Microtubule

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Lesson: Microtubule
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Microtubule

Introduction

Microtubules are important cytoskeletal elements along with microfilaments and intermediate filaments and are responsible for a number of functions within a cell. Microtubules are rigid, hollow, tubular structures having outer diameter of 25nm and wall thickness of 4nm. Microtubules are found in every eukaryotic cell and are composed of globular protein **tubulin.** A microtubule is a heterodimer of 55 kd protein **α- and β-tubulin** arranged in longitudinal rows forming **protofilament** (Fig. 1).

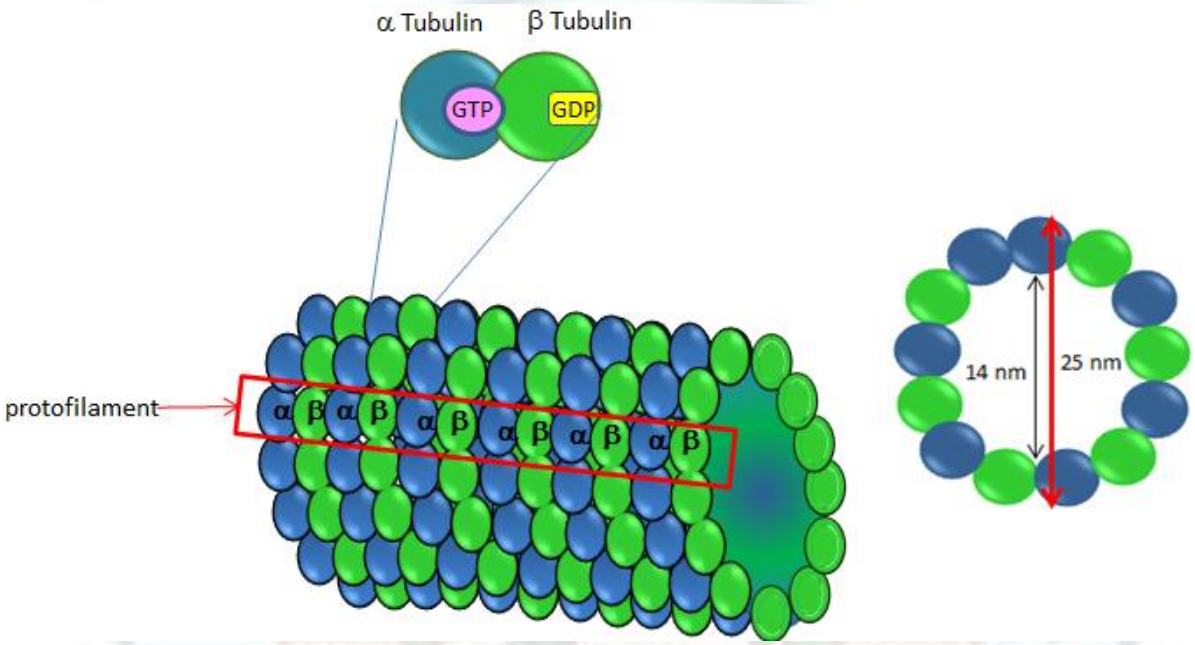

Fig. 1: Structure of Microtubules. Made up of α- and β-tubulin dimers, composed of 13 protofilament assembled around the core.

Each protofilament contains head to tail array of tubulin dimers. In a cross section, 13 protofilaments can be seen arranged in a circular pattern within each microtubule. All the protofilaments have same polarity and therefore the microtubules are also polar with definite end. One of the ends is designated is *plus* (+) end and other as *minus* (-) end. "+" end is fast growing end and has row of β-tubulin subunits while "-" end is slow growing end and has a row of α-tubulin. The plus end grows twice faster as compared to the minus end. Both α- and β-tubulin bind GTP. The GTP binding site on α-tubulin lies at the junction between α- and β-tubulin and therefore it binds GTP irreversibly and does not hydrolyze it. GTP is bound reversibly to β-tubulin and is hydrolyzed to GDP. Eukaryotes also contain a third type of tubulin protein **γ-tubulin** which is not the part of microtubule structure. γ-tubulin is particularly abundant in **centrosome** where it is thought to nucleate the assembly of α- and β-tubulin. The tubulin proteins of eukaryotes show homology with a 40 Kd bacterial GTPase FtsZ which plays an important role in cell division in bacteria. Usually the microtubule assembled in a cell is singlet having 13 protofilaments but it has been found that nematodes contain microtubules with 11 or 15 protofilaments. Cells also contain doublets (found in cilia and flagella) having two microtubules designated as A and B and

triplets (found in centrioles and basal bodies) having three microtubules designated as A, B and C (Fig. 2). In these structures, only A microtubule is complete with 13 protofilaments.

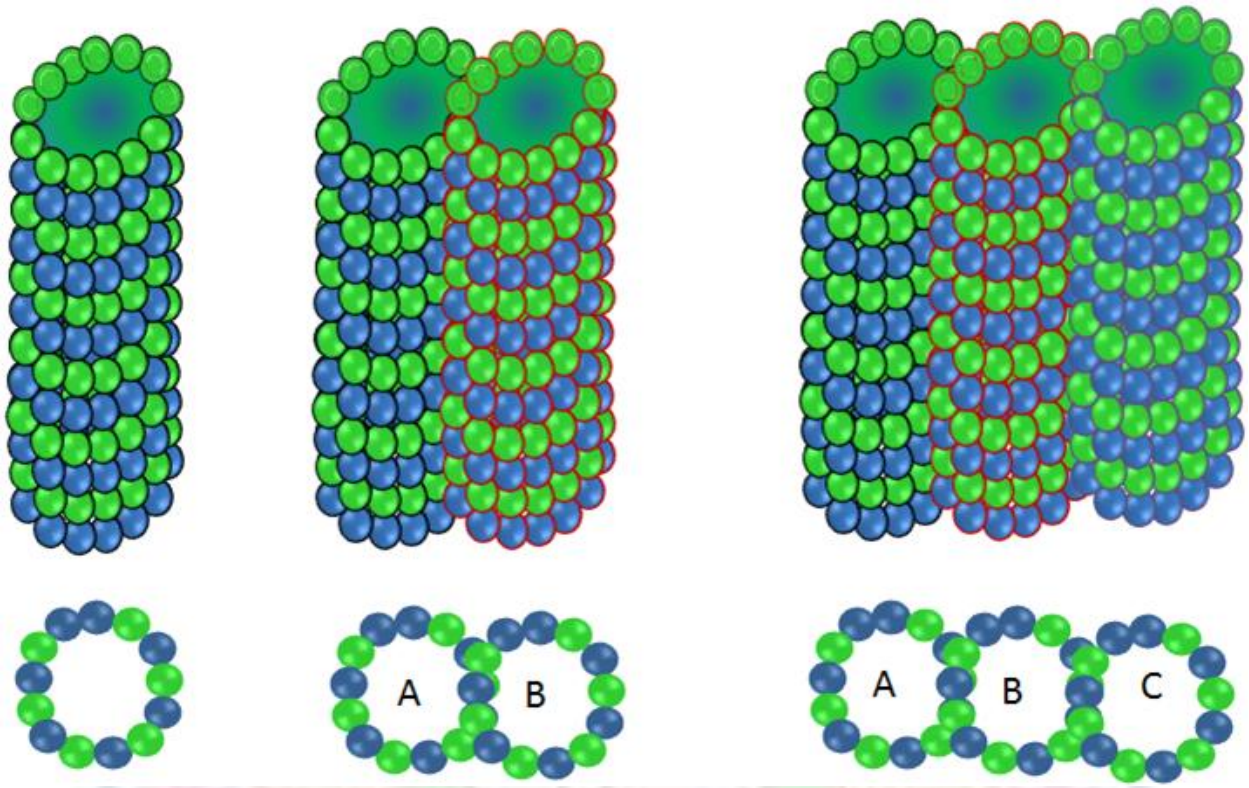

Fig. 2: Figure showing the arrangement of protofilaments in singlet, doublet, and triplet microtubules. In doublets and triplets, only A microtubule is complete with 13 protofilaments.

Microtubule assembly and disassembly

The assembly of microtubule take place by polymerization of αβ subunits.The assembly of microtubules from αβ tubulin dimers takes place in two phases: nucleation and elongation. These two phases can be understood in the three steps where first step corresponds with the nucleation phase and later steps with the elongation phase (Fig. 3). The three steps are:

1. Formation of protofilaments: αβ tubulin dimers associate in head to tail fashion forming protofilaments
2. Lateral association between protofilaments to form curved sheets
3. Growth of the microtubule by addition of the subunits at the ends

Microtubule

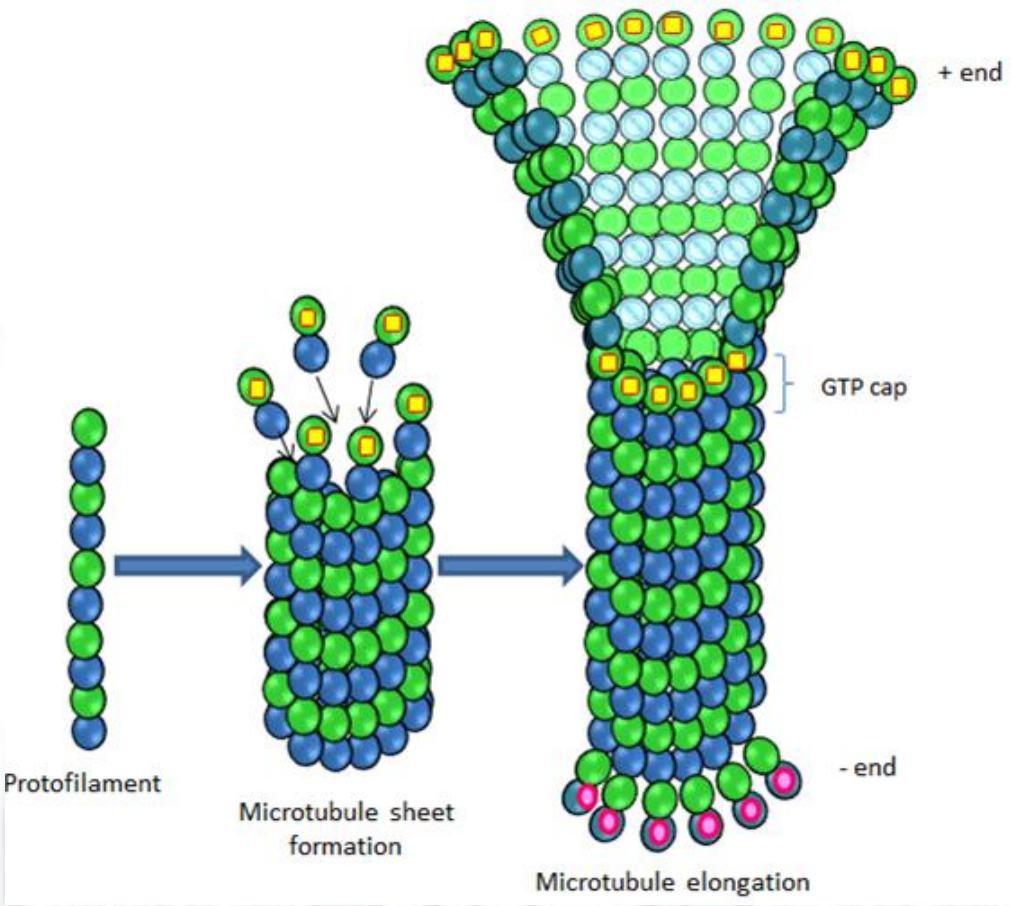

Fig. 3: Assembly of microtubules. αβ tubulin dimers associate in head to tail fashion forming protofilaments, lateral association between protofilaments results in forming curved sheets which grow by addition of the subunits at the ends.

