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Molecular Mechanism of Microtubules Dynamics and its Precise Regulation Inside Cells

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ABSTRACT

Microtubules are tubulin polymers that use nucleoside triphosphate (GTP) hydrolysis for polymerization. Microtubules (MTs) are involved in diverse and dynamic cellular functions like cell shape maintenance, cell division, cell migration, and signalling. Microtubules display dynamic behaviour of Treadmilling and microtubule dynamics, these processes are precisely regulated by microtubule associated proteins. Inside the cells, soluble and polymeric fraction of tubulin is in equilibrium state that is regulated by microtubule polymerizing and depolymerizing proteins.

The cytoskeleton of eukaryotic cells constitutes three distinct filamentsnamely microtubules, intermediate filaments and actin filaments. Microtubules and actin polymers use nucleoside triphosphate(NTP) hydrolysis[1] for polymerization, where as intermediate filaments use accessory proteins like kinases and phosphatases[2] to power polymer dynamics from chemical energy. Microtubules (MTs) are involved in the diverse and dynamic cellular functions like cell shape maintenance, cell division, cell migration, and signalling. Microtubules are polar hollow cylindrical structures of 25 nm diameter whose fibres consist of αβ-tubulin heterodimeric subunits[3]. There is 50% amino acid sequence similarity between α- and β-tubulin subunits and each subunit is of 50 Kd molecular weight[4]. The α-tubulin as well as β-tubulin monomers possess N-terminal nucleotide-binding domain, an intermediate domain and α-helical domain at the C-terminal end. Both tubulin monomers are capable of binding to GTP. In the αβ-tubulin heterdimer, the nucleotide at α-tubulin is buried at anintradimer interface where as β-tubulin nucleotide is exposed on the surface of dimer. However, upon polymerization αβ-tubulin heterodimers assemble in a head to tail fashion in such a manner that the exposed nucleotide in β-tubulin is buried at the interface between two heterodimers. The nucleotide on β-tubulin is hydrolyzed by coming in contact with amino acid residues from α-tubulin of incoming newly added tubulin dimer. During the polymerization process the GTP associated with β-tubulin (at the exchangeable or E-site) is hydrolyzed[5, 6]but the resulting E-site-GDP is not displaced, as long as it stays in the polymer. Depolymerization of microtubules release tubulin subunits which can now replace E-site GDP for GTP and this way tubulin subunits are replenished for another round of polymerization (figure1). Moreover, α-tubulin is also capable of binding GTP but this GTP is locked in non-exchangeable and nonhydrolyzable form, thus α-tubulin GTP binding site is designated as N-site [7].

