggrins cut by proteolysis from profilaggrin precursor; aggregates keratin	Cornified epithelia	
Lamin-associated		Binds laminin to nuclear envelope	Nuclei of animals	
LAP1	1	57–70 kD isoforms, integral membrane protein		
LAP2	1	50 kD, integral membrane protein		
LBR	1	73 kD, 8 transmembrane spans		
Emerin	1	34 kD protein of the inner nuclear membrane	Animal cells	Emery-Dreifuss muscular dystrophy
Plectin	1	>500 kD homodimer; cytoplasm, focal contacts, hemidesmosomes; binds IF, actin filaments, microtubules, spectrin, MAPs	Animal cells	Blistering skin with muscular dystrophy in mice and humans

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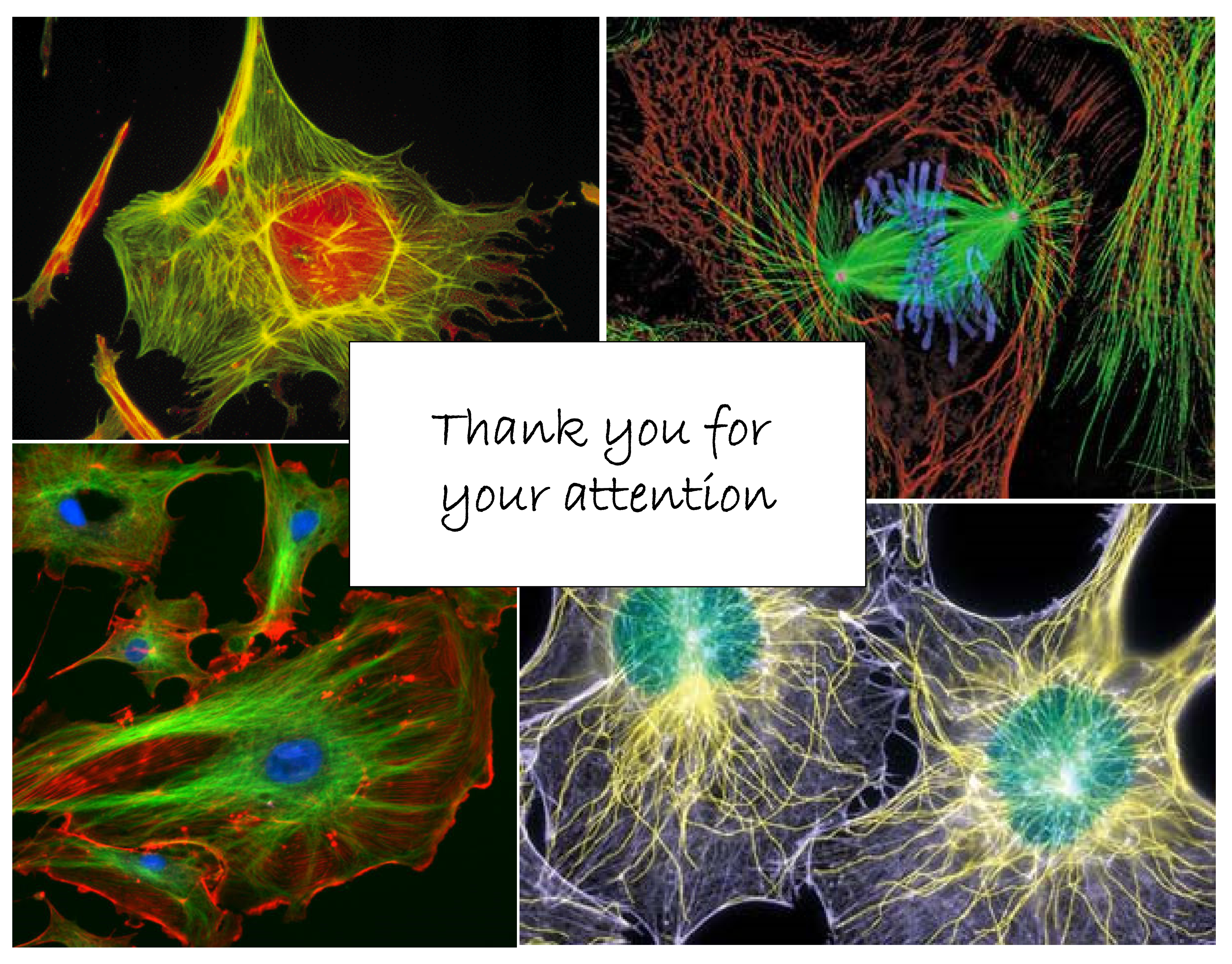
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