The polymerization of αβ tubulin dimers into microtubules was first studied in vitro by Richard Weisenbergin 1972. He used cell homogenates, Mg^{2+} and GTP at 37° C and found that the assembly and disassembly is dependent on the temperature. Now it is well established that the tubulin subunits in the presence of GTP undergo polymerization at 37° C. If the temperature is lowered to 4° C, the microtubule depolymerize to αβ tubulin subunits. The rate of polymerization also depends on the concentration of αβ subunits also known as critical concentration (C_c).So, the αβ subunit undergo polymerization at the concentration higher than critical concentration while undergo depolymerization at concentration lower than critical concentration. The C_c for polymerization also differs at both the ends. The αβ dimers preferably bind to the plus end at the concentrations higher than the C_c. Microtubules undergo a process known as **treadmilling** if the concentration of αβ subunit is higher than *C*c at the plus end but lower than *C*c at minus end. During this process, the αβ subunits are dissociated from minus end and are balanced by the addition of αβ subunits at the plus end (Fig. 4).

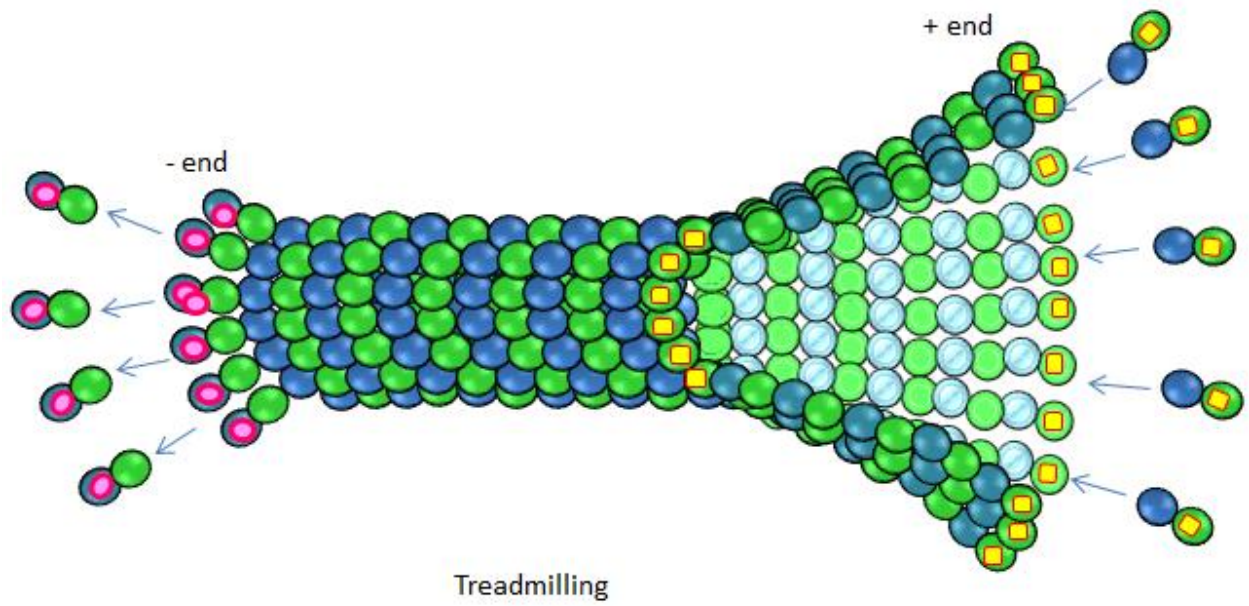

Fig. 4: Treadmilling in Microtubules. The αβ subunit are dissociated from minus end and are balanced by the addition of αβ subunit at the plus end. Results when the concentration of αβ subunit is higher than *C*c at the plus end but lower than *C*c at minus end.

As already mentioned, both α and β tubulin bind GTP. Soon after the polymerization, the GTP bound to β tubulin is hydrolyzed to GDP. If the rate of polymerization exceeds the rate of GTP hydrolysis, the plus end has a cap of GTP-bound subunits. The presence of this cap of GTP dimers is thought to favor the addition of more subunits resulting in growth of the microtubule (Fig. 3). The GTP hydrolysis weakens the association of the tubulin subunit resulting in their dissociation from the microtubule. Once dissociated and released from a microtubule the dimers enter the soluble pool where there is exchange of GDP with GTP so that these can be reincorporated into the microtubule. It has been found that the rate of dissociation of a GDP bound tubulin dimer is four orders of magnitude faster as compared to GTP bound tubulin dimer. If the rate of GTP hydrolysis is more as compared to the rate of polymerization, it results in the behavior known as **dynamic instability** (Fig. 5) first described by Timothy Mitchison and Marc Kirschner in 1984 while working at the University of California.

Do you know???

Temperature is an important factor affecting the stability of microtubules. The rapid changes in temperature has been used to isolate the tubulin and their associated proteins from cell. The temperature has also been used to study dynamic behavior of microtubules where the live cells were injected with fluorescent tubulin dimers. This was followed by rapid cooling to destabilize the microtubules and heating at 37^{o} C to allow incorporation of fluorescent tubulin dimers. The study showed that some microtubules grow, some shrink while some alternate between growth and shrinkage displaying the dynamic nature.

Microtubule

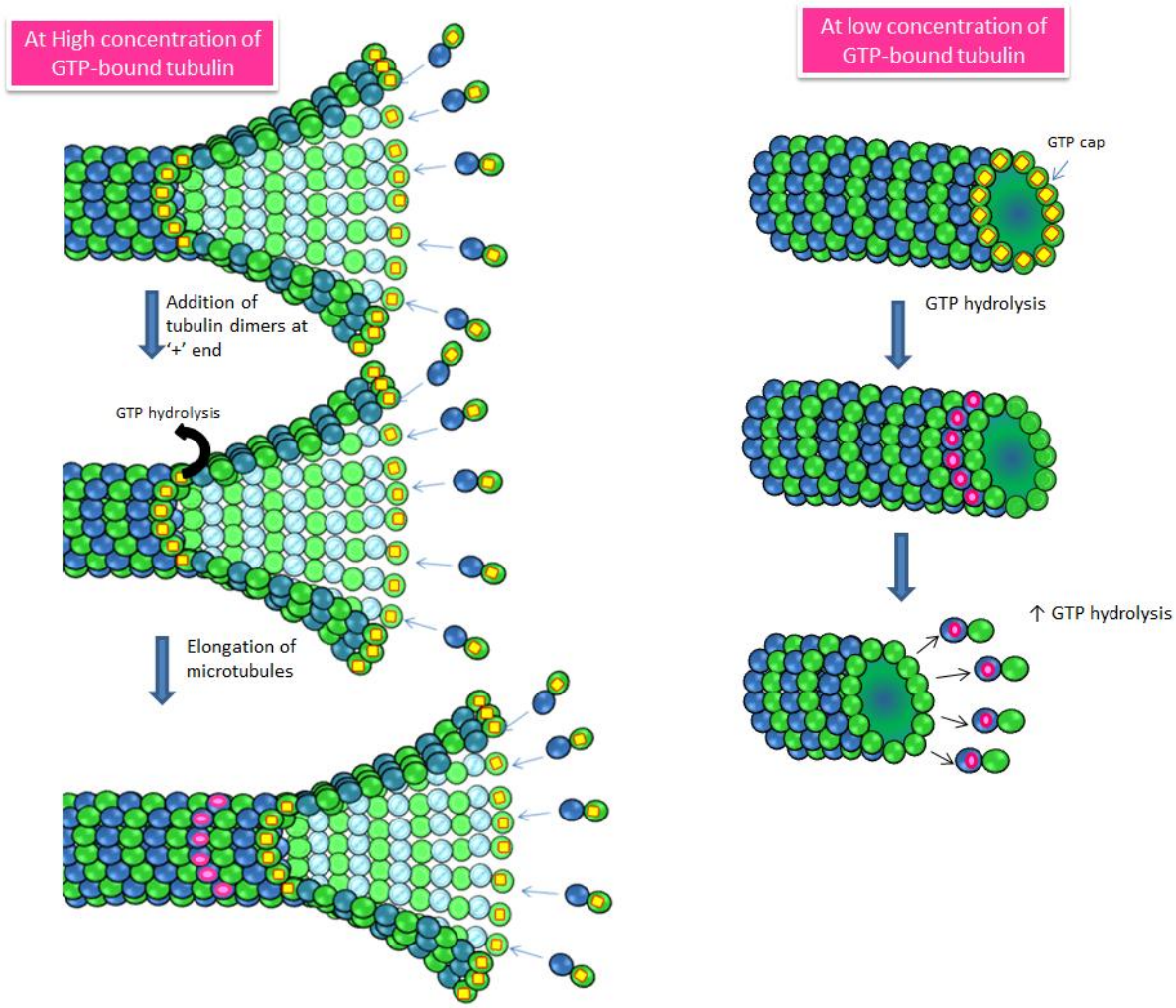

Fig. 5: Dynamic instability in microtubules. It results from the hydrolysis of GTP bound to β-tubulin soon after polymerization.

Dynamic instability is the inherit property of microtubules because of the hydrolysis of bound GTP. The stability of microtubules depends on two factors:

1. **Critical concentration (C_c):** As already mentioned if the concentration of αβ dimers is more than C_c, microtubules undergo polymerization or growth and if the concentration of αβ dimers is less than C_c, microtubules undergo depolymerization or shrinkage. At C_c some show growth while other show shrinkage.
2. **Presence of GTP at β-tubulin:** As already mentioned GDP bound tubulin dimer dissociates faster as compared to GTP bound tubulin dimer. If the rate of GTP hydrolyses is more than the rate of subunit addition, the plus end has a cap of GDP-bound subunits which results in instability and rapid shrinkage of microtubules.

Microtubule

The factors which promote disassembly of microtubules are low temperature, increased Ca^{2+} concentration and various chemicals like colchicine.

Do you know???

Many drugs bind microtubule and affect their assembly. These drugs include colchicine, colcemid, vinblastine, vincristine, nocodazole, podophyllotoxin and taxol. Colchicine, colcemid, vinblastine and vincristine inhibit microtubule polymerization and therefore block mitosis. Taxol stabilizes microtubule by preventing its disassembly and also the cell from assembling new microtubule. These drugs are therefore used as chemotherapeutic agents against cancer.