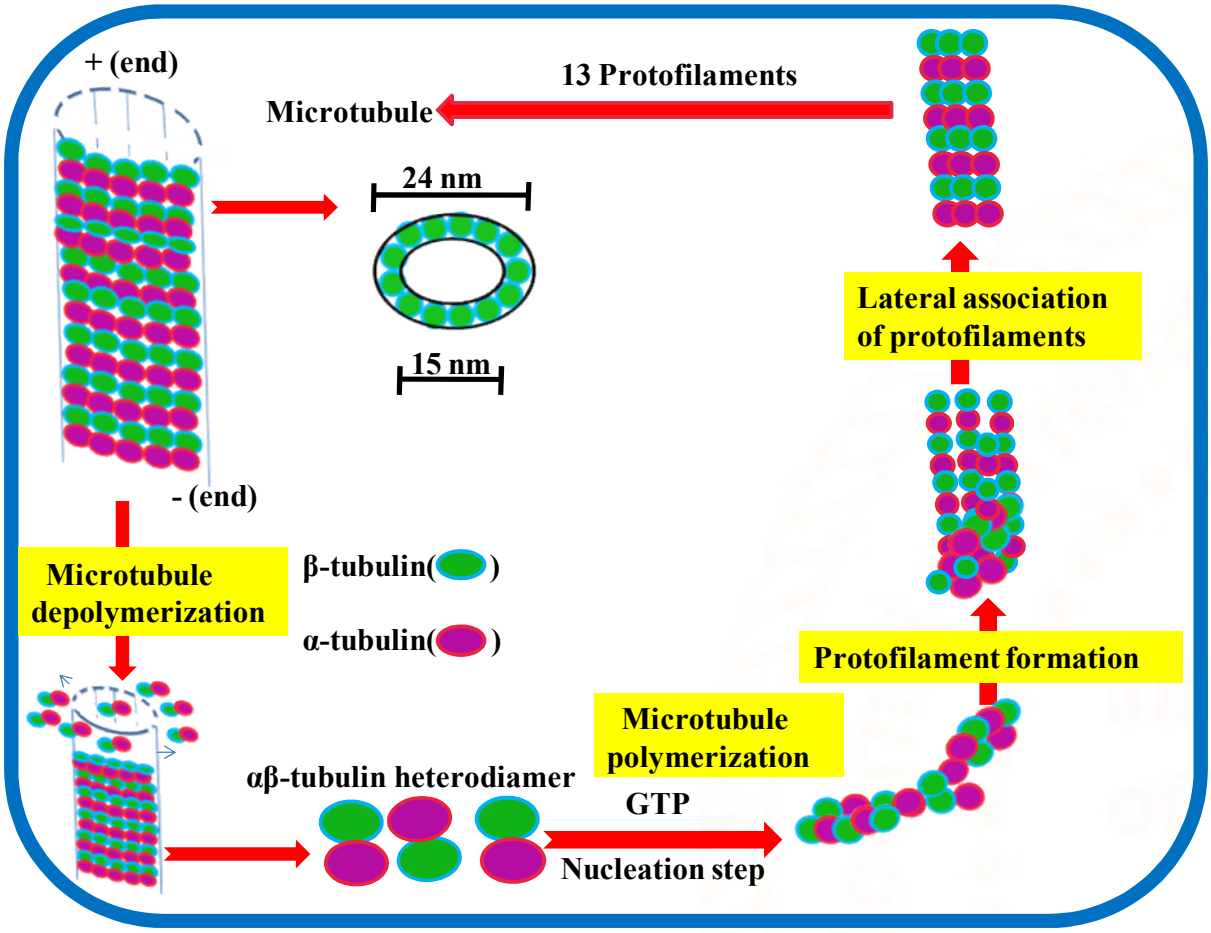


Figure 1: Microtubule disassembly and reassembly: The end of microtubule containing β-tubulin subunit is designated as plus end and opposite side is denoted as minus end of the microtubule. Microtubules are dynamic polymers which once undergo depolymerization release heterodimeric αβ-tubulin subunits. The disassembled αβ-tubulin subunits released from microtubules are replenished with GTP and now they can act as building blocks for new microtubule formation. This microtubule polymerization process is a nucleation mediated phenomenon in which ultimately 13 protofilaments composed of αβ-tubulin subunits combine to constitute a microtubule. There are non-covalent lateral interactions among 13 protofilaments, which combine together to make a hollow cylindrical microtubule, with internal diameter of 15 nm and outer diameter of 24 nm.