Microtubule organization within the cell

In interphase animal cells, microtubules radiate and extend from the center towards the cell periphery from the structures known as **centrosomes**. Thus centrosome acts as MTOC (Microtubule organizing center). The minus end of the microtubule is anchored within the centrosome while the plus end lies towards the cell periphery establishing polarity within the cell (Fig. 6a).

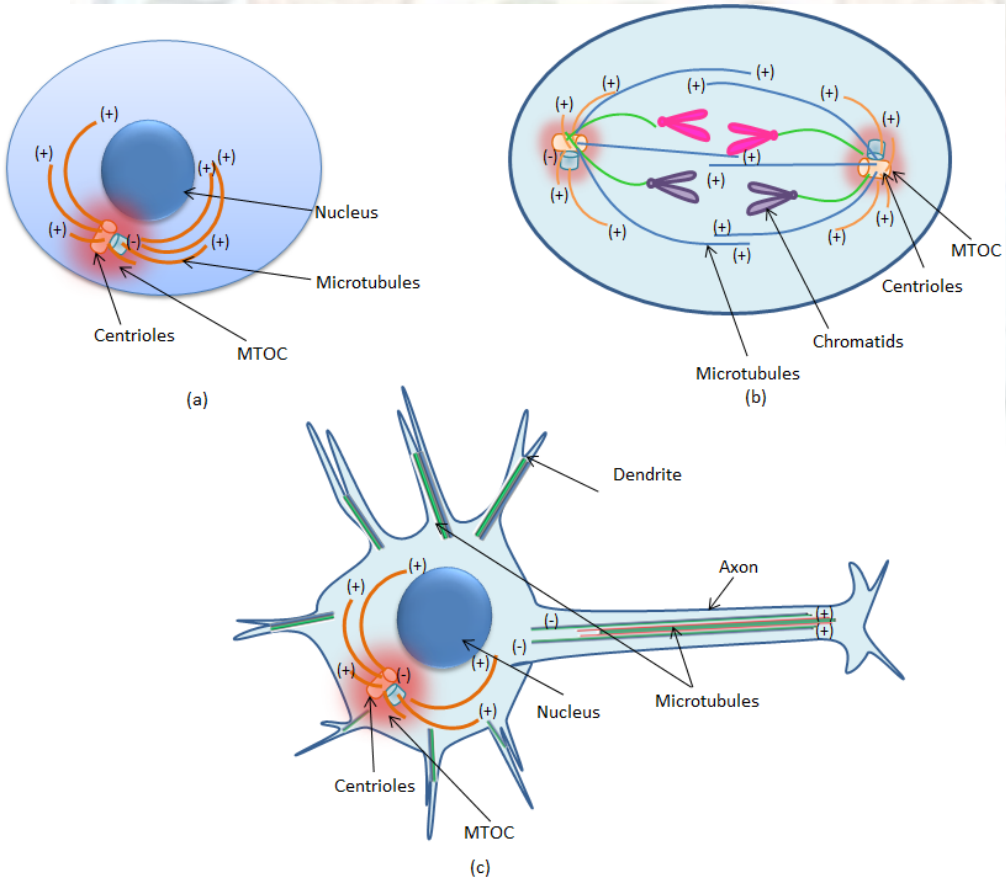

Fig. 6: Organization of microtubules in (a) an interphase cell (b) mitotic cell (c) nerve cell.

The whole microtubule network of a cell is reorganized during mitosis (Fig. 6b). Nerve cells present an excellent example of the organization of microtubules where the microtubules are oriented with their plus end away from the cell body and minus end towards the cell body (Fig. 6c).

The centrosome contains microtubule-associated proteins and a pair of **centrioles** which are 0.2 µm in diameter and held perpendicular to each other, surrounded by electron-dense peri-centriolar material. The pericentriolar material contains many proteins essential for initiating the assembly of microtubules. Centrioles contain nine triplet of microtubules designated as A, B, and C tubules, out of which only A is complete with 13 protofilaments. The centrioles contain a unique protein δ-tubulin which forms the part of microtubule triplet. The centrioles also have various extensions that project into the centrosome (Fig. 7). The two centrioles are connected by means of centrin fibers. The centrioles are not required for microtubule formation and are absent from the MTOCs of plants and fungi. During mitosis centrosomes duplicate and migrate towards opposite pole where they organize mitotic spindles required for the segregation of chromosomes into the daughter cells.

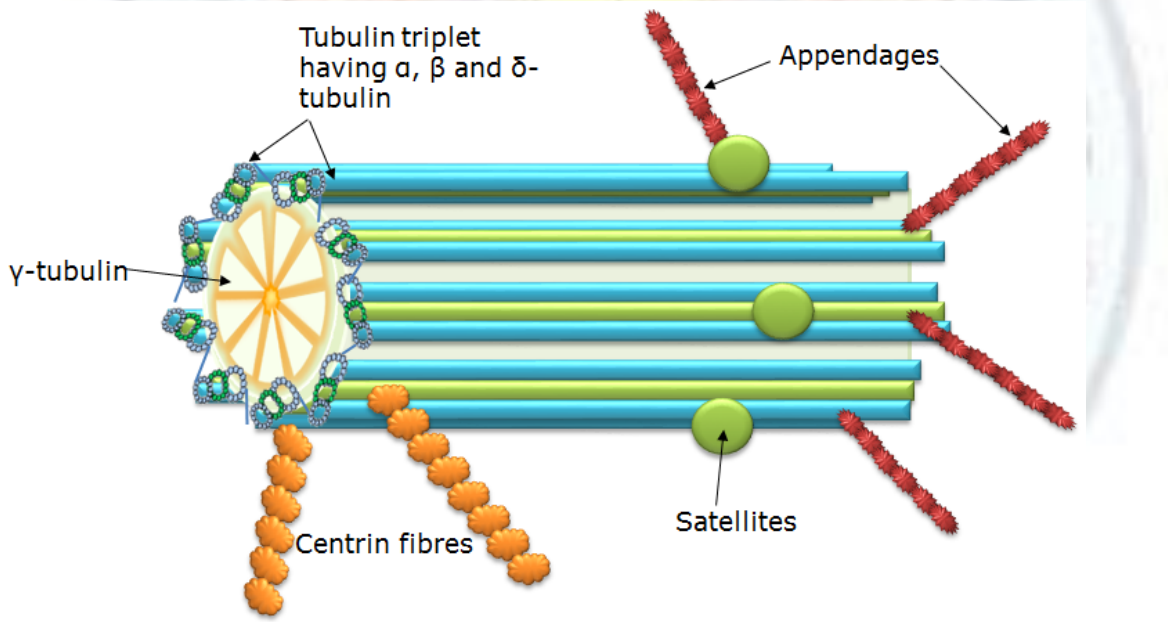

Fig. 7: Structure of centriole having outer triplet of microtubules. The two centrioles are connected with each other through centrin fibers.

Centrosomes are not the only MTOC found in cells. Basal bodies which give rise to cilia and flagella are similar to centrioles in structural organization. Basal bodies and centrioles can give rise to one another as is seen in the sperms where the basal body is derived from the centriole of spermatocyte.

The significant protein found in the centrosome is γ-tubulin which plays an important role in initiating the assembly of microtubules. γ-tubulin was first identified in the mid-1980s during genetic studies in fungi along with a lattice protein pericentrin. γ-tubulin along with eight proteins form a ring-like complex known as **γ-tubulin ring complex**(γ-TuRC) which has diameter of 25nm. γ-tubulin and pericentrin have also been found in the MTOCs of the organisms that lack centrioles. In plant cell γ-tubulin is found to be concentrated near the periphery of nucleus and nucleates the assembly of microtubule from the periphery of nucleus.

Microtubule-Associated Proteins

Within a cell the stability and assembly of microtubules is affected by a number of proteins referred to as Microtubule-associated proteins (MAPs). The MAPs can be classified in two categories: stabilizing MAPs which stabilize microtubule structure and destabilizing MAPs which destabilize microtubule structure. The MAPs which stabilize the structure of microtubules like MAP2 were first to be identified and are referred to as "classical MAPs". These MAPs have two domains:

1. **Basic microtubule-binding domain:** it consists of conserved positive charge amino acids which bind to the negatively charged C-terminal of tubulin and stabilize the structure by preventing the repulsion between tubulin subunits.
2. **Acidic projection domain:** it binds to other microtubules and intermediate filaments and controls the spacing between microtubules.

Different MAP have been identified and isolated including MAP1A, MAP1B, MAP2, MAP4, Tau, and CLIP170. MAP1A and MAP1B are found in neuronal and non-neuronal cells and are the products of proteolytic cleavage of same polypeptide. MAP2 is found in dendrites and MAP4 in found in all non-neuronal cells. Tau is one of the most widespread MAP and cross-links microtubules into thick bundles. Tau is also most extensively studied protein as it has been implicated in the development of Alzheimer's disease which is characterized by *neurofibrillary tangles*. These tangled filaments contain tau protein which are unable to bind to the microtubules. CLIP170 cross-links microtubules to chromosomes. The binding of stabilizing MAPs prevents the dissociation of tubulin subunits thereby stabilizing the microtubule structure. The binding of MAPs to microtubules is regulated by phosphorylation of the projection domain by MAP kinase. MAPs once phosphorylated cannot bind to the microtubules resulting in their disassembly.

Destabilizing MAPs promote microtubule disassembly by either breaking them or by increasing the rate of disassembly at the ends. The proteins that break microtubule include katanin which require ATP for fragmentation of microtubule. Some proteins like Op18 or stathmin increase the rate of depolymerization causing disassembly of microtubules. It is also thought to bind tubulin dimers preventing their re-association into the microtubule.

Microtubule

Do you know???

Microtubules of the mitotic spindle, cytoskeleton are quite unstable while microtubules of neurons, centrioles, cilia and flagella are highly stable.

Microtubule function

Microtubules perform a number of functions in a cell some of these are:

1. Intracellular transport of vesicles
2. Intracellular transport and positioning of various cell organelles
3. Cell movement by cilia and flagella
4. Separation and movement of chromosomes during mitosis

The intracellular transport of vesicles and positioning of organelles require motor proteins which hydrolyze ATP to move along the microtubule tracks.

Microtubule motor proteins

Two key motor proteins are identified: **kinesin** and **dynein** which move along the microtubules in opposite directions. Most kinesins move towards plus end, and all dyneins towards negative end of the microtubule.

The first microtubule motor protein to be identified was dynein, isolated from cilia in 1965 by Ian Gibbons of Harvard University. This form of dynein was called **axonemal dynein**. Another protein previously identified as microtubule-associated protein MAP-1C was found to be related to axonemal dynein and referred to as **cytoplasmic dynein**. It took almost 20 years to identify the cytoplasmic dynein that was isolated from mammalian brain. The second motor protein kinesin was isolated in 1985 by Ronald Vale, Thomas Reese and Michael Sheetz from the cytoplasm of squid giant axons. This kinesin is now known as kinesin I or conventional kinesin which was found to move towards the plus end of the microtubule and therefore is called plus end-directed microtubular motor. The dyneins on the other hand move towards the minus end of the microtubule and called minus end-directed microtubular motor.