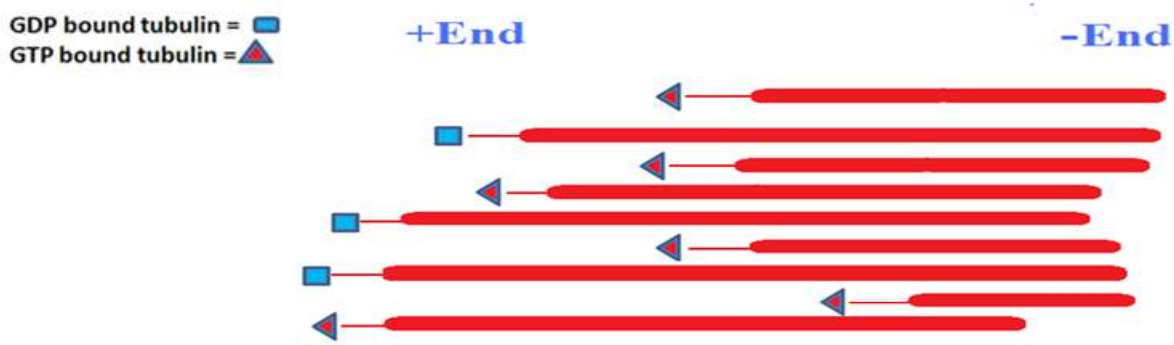
Microtubules display two main dynamic properties: dynamic instability and treadmilling (figure 2 and figure 3)[8, 9]. Dynamic instability is a process where microtubule ends switch between the phases of growth and shortening[8]. Microtubule dynamics is characterized by four main parameters: growth and shortening rates, catastrophe and rescue frequency. The parameter called 'dynamicity' is used to describe the overall rate of tubulin subunits exchange at microtubule ends. The dynamic instability model[8] of microtubule assembly suggests that the individual microtubules exist either in an elongation state or a rapidly shortening state, with abrupt and random transitions between these two states. The transition between growth and shrinkage has been revealed to be controlled by the structure of the microtubule ends. One end of microtubule which is referred as (+) end grows more than other end designated as (-) end. As tubulin dimers add to the growing (+) end, the β-tubulin-bound GTP is hydrolyzed so that at a particular time only a short stretch of β-tubulin-GTP is present at the tip of microtubules, which creates a 'GTP cap' that prevents microtubules from depolymerization. The GTP-bound end of a growing microtubule forms an open sheet that closes to form a

tube like structure where it joins the microtubule shaft[10, 11]. However, if the subunit addition is slower than the rate of GTP hydrolysis, the microtubule end will contain only GDP-bound β-tubulin, which further results inprotofilament

unwinding and microtubule catastrophe. Although both ends of microtubule are capable of growth and shortening, the changes in length at the plus end is much greater than the other end (figure 2). Microtubules exhibit another important dynamic behaviour called treadmilling which corresponds to a polymer mass steady state resulting from the growth of microtubule at one end and simultaneous and equalshortening of microtubule at the opposite end. In other words, treadmilling is a process by which tubulin subunits continuously flux from one end of the polymer to the other, due to net differences in the critical concentrations at the opposite microtubule ends (figure 3). Dynamic instability of microtubules can be depicted in a graphical form as represented in figure 2b. The different parameters of microtubule dynamics are shown. Microtubules increase in length for some time and this phase is referred as growing phase of microtubule and slope of this phase represent growth rate. In the course of microtubule growth, it stops growing or shortening for some time and this phase is represented as pause stage. Although it is believed that mild addition or removal of tubulin dimers might occur during this phase but our microscopic techniques are limited to visualise it due to poor resolution. The phase in which microtubule depolymerizes is called as shortening or catastrophic phase and its slope represent shortening rate. The frequency of transition from growth or pause to shortening is called as catastrophe, whereas the frequency of transition from shortening to growth or pause is called as rescue. The total change in the microtubule length (overall dimer exchange) per minute is known as microtubule dynamics [12]. Our understanding of microtubule dynamics was complemented by different techniques like electron microscopy and other fluorescence microscopy techniques along with optimization of buffering conditions helped to examine this important phenomenon at individual microtubule level both in *in vivo* and *in vitro* conditions [8, 12-19]. Sea urchin sperm axonemes are stable microtubule nucleating filaments which helped us to monitor microtubule dynamics in the *in vitro* system. The newly originating microtubules from axonemes could be videoed and different events of microtubule dynamics can be easily studied [8, 13]. In cells, the expression of GFP-tubulin, EGFP-tubulin or microinjection of rhodamine or biotin labelled tubulin have emergedas important strategic tools to understand microtubule dynamics and the role played by microtubule dynamics in cell functioning [19-21].Time lapse imaging of GFP-tagged visible microtubules is recorded by using confocal or a total internal reflection fluorescence microscope, which is coupled with a CCD camera and a temperature controller. An image of same region of cell is taken at a fixed interval of time and a video is made. Then the growth of individual microtubule is noted by tracking the tip of microtubules in each time frame to locate the specific location of microtubules in x-y plane. The change in microtubule length over time is plotted to obtain life history tracks of microtubules and from which different microtubule dynamic instability parameters are calculated. Further, the intrinsic dynamic characteristic of microtubule is important for assembly of mitotic spindle, proper attachment of microtubules with kinetochore and segregation of chromosomes.