Kinesin I isolated from squid giant axons is a molecule of about 380 Kd and is a dimer of two heavy chains (about 120Kd) and two light chain (about 64 Kd). The N-terminus of heavy chain forms large globular head domains which forms the motor domain (Fig. 8). The motor domain binds with the microtubule and ATP which provides energy for the movement. The heavy chains also have long α-helical regions wind around each other forming coiled-coil structures and small globular tail domains. The tail domain binds the light chains and the cargo to be transported through the kinesin light chain .Light chains of kinesin I contains a sequence known as tetratrico peptide sequence which is thought to interact with membrane receptor proteins of cargoes to be transported. The motor domains from

different kinesins are found to share similar amino acid sequences while the tails domains have various sequences consistent with their role in binding different cargoes.

Do you know???

Nerve cells give an excellent example of the role of microtubules and associated motor proteins in transport of materials. Protein synthesis takes place in the cell body and the products must be transported to the tip of axon (*Anterograde* transport). The endocytotic vesicles must be transported back to the cell body for refilling with neurotransmitter like acetylcholine (retrograde transport). In nerve cells, the microtubules are oriented with their plus end away from the cell body i.e. towards the tip of the axons. The movement of cargo in the cell-free system can be visualized using video microscopy. In the nerve cells, kinesin I transport the secretory vesicles containing neurotransmitter andorganelles from cell body to the axon tip while dyneins and other minus end directed kinesins carry empty vesicles from axonal tip to the cell

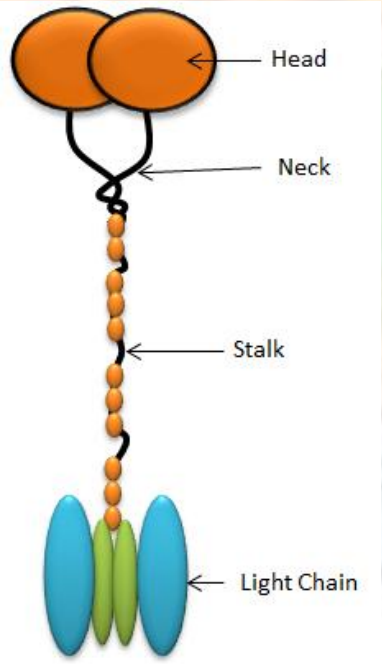

Fig. 8: Structure of kinesin showing heavy and light chains.

The motor domain of kinesin, though smaller as compared to myosin, share significant homology with latter suggesting a common ancestor of both motor proteins. Till date about 10 different kinesin subfamilies have been identified, all containing globular head but vary in the tail domain. Most of these kinesins move towards +end like kinesin I while others move towards –end like members of small family kinesin-14 which includes Ncd protein of

Drosophila and some do not move at all like kinesin-13 family. All kinesins that move towards +end have motor domain at the N-terminal (N-type), those which move towards –end have motor domain towards C-terminal (C-type), while some which show do not move have motor domain at the middle of the heavy chain (M-type, also called middle motor kinesins). Some M-type move towards +end like N-type motor. Those kinesins that do not move along microtubules play an important role in depolymerization and are called **microtubule depolymerases**. Majority of kinesins have two heavy chains like kinesin I, some like KIF1have a single heavy chain while some like BimC have four heavy chains and is bipolar.

Based on the type of cargo, transported kinesins can be classified as cytosolic and mitotic kinesins. Cytosolic kinesins transport vesicles and orgenelles. These kinesins include kinesin I which transports lysosomes, KIF1B transports mitochondria and KIF1A transports synaptic vesicles to axon terminals. Mitotic kinesins take part in the assembly of spindle and separation of chromosome. These kinesins include CENP-E which is a kinetochore-associated protein and BimC.

It has been found that kinesins bind mostly to β-tubulin and move along a single protofilament at maximum velocity of 1μm per second using ATP hydrolysis. Hydrolysis of a single ATP molecule results in a single step of 8nm in length which corresponds to the length of one tubulin dimer.

Dyneins are large molecules with molecular weight more than 2000 Kd and composed of two or three heavy chains complexed with variable number of intermediate and light chains (Fig. 9). The number of intermediate and light chains are poorly known.

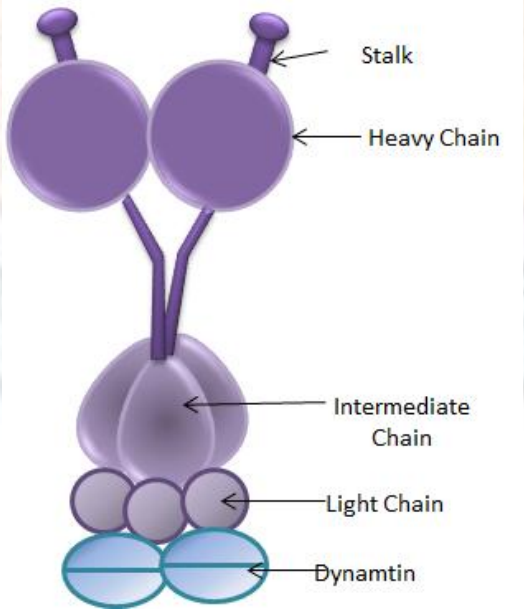

Fig. 9: Structure of dynein showing heavy, intermediate and light chains.

The heavy chains contain N-terminal head which forms the motor domain responsible for binding microtubules and ATP; and an elongated projection called stalk. The stalk contains microtubule binding site. The tail also known as stem binds intermediate and light chains. The head domain of dyneins is larger as compared to kinesins. Dyneins are divided into two classes: (a) **cytosolic dyneins** which transport vesicles and play important role in the movement of chromosomes during mitosis and **axonemal dyneins** found in cilia and flagella and responsible for their beating. Unlike kinesins, cytoplasmic dyneins cannot bind and transport cargo by themselves. They require additional multi protein complex known as **dynactin** which consists of eight subunits including proteins like **Glued** which binds microtubules, **dynamtin** which interacts with dynein light chains and **Arp1** which binds spectrin.

Cargo transported by kinesins and dyneins

Like nerve cells, many non-neuronal cells have definite arrangement of microtubules providing the cell fixed polarity. In a cell the microtubules are oriented with their plus end towards the cell periphery and negative end embedded in the centrosome. The members of kinesin family move vesicles and organelles (e.g., peroxisomes and mitochondria) from the center towards the cell periphery i.e. towards the plasma membrane. On the other hand, cytoplasmic dynein transport organelles from the periphery towards the cell center (Fig. 10).

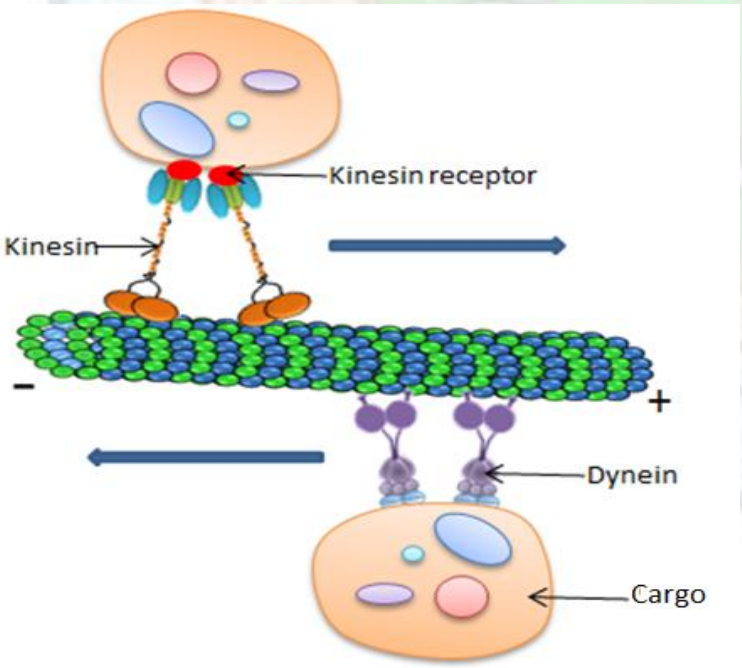

Fig. 10: Intracellular transport of kinesins and dyneins. The members of kinesin family move vesicles and organelles towards the cell periphery while cytoplasmic dynein transport organelles from the periphery towards the cell center.

The motor proteins also play an important role in positioning the organelles within a cell. Kinesin I and other plus end directed kinesins help in positioning of ER, mitochondria, lysosomes and peroxisomes towards the cell periphery (Fig. 11). Treating the cell with drugs that depolymerize the microtubule network result in retracting the ER towards the cell center. Similarly cytoplasmic dyneins play important role in positioning the Golgi bodies after mitosis.

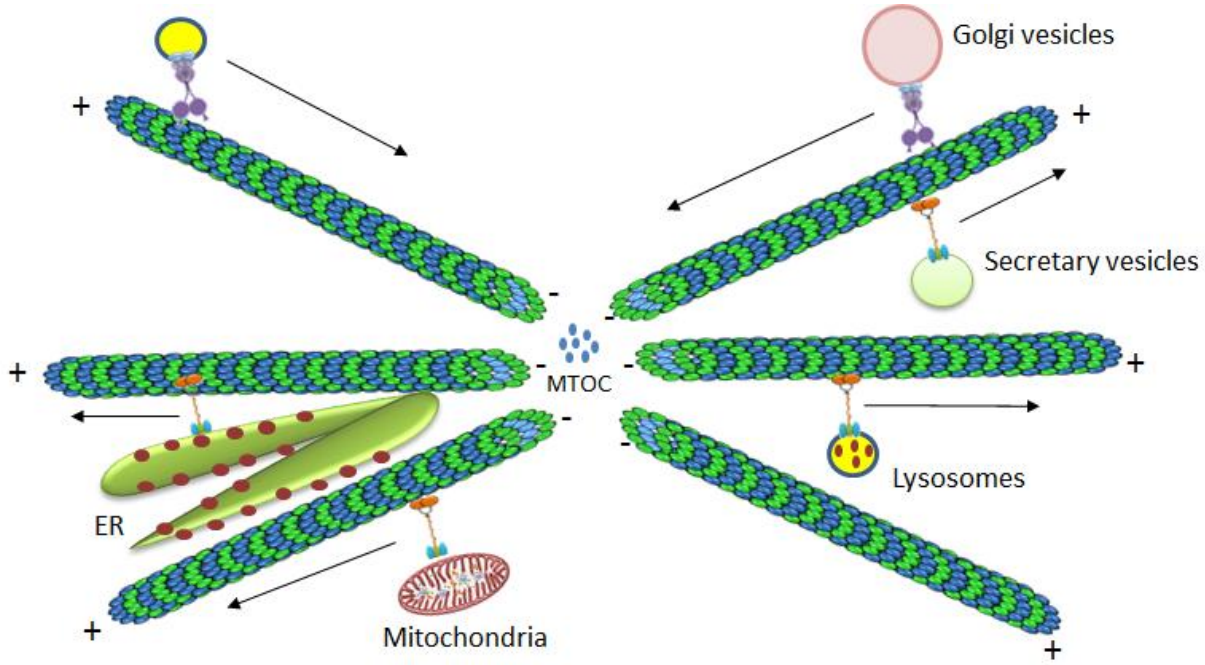

Fig. 11: Role of kinesins and dyneins in positioning of orgenelles within the cell.Kinesin I and other plus end directed kinesins help in positioning of ER, mitochondria, lysosomes and peroxisomes towards the cell periphery while cytoplasmic dyneins play important role in positioning the Golgi bodies after mitosis.

Do you know???

Cytoplasmic dyneins are thought to play role in transporting the HIV virus into the cell nucleus.

Cilia and Flagella

Microtubule

Cilia and flagella are hair-like projections of the plasma membrane which are supported by microtubules. Cilia and flagella are about 0.25 µm in diameter and are found in many eukaryotic cells. Cilia are usually 10 µm in length and beat back and forth motion to bring about cell movement like in ciliated protozoans, and propel mucous and fluid over the surface of epithelium cells like in respiratory tract. The movement of cilia can be compared to that of an oar. The cilia beating has a power stroke followed by recovery stroke. During power stroke the cilium pushes against the surrounding medium propelling the cell forward while in recovery stroke it moves back and does not pushes the medium. The cilium is more rigid during the power stroke and becomes flexible during the recovery stroke. Many cells contain a single non-motile cilia which is thought to have sensory function. Flagella differ from cilia in length (flagella are about about 200 µm in length) and wavelike pattern of beating.

Structure

Both cilia and flagella are similar in structures made up of microtubules and other associated proteins. The basic structure of both cilia and flagella is known as **axoneme** first described in 1952 by Irene Manton (in plants) and Don Fawcett and Keith Porter (in animals).An axoneme is composed of outer nine doublet of microtubules surrounding the central singlet pair giving characteristic "9+2" pattern (Fig. 12a). The outer doublet consists of one complete microtubule having 13 protofilaments (A tubule) and one incomplete microtubule having 10-11 protofilaments (B tubule) fused with the A tubule.

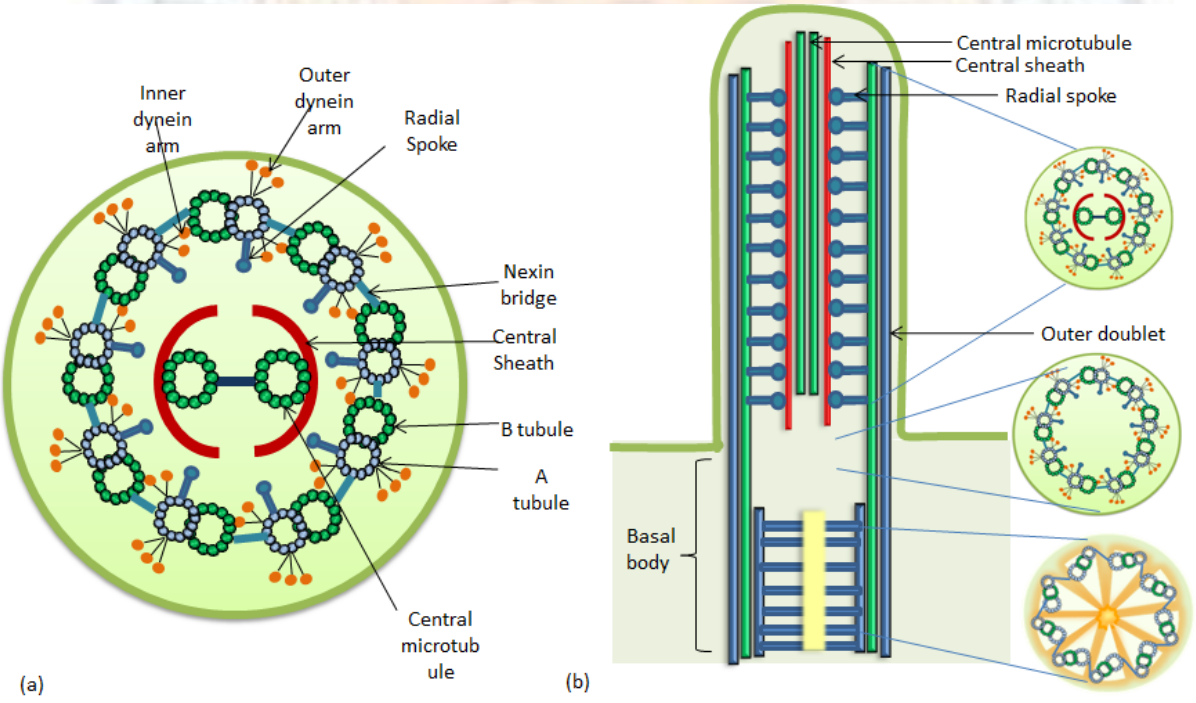

Fig. 12: Structure of axoneme from cilia and flagella. a) The axoneme consists of 9+2 pattern of arrangement of microtubules, nine outer doublets and central singlet pair. In doublet only A tubule is

complete with 13 protofilaments. b) Diagrammatic representation differences in arrangements of microtubules in basal body and axoneme of cilia and flagella.

All microtubules in the axoneme have same polarity with their plus end at the tip and minus end anchored in the basal body. A basal body is similar to centriole and contain nine triplet of microtubules (Fig. 12b). Basal body arises from centriole and play an important role in initiating the growth of axonemal microtubules and also anchor cilia and flagella. The central singlet microtubules is surrounded by inner sheath which is connected to the A tubules of the outer doublet microtubules by radial spokes. The A tubule consists of a pair of dynein arms referred to as inner and outer dynein arm. The outer doublets are connected to one another by an elastic protein nexin.

Do you know???

Both cilia and flagella arise form basal body. If a cilium or flagellum is cut, basal body starts its regeneration by addition of tubulin dimers to the plus end of the microtubule which is the outer end. The transport of the molecules takes place in the space between the outer doublet and the plasma membrane in a process known as intra-flagellar transport (IFT). The IFT requires both kinesins and dyneins. The movement of these motor proteins takes place along the protofilaments of the outer doublet microtubules.

Mechanism of beating of cilia and flagella

The beating of cilia and flagella results from the sliding of the outer doublet microtubules relative to one another. The dynein arms are known to play an important role in this process. The dynein tail along with intermediate and light chains binds to the outer surface of the A tubule while the motor heads domains project towards the B tubule of the adjacent doublet. During the beating of cilia or flagella, the dynein motor heads domains bind to the B tubule of the adjacent doublet and move towards the minus end resulting in the sliding of the A tubule towards the basal end or the minus end of the adjacent B tubule (Fig. 13).

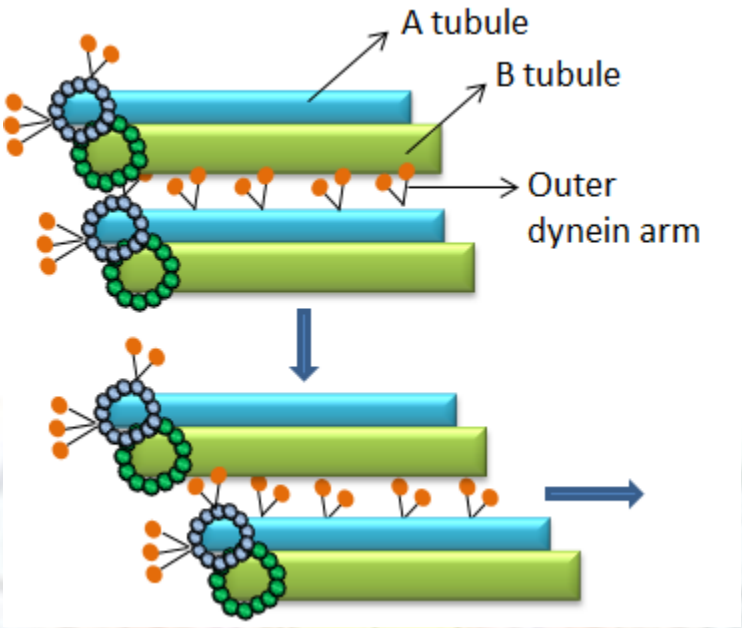

Fig. 13: Mechanism of movement of cilia and flagella. The dynein arms attached to the outer A microtubule play an important role in sliding of the microtubules past each other.

The sliding requires ATP and also involves conformational change in the head domain. This causes the bending of the microtubules resulting in the beating movements. The dynein arms then detach from the adjacent B tubule and reattach to begin the new cycle. The nexin protein links play an important role in beating of cilia and flagella by limiting the amount by which the adjacent doublets can slide past one another. Though this model is accepted widely the exact mechanism underlying this process is still poorly understood.

Mitosis and role of motor proteins

Mitosis is the last phase of cell cycle and involves separation and movement of chromosomes to the daughter cells. The movement of chromosomes involves the microtubules and associated motor proteins. The mitotic spindle is made up of microtubules which undergo changes during mitosis. During G1 phase, the centrioles and other components of centrosomes are duplicated but remain together. During the beginning of mitosis the two pairs of centrioles move towards the opposite sides of the nucleus. In an interphase cell, the microtubules are oriented with their plus end towards the cell periphery. With the onset of mitosis the microtubule dynamics change significantly and there is complete reorganization of the microtubule. The interphase microtubules disappear and there is formation of astral and spindle microtubules. The centrosomes migrate to the opposite ends establishing two poles of the mitotic spindle. The four different types of microtubules radiate from the centrosomes: **kinetochore microtubules, chromosomal microtubules, polar microtubules** and **astral microtubules** (Fig. 14). Out of these four, kinetochore microtubules, chromosomal microtubules and polar microtubules form the mitotic spindle. The minus end of all these microtubules is found within the centrosomes.