Microtubule dynamics is regulated by a family of cellular proteins which though perform distinctly different cellular functions like some proteins act as oncogenes, tumor suppressors or apoptotic regulators etc, but together these proteins modulate microtubule dynamics for proper cell functioning. Over-expression of some of the MAPs in certain tumours not only imparts resistance to microtubule drug targeting therapy, but it is also responsible for disease progression. The mechanism by which MAPs render MTAs unsuccessful could be exploited for rational drug design. The alteration of microtubule dynamics by microtubule targeting agents is well known strategy to cure cancer growth and metastasis. The combination of siRNA against specific MAPs along with use of MTAs can be utilized to generate cancer specific cytotoxic effects without harming normal cells via using specific drugs or siRNA targeting or delivery strategy. Importantly, the vinblastine and taxol binding agents are widely used successful anticancer agents.

(A)



(B)

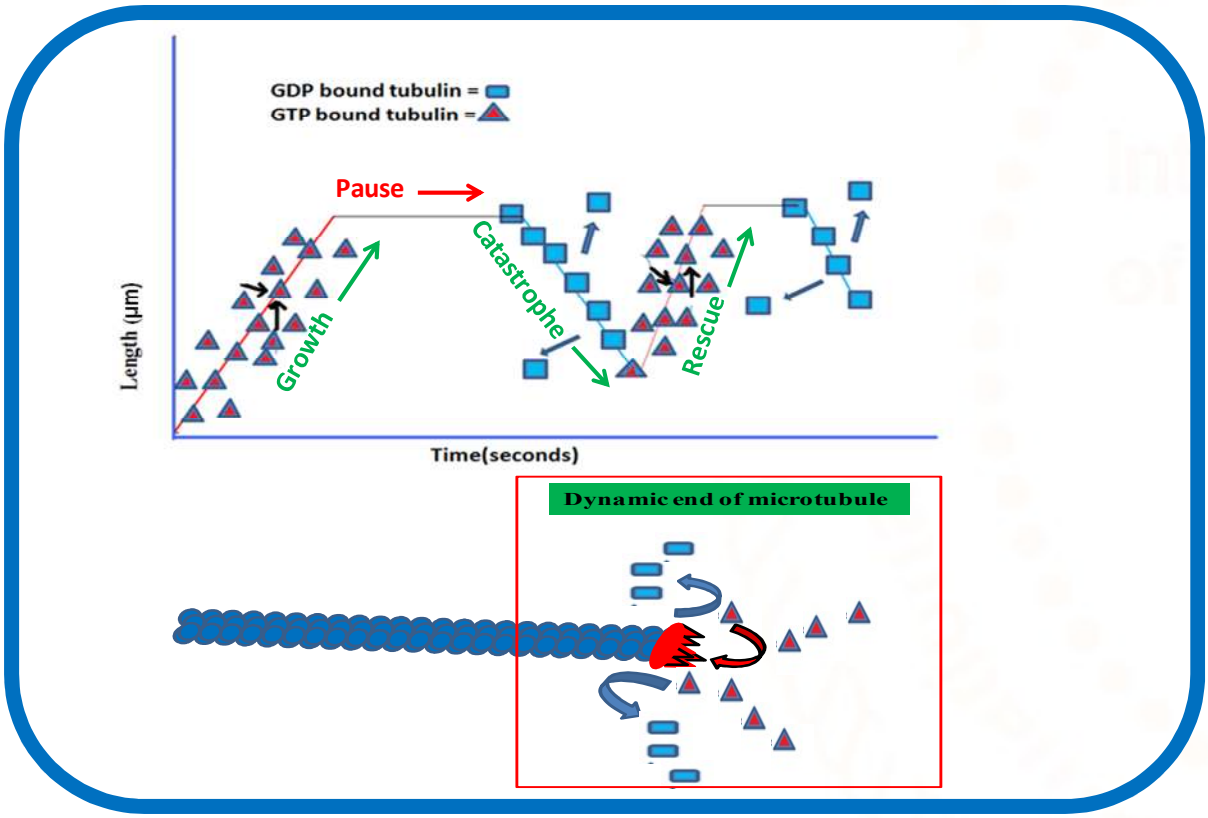


Figure 2: Dynamic instability of microtubules: **(A)** Microtubules undergo continuous rounds of growth and catastrophe depending on the presence of GTP or GDP cap at the tips of microtubules. As long as GTP cap is present at the tip of microtubule, it will grow in length and then at the sometime it loses GTP cap and undergoes catastrophe. During the course of depolymerization microtubule can regain GTP cap and resume growth. (B) The pictorial representations of microtubule length change over time, showing addition of GTP bound αβ-tubulin subunits during growth phase of microtubule and during depolymerization or catastrophe GDP bound αβ-tubulin subunits are released. Pause state represents a phase of microtubule where no net change in microtubule length is visualised. It is to be noted that all the additions and removal of GTP or GDP bound tubulin occur only at the ends of microtubules as shown in the representative microtubule.

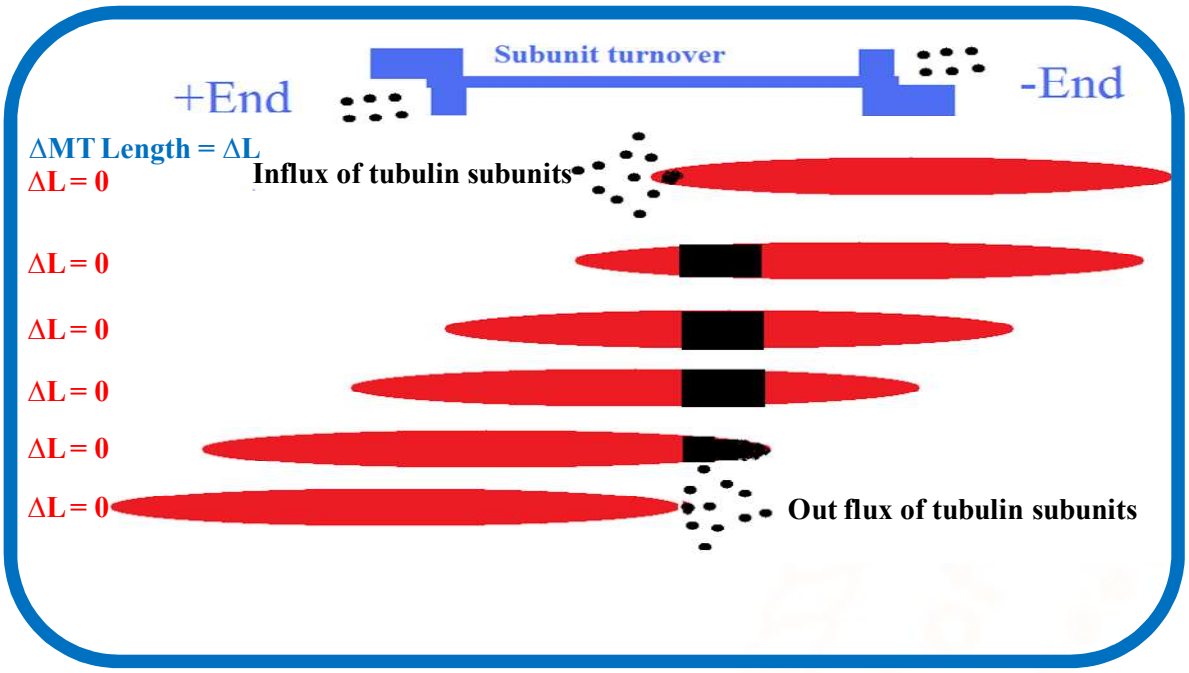


Figure 3: Treadmilling: It represents net polymer mass steady state, which is outcome of microtubule growth at one end and shortening from opposite end. In other words, treadmilling is a process by which tubulin subunits continuously flux from one end of the polymer to the other, due to net differences in the critical concentrations at the opposite MT ends. Flux of αβ-tubulin subunits is represented in black color and tubulin subunits treadmill through microtubule length and are released through (-) end of microtubule.

Microtubule dynamics and its regulation inside cells

Although microtubules are intrinsic dynamic polymers but programmed regulation of microtubule dynamics through different phases of cell cycle is modulated by numerous proteins known as microtubules associated proteins (MAPs) and mitotic kinases[22, 23]. Various MAPs present inside the cell maintain a balance between polymeric and soluble pool of tubulin and are also responsible for reorientation of microtubule cytoskelton (figure 4). There are three classes of MAPs, microtubule stabilizing proteins, end binding proteins and microtubule depolymerizing MAPs. Microtubule stabilizing proteins aid in tubulin polymerization as well as stabilization of microtubules by shifting equilibrium towards polymerization state. Whereas, microtubule stabilizing MAPs reduce the catastrophe frequency and increase the growth rate of the microtubules [24-26]. Moreover, some of the remarkable examples of this class of MAPs are MAP 1, MAP 2, MAP 4, MAP 7 and tau, all of them are known to bind along the microtubule lattice and regulate the microtubule dynamics by stabilizing and promoting microtubule bundle formation(figure 4). MAPs