Microtubule

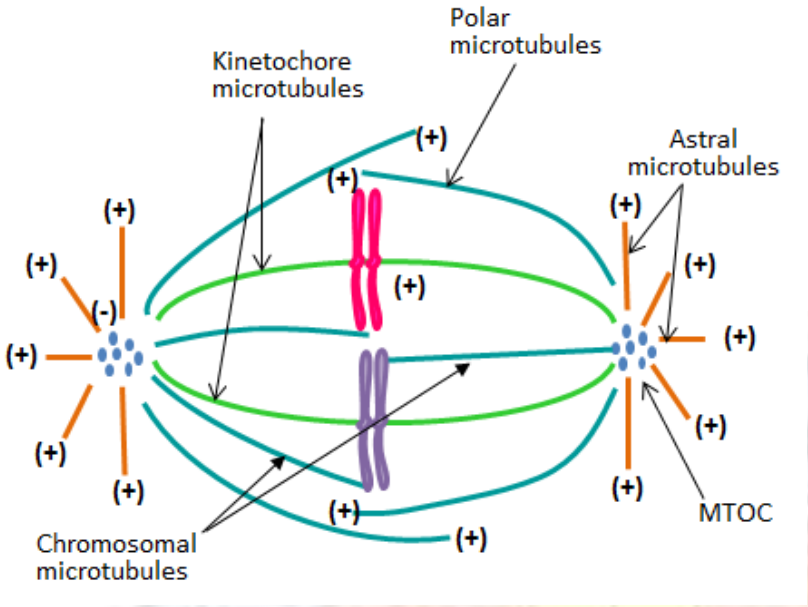

Fig. 14: Different types of microtubules forming the mitotic apparatus. The minus end of microtubule is within the spindle pole.

The kinetochore microtubules attach to chromosomes at their centromere. The centromere is associated with different proteins forming **kinetochore.** The location of the centromere and kinetochoreis controlled centromeric DNA. Kinetochore has associated microtubule-binding proteins like CENP-E and microtubule motor proteinslike mitotic kinesin MCAK and cytosolic dynein. These associated proteins play an important role in attaching the kinetochore to the kinetochore microtubule. The kinetochore also plays an important role in regulating chromosome separation and translocation.

The kinetochore in animal cells consist of an inner and outer layer place in a **fibrous corona** (Fig. 15). The inner layer consists of the proteins that bind centromeric DNA while outer layer binds the kinetochore microtubules. The microtubule-binding proteins and microtubule motor proteins are found in the outer layer and fibrous corona.

The chromosomal microtubules bind to the chromosomal ends via chromokinesin. The polar microtubules do not bind chromosomes, rather they overlap with other polar microtubules from the opposite pole at the cell center. The astral microtubules radiate from the centrosomes towards the cell cortex and have free plus ends. Astral microtubules play an important role in positioning the mitotic apparatus.

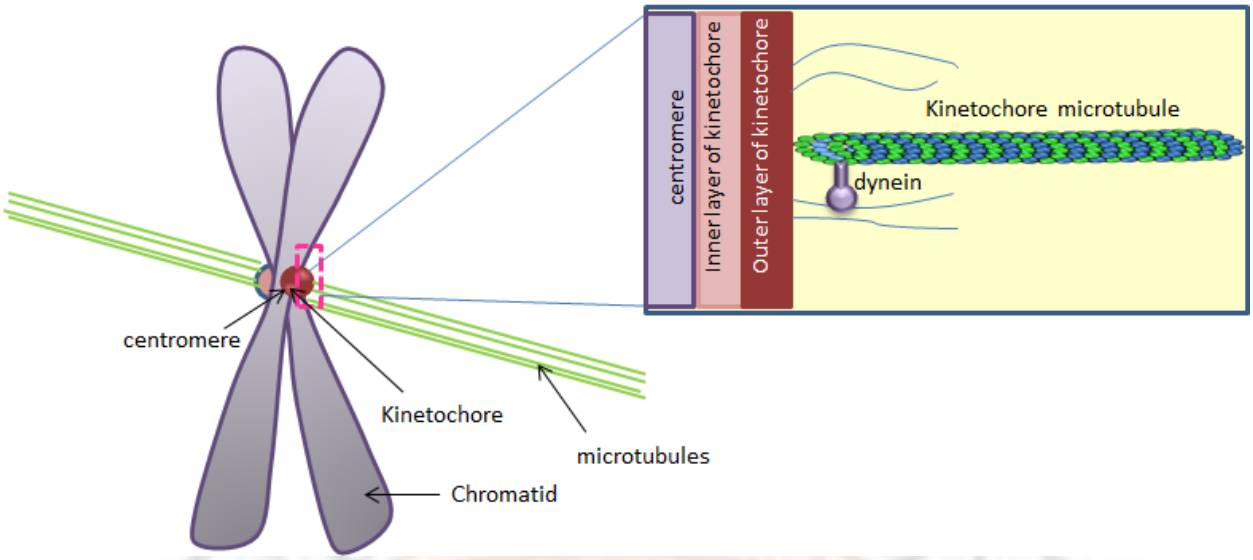

Fig. 15: The structure of kinetochore in animal cells. The kinetochore consists of an inner layer and an outer layer. The inner layer consists of the proteins that bind centromeric DNA while outer layer binds the kinetochore microtubules. The microtubule-binding proteins and microtubule motor proteins are found in the outer layer and fibrous corona.

The two spindle halves are held together by the lateral interactions between the polar microtubules whose plus ends overlap at the cell center and end-on interactions between the kinetochores and the kinetochore microtubules.

Events during mitosis

One the centrosomes are duplicated and migrated to opposite poles there is change in the microtubule dynamics. New microtubules radiate from the centrosomes which form the spindle microtubules and astral microtubules. The spindle microtubules show dynamic behavior in late prophase which is essential to capture the chromosomes. The chromosomes do not always interact with the microtubule ends. Some chromosomes first bind with the sides of the microtubule and then slides to the end with the help of plus end–directed motor proteins like MCAK present at the kinetochore (Fig. 16).

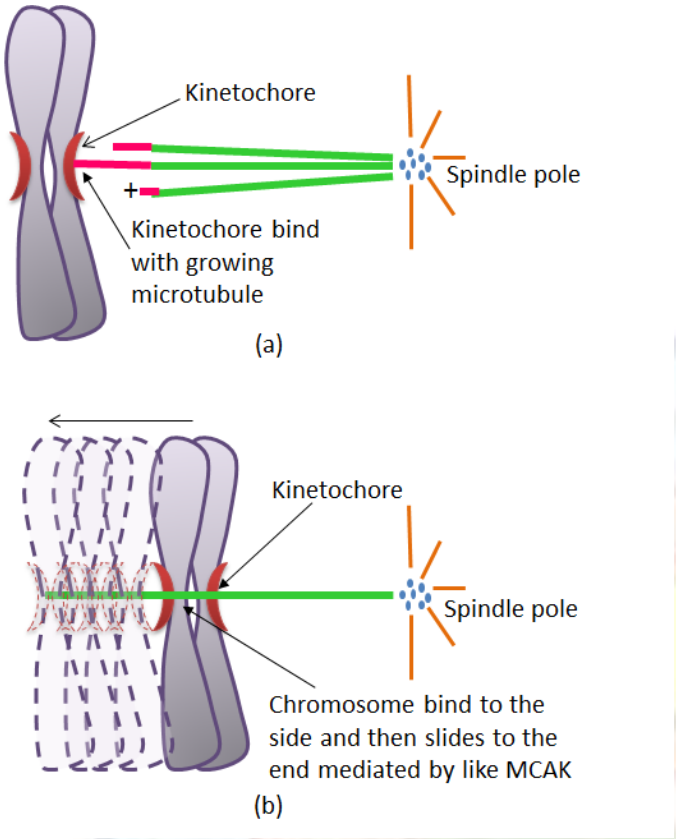

Fig. 16: Binding of microtubules to the chromosomes during late prophase. (a) kinetochore can directly bind to the + end of the kinetochore microtubule (b) sometimes chromosomes bind with the sides of the kinetochore microtubule and then slide to the + end. Sliding is mediated by plus end–directed motor proteins like MCAK present at the kinetochore.

During the beginning of the metaphase the chromosomes attached to the kinetochore microtubules move to the equator of the spindle. These movements require coordinated activities of minus (-) end directed motor proteins like cytoplasmic dynein present at the kinetochore and plus end directed motor proteins present at the spindle pole and chromokinesin present at the chromatid arms (Fig. 17).

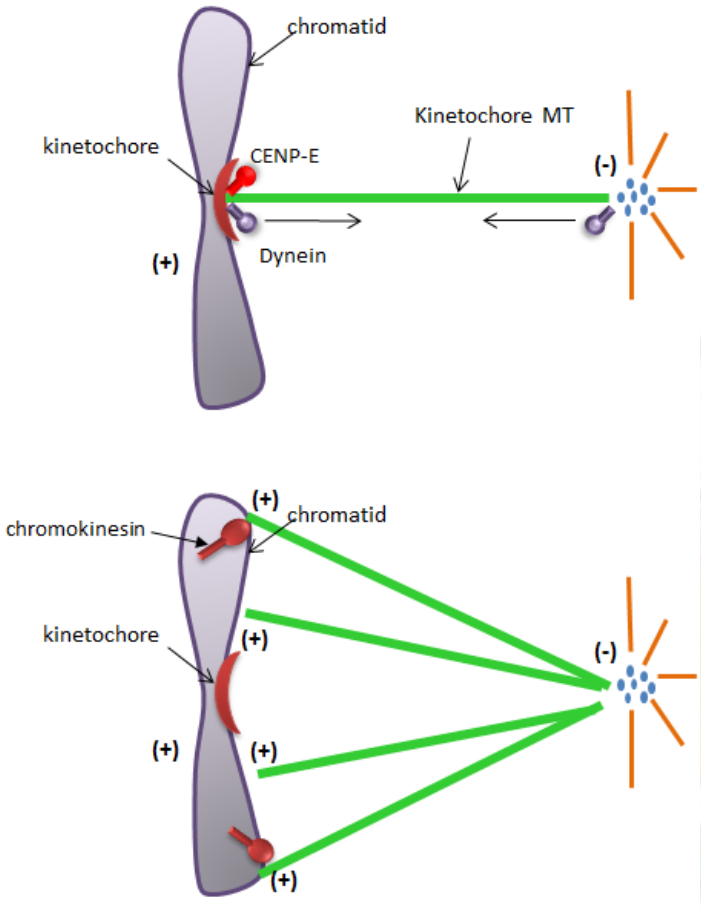

Fig. 17: Movement of chromosomes to the equatorial plate during metaphase. The chromosomal movements require coordinated activities of (a) minus (-) end directed motor proteins like cytoplasmic dynein present at the kinetochore and plus end directed motor proteins present at the spindle pole and (b) chromokinesin present at the chromatid arms.

During anaphase, the sister chromatids are separated and move to the opposite poles. The forces that are responsible for capturing and aligning the chromosomes at late prophase and metaphase respectively are responsible for movement towards opposite poles. Anaphase can be divided into **anaphase A** which involves the disassembly of kinetochore microtubules at plus ends and **anaphase B** which involves movement of two poles apart.

In anaphase A, the disassembly of kinetochore microtubules at their plus end drives the movement of chromosomes towards the pole (Fig. 18). As already mentioned the kinetochore contains mitotic kinesins like MCAK which promote disassembly at the plus end. Another kinetochore-associated kinesin CENP-E helps binding the kinetochore to the gradually shortening end of the kinetochore microtubule. In vitro studies done using purified microtubules and purified anaphase chromosomes without ATP indicate that the chromosomes move towards the minus end. Since the movement by motor protein require ATP, the chromosome movement towards minus end without ATP suggests that the chromosome movement is not mediated by motor proteins. This clearly indicates that disassembly of microtubules is necessary for poleward movement of the chromosomes.