have specific microtubule binding domains by which they bind to microtubules. The distribution of these MAPs could be specific to particular type of cells or they may be randomly present in all cells. Notably, the example is tau, which is particularly present in the axonal cells, whereas MAP-2 is present in the dendrite cells [24-26]. Tau a neuronal protein is one of the extensively studied proteins. Tau stabilizes neuronal microtubules and its affinity with microtubules is regulated through phosphorylation. The altered phosphorylation of tau is responsible for Alzhimers disease and other tauopathies. Tau regulates microtubule dynamics by reducing the rates of growth and shortening with simultaneous increase in time spent by microtubules in the pause state[27]. There are several other post-translational modifications which can occur with tau protein like phosphorylation, glycation, ubiquitination, acetylation, nitration, truncation, glycosylation and polyaminations. However, the most predominant one is tau phosphorylation which regulates its interaction with the microtubules. Tau undergoes phosphorylation on serine and threonine residues.The hyperphosphorylated serine and threonine residues of tau proteins could be a cause for neurodegenerative diseases by destabilizing microtubules due to electrostatic interference in polymerization. Moreover, the phosphorylation of tau also interfers

with binding ability of tau to microtubules as compared to non-phosphorylated tau. Glycogen synthase kinase 3(GSK-3) is responsible for tau phosphorylation;it phosphorylates tau on serine and threonine residues. GSK-3 is also linked with β-amyloid formation in neurons, thus it could be an important contributor in neurodegeneration[28].

However, the other important regulators of microtubule dynamics are tracking proteins called as plus end binding proteins (+TIPS) which consists of EB family of proteins, CLIP family of proteins like CLIP-170 (cytoplasmic linker protein170), CLASP(cytoplasmic linker associated proteins), APC (adenomatous polyposis coli), XMAP215 (Xenopus microtubule associated protein 215) anddynamitin. Plus end tracking proteins are known for specific binding and recognization of GTP cap structure present at microtubule plus end. These proteins have been reported to regulate microtubule growth and dynamics. Specific domains are involved for tracking and binding of +TIPs proteins at the plus end of the microtubules. The important examples are end binding proteins which bind through calponin homology domain (CH), XMAP215 bind through TOG (tumor overexpressed genes) domain and CLIP-170 binding involves CAP-Gly domain[29]. EB1 an important member of EB family of proteins is a +TIP which binds at the tip of dynamic microtubules and form a comet like shape at the growing end[30, 31]. The EB1 forms a comet and thus regulates microtubule dynamics also. This comet formation also help in important processes like cargo transport and cell signaling during cell migration. Catastrophe frequency of microtubules is decreased by EB1[32] and it promotes tubulin polymerization. The importance of GTP hydrolysis for plus end tracking of EB1 was established by using non-hydrolysable analogue of GTP (GMPPCP) which hampered EB1 tracking ability, suggesting EB1 recognization of some important structural feature in GTP-cap[33]. Regulation of microtubule dynamics during mitosis by XMAP-215 and mitotic kinases like aurora kinases and polo like kinases help in proper chromosomal segregation and cell division [34-36]. Kinesins are minus end directed motor proteins and dyneins are directed towards plus end of microtubule, these cargo carrying proteins also regulate microtubule dynamics[37]. The kinesins and dyneins use microtubules as tracks on which they carry different cargoes from one compartment of cell to another. An individual component of dynein motor called as LC8 was found to interact and stabilize microtubules indicating possible regulation of microtubule dynamics by LC8 association with microtubules[38]. In addition to microtubule polymerization promoting MAPs, there are microtubule depolymerizing MAPs which upon binding to microtubule depolymerizes it [39-44], important examples are Kinesin-13 family proteins (Kif2A, Kif2B, MCAK) which upon binding to microtubules cause depolymerization. Kinesin-13 family proteins also sequester the tubulin monomers and increase catastrophe frequencies of microtubules[40, 41]. Length based microtubule depolymerization is carried over by Kinesin 8 family proteins [42]. Kinesin proteins are known for their important functions during mitosis as they help in proper bipolar spindles orientation and hence chromosome segregation. Katanin depolymerizes microtubules by forming a ring like structure on microtubule lattice. The activation of ATPase activity of katanin leads to severing of microtubules[43]. End binding followed by depolymerization is carried out by Kin I kinesin family members like XKCM1 and MCAK[45]. Op18 (oncoprotein 18 or stathmin) forms a ternary complex with tubulin and sequesters the tubulin heterodimer, thereby making them unavailable for polymerizationthus, shifting the equilibrium of microtubule assembly towards depolymerization state[44].