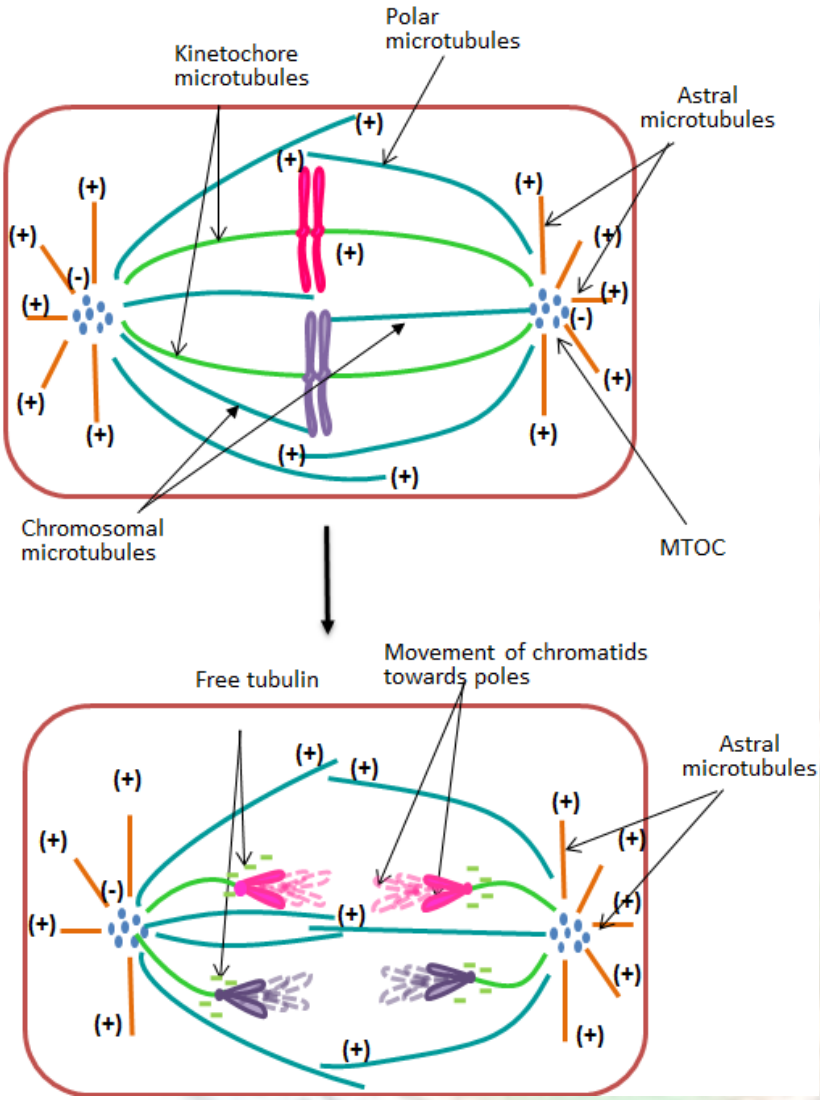

Fig. 18: Separation and movement of chromosomes during Anaphase A. The disassembly of kinetochore microtubules at their plus end mediated by mitotic kinesins like MCAK, drives the movement of chromosomes towards the pole. Kinetochore-associated kinesin CENP-E helps binding the kinetochore to the gradually shortening end of the kinetochore microtubule.

In contrast to anaphase A, the anaphase B involves the participation of motor proteins which power the separation of spindle poles. This was confirmed in the experiments with metaphase cells where depletion of ATP does not affect the movement of chromosomes towards pole but hampers the separation of spindle poles. The anaphase B involves the sliding of polar microtubules past each other mediated by kinesins (Fig. 19). One model assumes that the bipolar kinesin BimC, moves towards the plus end of the antiparallel polar microtubules sliding them apart.

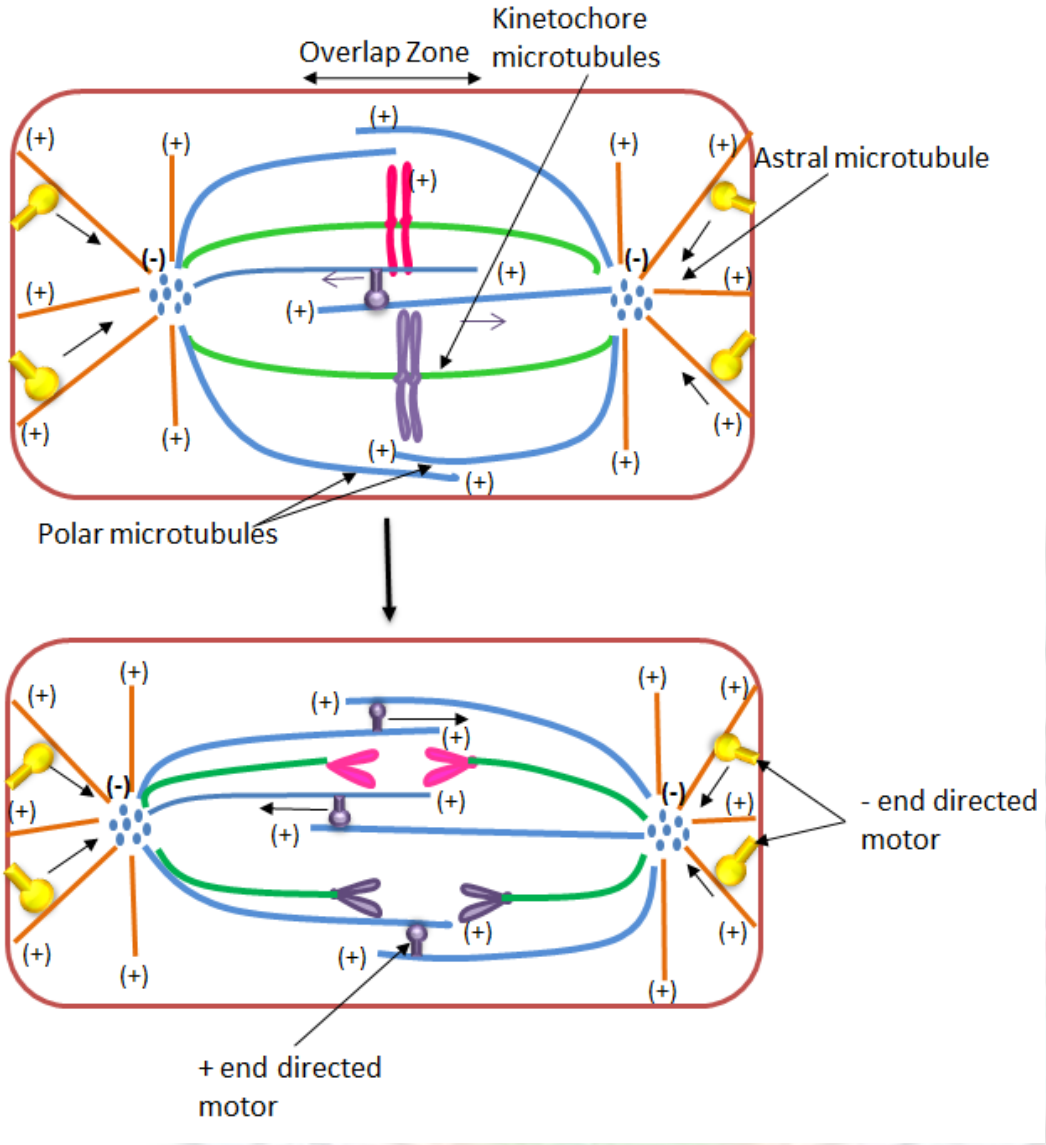

Fig. 19: Separation of spindle poles in Anaphase B. It involves the sliding of polar microtubules past each other mediated by kinesins and pulling of spindle poles mediated by cortex-associated cytosolic dynein.

Anaphase B also involves the cortex-associated cytosolic dynein which move towards the minus end of the astral microtubules pulling the spindle poles apart. This is accompanied by the disassembly of the astral microtubules thus pulling the spindle poles towards the cell periphery.

Summary

Microtubules are rigid, hollow, tubular structures having outer diameter of 25nm with wall thickness of 4nm and are composed of globular protein tubulin. A microtubule is a

heterodimer of 55 kd protein α- and β-tubulin arranged in longitudinal rows forming protofilament. In a cross section, 13 protofilaments can be seen arranged in a circular pattern within each microtubule. Microtubules are polar structures with *plus* (+) end and *minus* (-) end. The plus end grows twice faster as compared to minus end. Usually the microtubule assembled in a cell is singlet but cells also contain doublets and triplets. Both α- and β-tubulin bind GTP but only the GTP bound to β-tubulin can be hydrolyzed to GDP. Eukaryotes also contain a third type of tubulin protein γ-tubulin which is abundant in centrosome.

The assembly of microtubules from αβ tubulin dimers takes place in two phases: nucleation and elongation. The rate of polymerization depends of the concentration of αβ subunits also known as critical concentration (C_c). The αβ subunit undergo polymerization at the concentration higher than critical concentration while undergo depolymerization at concentration lower than critical concentration. Microtubules undergo treadmilling if the concentration of αβ subunit is higher than *C*c at the plus end but lower than *C*c at minus end. During this process, the αβ subunit are dissociated from minus end and are balanced by the addition of αβ subunit at the plus end. Soon after the polymerization, the GTP bound to β tubulin is hydrolyzed to GDP. If the rate of polymerization exceeds the rate of GTP hydrolysis, the plus end has a cap of GTP-bound subunits. If the rate of GTP hydrolyses is more as compared to the rate of polymerization, it results in dynamic instability. Dynamic instability is the inherit property of microtubules and depends on Critical concentration (C_c) and presence of GTP at β-tubulin. The factors which promote disassembly of microtubules are low temperature, increased Ca^{2+} concentration and various chemicals like colchicine.

In interphase animal cells, microtubules radiate and extend from centrosomes which act as MTOC (Microtubule organizing center) within a cell. The centrosome contains microtubule-associated proteins and a pair of centrioles surrounded by electron-dense peri-centriolar material. Centrioles contain nine triplet of microtubules and various extensions that project into the centrosome. The two centrioles are connected by means of centrin fibres. The centrioles are absent from the MTOCs of plants and fungi. The significant protein found in the centrosome is γ-tubulin which plays an important role in initiating the assembly of microtubules. γ-tubulin along with eight proteins form a ring-like complex known as γ-tubulin ring complex (γ-TuRC). In plant cell γ-tubulin is found to be concentrated near the periphery of nucleus and nucleates the assembly of microtubule from the periphery of nucleus.