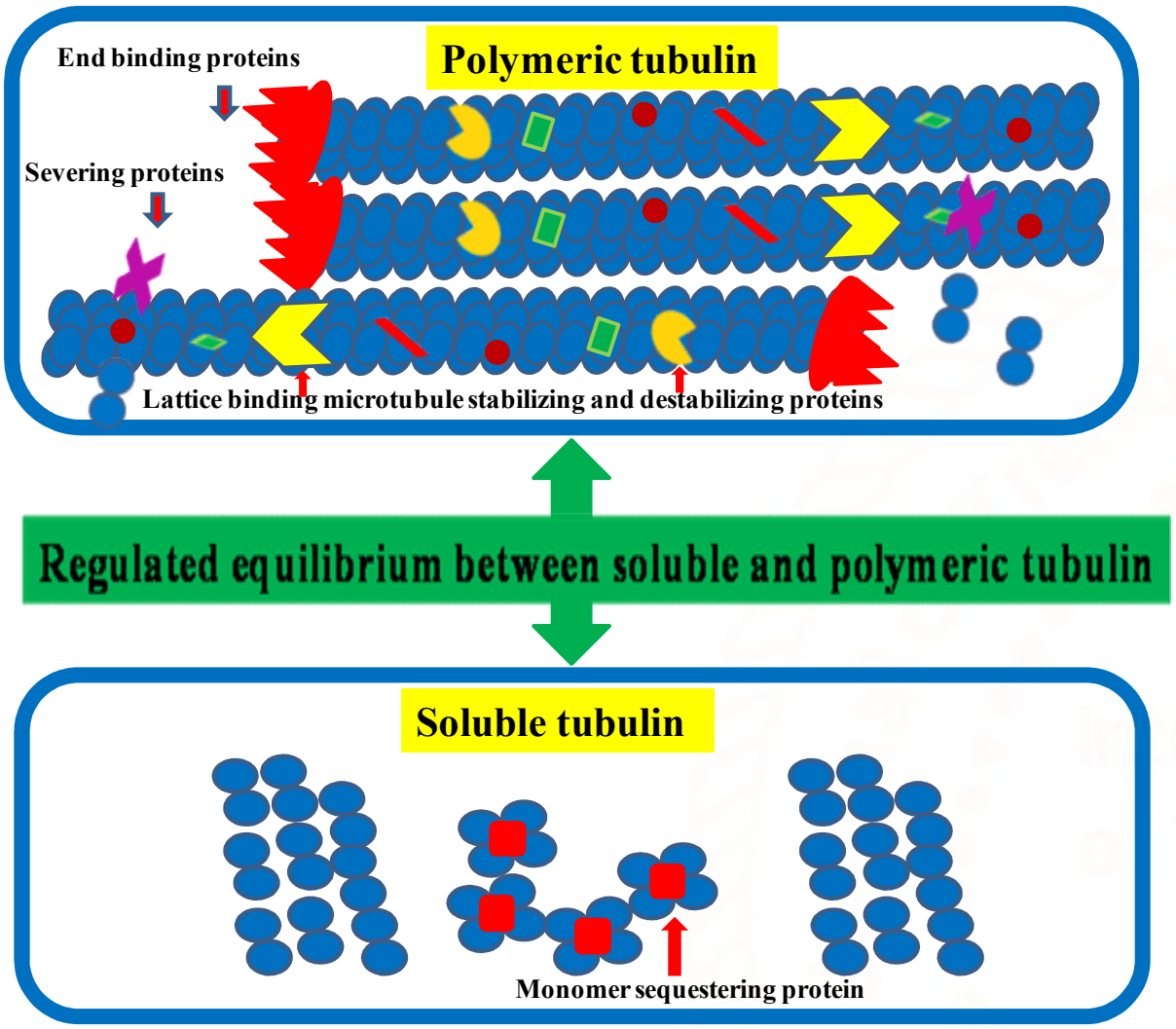


Figure 4: Polymerization equilibrium: Inside the cells, cytosolic and polymeric fraction of tubulin is in an equilibrium state. Microtubule polymer dynamics is maintained by continuous addition and removal of tubulin subnits. There are broadly four categories of proteins regulating microtubule polymerization. End binding proteins like CLIP-170, EB1 bind at the ends of microtubules and regulate microtubule dynamics and polymerization. Microtubule lattice binding proteins are either microtubule stabilizers like Tau, MAP4 or microtubule depolymerizing proteins like MCAK. There are microtubule severing proteins also likekatanin, which binds to microtubule and forms a ring like structure around the microtubules and ultimately resulting in the cleavage of microtubules. Microtubule polymerization is also regulated by sequestration of tubulin subunits by stathmin (Op18).

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