Within a cell the stability and assembly of microtubules is affected by a number of proteins referred to as Microtubule-associated proteins (MAPs). The MAPs can be classified in two categories: stabilizing MAPs which stabilize microtubule structure and destabilizing MAPs which destabilize microtubule structure. MAP1A and MAP1B are found in neuronal and non-neuronal cells, MAP2 is found in dendrites, MAP4 in found in all non-neuronal cells, CLIP170 cross-links microtubules to chromosomes and Tau cross-links microtubules into thick bundles. Destabilizing MAPs promote microtubule disassembly by either breaking them or by increasing the rate of disassembly at the ends.

Microtubules perform a number of functions in a cell some like intracellular transport of vesicles and cell organelles, cell movement by cilia and flagella and separation and movement of chromosomes during mitosis.

Microtubule

The intracellular transport of vesicles and positioning of organelles require motor proteins which hydrolyze ATP to move along the microtubule tracks. Two key motor proteins are kinesin and dynein which move along the microtubules in opposite directions. Most kinesins move towards plus end, and all dyneins towards negative end of the microtubule. Kinesin I is a molecule of about 380 Kd and is a dimer of two heavy chains (about 120 Kd) and two light chain (about 64 Kd). The N-terminus of heavy chain forms large globular head domainswhich forms the motor domain. The motor domain binds with the microtubule and ATP which provides energy for the movement. The tail domain binds the light chains and the cargo to be transported through the kinesin light chain. Most kinesins move towards +end like kinesin I while others move towards –end like members of small family kinesin-14 which includes Ncd protein of *Drosophila* and some do not move at all like kinesin-13 family. Kinesins can be classified as cytosolic and mitotic kinesins. Cytosolic kinesins transport vesicles and orgenelles while mitotic kinesins take part in the assembly of spindle and separation of chromosome. Dyneins are large molecules with molecular weight more than 2000 Kd and composed of two or three heavy chains complexed with variable number of intermediate and light chains. The heavy chains contain N-terminal head which forms the motor domain responsible for binding microtubules and ATP; and an elongated projection called stalk. Dyneins are divided cytosolic dyneins which transport vesicles and play important role in the movement of chromosomes during mitosis and axonemaldyneins found in cilia and flagella and responsible for their beating.

Cilia and flagella are hair-like projections of the plasma membrane which are supported by microtubules. Cilia and flagella are about 0.25 μm in diameter and are similar in structures made up of microtubules and other associated proteins. The basic structure of both cilia and flagella is known as axoneme which is composed of outer nine doublet of microtubules surrounding the central singlet pair giving characteristic "9+2" pattern. The central singlet microtubules is surrounded by inner sheath which is connected to the A tubules of the outer doublet microtubules by radial spokes. The A tubule consists of a pair of dynein arms referred to as inner and outer dynein arm. The outer doublets are connected to one another by an elastic protein nexin. The beating of cilia and flagella results from the sliding of the outer doublet microtubules relative to one another mediated by the dynein arms.

The movement of chromosomes during mitosis involves the microtubules and associated motor proteins. The mitotic spindle is made up of microtubules which undergo changes during mitosis. With the onset of mitosis the microtubule dynamics change significantly. The interphase microtubules disappear and there is formation of astral and spindle microtubules. The spindle microtubule is made up of kinetochore microtubules, chromosomal microtubules and polar microtubules. During the beginning of the metaphase the chromosomes attached to the kinetochore microtubules move to the equator of the spindle. These movements require coordinated activities of minus (-) end directed motor proteins like cytoplasmic dynein; and plus end directed motor proteins and chromokinesin. During anaphase, the sister chromatids are separated and move to the opposite poles. Anaphase can be divided into anaphase A and anaphase B. In anaphase A, the disassembly of kinetochore microtubules at their plus end drives the movement of chromosomes towards the pole. The anaphase B involves the sliding of polar microtubules past each other mediated by kinesins and also involves the cortex-associated cytosolic dynein which move towards the minus end of the astral microtubules pulling the spindle poles apart.

Microtubule

Exercise/ Practice

A. Multiple choice questions:

1. The + end is fast growing end and is characterized by the row of
   (a) α-tubulin (b) β-tubulin (c) γ-tubulin (d) δ-tubulin

2. Which of the following is not the characteristic feature of centrioles
   (a) 0.2 μm in diameter (b) surrounded by peri-centriolar material
   (c) microtubule doublet (d) present within centrosome

3. An example of destabilizing MAP is
   (a) MAP4 (b) Tau (c) katanin (d) CLIP170

4. Microtubules perform a number of functions in a cell except
   (a) Intracellular transport of vesicles
   (b) positioning of various cell organelles
   (c) Separation and movement of chromosomes during
   (d) Support nucleus by forming nuclear lamina

5. Anaphase A is mediated by
   (a) Polar microtubules (b) chromosomal microtubules (c) Kinetochore microtubule (d) Astral microtubule

6. Axoneme is characterized by
   (a) Outer doublet with "9+2" pattern (b) Outer doublet with "9+0" pattern (c) Outer triplet with "9+2" pattern (d) Outer triplet with "9+0" pattern

7. The first microtubule motor protein to be identified was
   (a) axonemal dynein (b) cytoplasmic dynein (c) kinesin I (d) myosin

8. The assembly of microtubules does not depend on
   (a) Critical concentration (b) colchicine (c) bound GTP (d) motor proteins

9. Microtubules are made up of
   (a) αβ dimer (b) δ-tubulin (c) γ-tubulin (d) α-tubulin

10. MTOC in most interphase animal cells is
    (a) centrin (b) centromere (c) centrosome (d) centriole

B. Fill in the blanks:

1. A microtubule is a heterodimer of _____
2. Microtubules have outer diameter of_____
3. γ-tubulin is abundantly found in _____
4. The examples of structures having doublets microtubules are _____ and _____

5. The examples of structures having triplet microtubules are _____ and _____
6. During _____ the αβ subunit are dissociated from minus end and are balanced by the addition of αβ subunit at the plus end.
7. The drug _____ stabilizes microtubule by preventing its disassembly and also the cell from assembling new microtubule.
8. The MTOC present in interphase animal cells is_____
9. The two centrioles present in centrosome are connected by means of _____
10. The MAP which has been implicated in the development of Alzheimer's disease _____
11. Motor proteins that move towards the negative end of the microtubule are _____ dyneins
12. The kinesin found in kinetochore is _____
13. Cytoplasmic dyneins require additional multiprotein complex known as _____ for transporting cargo.
14. The basic structure of both cilia and flagella is known as _____
15. Both cilia and flagella have characteristic _____ pattern of microtubules.
16. The beating of cilia and flagella is coordinated by _____
17. The anaphase characterized by the disassembly of kinetochore microtubules at their plus end is _____

C. True/False

1. The minus end of microtubule grows twice faster as compared to the plus end.
2. Within each microtubule 13 protofilaments are arranged in a circular pattern.
3. The assembly and disassembly of microtubule is dependent on temperature.
4. Centrioles are required for microtubule formation in a cell.
5. In sperms the basal body is derived from the centriole of spermatocyte.
6. Microtubule-associated proteins are responsible for stability of microtubule in a cell and do not promote unstability.
7. All known kinesins move towards +end like kinesin I.
8. The axoneme consist of outer doublet of microtubules where only one is complete with 13 protofilaments.
9. Astral microtubules play an important role in positioning the mitotic apparatus.
10. Anaphase is dependent only on the activity of motor proteins for separation and movement of chromosomes.

D. Expand the following

1. C_c
2. MTOC
3. γ-TuRC
4. MAP

Microtubule

E. Match the following scientist with their contributions

A	B
A. Richard Weisenberg	A. describe structure of axoneme
B. Timothy Mitchison and Marc Kirschner	B. isolate axonemal dynein from cilia
C. Ian Gibbons	C. isolate kinesin from squid giant axons
D. Ronald Vale	D. first attempts to assemble microtubules in vitro

Glossary

Anaphase A: anaphase involving poleward chromosomal movement mediated by disassembly of kinetochore microtubules at their plus end

Anaphase B: anaphase involving the separation of spindle poles mediated by sliding of polar microtubules past each and pulling tf the spindle poles apartby cortex-associatedcytosolic dynein

Astral microtubules radiate from the centrosomes towards the cell cortex and play an important role in positioning the mitotic apparatus

Axoneme: basic structure of cilia and flagella, composed of outer nine doublet of microtubules surrounding the central singlet pair giving characteristic "9+2" pattern

Centriole: cylindrical structures consisting of nine triplet of microtubules and found in the centrosome

Centrosome: Microtubule organizing center found in most interphase cells, nucleate the assembly of microtubules

Chromosomalmicrotubules: spindle microtubules that bind to the chromosomal ends via chromokinesin

Critical concentration (C_c): the concentration of αβ tubulin dimers required for polymerization

Dynactin: additional multiprotein complex required by cytoplasmic dyneins to carry cargo, composed of eight subunits

Dynein:microtubule motor protein which moves towards the minus end of the microtubule

Kinesin: microtubule motor protein which moves towards the plus end of the microtubule

Kinetochore microtubules: spindle microtubules thatattach to chromosomes at their centromere

Kinetochore: a complex involvingcentromere and associated proteins, help binding the chromosomes with spindle microtubule

Microtubule depolymerases: kinesins incapable of movement along microtubules but play an important role in microtubule depolymerization

Microtubule-associated proteins: proteins that bind microtubules and regulate their stability

Polar microtubules: do not bind chromosomes and overlap with other polar microtubules from the opposite pole

Treadmilling: a dynamic behavior of microtubules where the αβ subunit are dissociated from minus end and are balanced by the addition of αβ subunit at the plus end.

Tubulin: globular proteinwhich is the building block of microtubule, found as heterodimer of α- and β-tubulinin the microtubule

γ-tubulin ring complex: A complex of γ-tubulin along with eight different proteins required for assembly of microtubules

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Answers

A. Multiple choice questions

1. (b) β-tubulin
2. (c) microtubule doublet
3. (c)katanin
4. (d) Support nucleus by forming nuclear lamina
5. (c) Kinetochore microtubule
6. (a) Outer doublet with "9+2" pattern
7. (a) axonemal dynein
8. (d) motor proteins
9. (a) αβ dimer
10. (c) centrosome

B. Fill in the blanks

1. α- and β-tubulin
2. 25 nm
3. centrosome

4. cilia and flagella
5. centrioles and basal bodies
6. Treadmilling
7. Taxol
8. centrosome
9. centrin fibers
10. Tau
11. dyneins
12. CENP-E
13. dynactin
14. axoneme
15. 9+2
16. dynein arms
17. anaphase A

C. True/False

1. False; plus end is the faster growing end.
2. True
3. True
4. False; they are not essential and are absent from MTOC of plants and fungi
5. True
6. False; they are responsible for both stability and unstability
7. False; Some move towards – end as well
8. True
9. True
10. False; it also depends on the disassembly of the kinetochore microtubules

D. Expand the following

1. critical concentration
2. Microtubule organizing center
3. γ-tubulin ring complex
4. Microtubule-associated protein

E. Match the following

1. (d)
2. (e)
3. (b)
4. (c)
5. (a)