Lessons from bacterial homolog of tubulin, FtsZ for microtubule dynamics

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Abstract

FtsZ, a homolog of tubulin, is found in almost all bacteria and archaea where it has a primary role in cytokinesis. Evidence for structural homology between FtsZ and tubulin came from their crystal structures and identification of the GTP box. Tubulin and FtsZ constitute a distinct family of GTPases and show striking similarities in many of their polymerization properties. The differences between them, more so, the complexities of microtubule dynamic behavior in comparison to that of FtsZ, indicate that the evolution to tubulin is attributable to the incorporation of the complex functionalities in higher organisms. FtsZ and microtubules function as polymers in cell division but their roles differ in the division process. The structural and partial functional homology has made the study of their dynamic properties more interesting. In this review, we focus on the application of the information derived from studies on FtsZ dynamics to study microtubule dynamics and vice versa. The structural and functional aspects that led to the establishment of the homology between the two proteins are explained to emphasize the network of FtsZ and microtubule studies and how they are connected.

Key Words

- ▶ FtsZ assembly
- ▶ microtubules
- ▶ cell division
- ▶ anticancer drugs
- ▶ antibacterial drugs

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Introduction

Microtubules are major cytoskeletal protein structures of the eukaryotic system involved in cell division forming the primary components of the mitotic apparatus. Microtubules also form flagellar and ciliary structures for cell transport, maintain the cell polarity and are involved in cell signaling (Nogales 2001). Defective microtubules would lead to abnormal morphology of the cell and ultimately cell death if the division machinery is impaired. Microtubules are made up of protofilaments of tubulin subunits, which assemble to form the highly dynamic structures. Microtubule assembly is a highly complex process that has been studied for the past five decades and yet, so many mysteries are to be unraveled. However, numerous studies have made significant

progress in understanding the mechanism of assembly and the various associating factors that play important roles in it.

Microtubules play a significant role in metaphase and anaphase of mitosis. They are the key components of the mitotic spindle apparatus, which is responsible for the segregation of chromosomes (Kline-Smith & Walczak 2004, Kwon & Scholey 2004). During the metaphase, the plus ends of microtubules undergo elongation and shortening for probing and binding to the kinetochores. This is called 'search and capture' function (Rieder *et al.* 1986). The chromosomes are then translocated to the equatorial plane, during which, the highly dynamic microtubules are stabilized to separate the chromosomes

in the following anaphase (Higuchi & Uhlmann 2005). Chromosomes get pulled toward the poles due to the shortening of microtubules (Rieder *et al.* 1994). This entire process is dependent on the dynamicity of microtubules and an obstruction would lead to the arrest of the cell division at the spindle checkpoint (Logarinho *et al.* 2004). Thus, agents that target the microtubules and hamper their dynamics can be potential anticancer, anti-fungal and anti-parasitic drugs (Jordan & Wilson 2004, Singh *et al.* 2008b). The use of anti-microtubule agents as anticancer drugs has been studied extensively (Jordan & Wilson 2004). In addition, it has been indicated that cell stress response is mediated by microtubules and their interacting partners (Parker *et al.* 2014). In several types of tumors, alterations in the expression of tubulin isotypes and microtubule-associated proteins and post-translational modifications have been observed (Parker *et al.* 2014). The understanding of signaling pathways and response mechanisms with respect to microtubules will help in designing new chemotherapeutic drugs.

Microtubule dynamics has been a subject of extensive research since the discovery of tubulin, yet, the mechanism is not fully understood. Complications in studying microtubules arise from the inability to express the protein in bacterial expression systems. Post-translational modifications of tubulin are not possible inside bacterial cells due to the lack of chaperones and protein co-factors. Thus, the extraction and isolation of tubulin from eukaryotic cells like yeast is the preferred method of purifying the protein. This triggered the search for the homolog of tubulin in prokaryotes.

Many bacterial species such as *cyanobacteria*, *azotobacteria*, *mycoplasmas*, enteric bacteria and archaeobacteria were found to contain cytoplasmic tubules, but whether or not these were related to microtubules and tubulin was a question of interest (Bermudes *et al.* 1994). About 20 years after the discovery of tubulin, FtsZ, initially thought to be the homolog of actin, arose as the candidate for being the tubulin homolog in prokaryotes (Erickson 1995). FtsZ is the first of several proteins that gets assembled at the division site in prokaryotes, to form the Z-ring, which is in association with other proteins forms the divisome (Margolin 2005). Over the years, numerous studies have been done on this bacterial cell division protein and several reports indicated striking similarities as well as differences between FtsZ and tubulin.

Since the discovery of FtsZ, studies on FtsZ dynamics have influenced the study of microtubule dynamics and vice versa. In this review, we focus on the similarities and differences between the two proteins, believed to have a

common ancestor. Structural aspects have been covered in the first section. The dynamics of the two proteins have been compared in the following section. In the next section, some proteins belonging to FtsZ/tubulin superfamily, which act as connecting links between the two proteins have been mentioned. A section on microtubule-targeting agents and FtsZ-targeting agents and a comparison of their binding sites has been included to emphasize the significance of such interrelated studies between FtsZ and microtubules.

Discovery of FtsZ and establishing homology with tubulin

Several genes in the *fts* regions of the *E. coli* chromosome were identified while studying the effects of DNA damage on cell such as the elongation of cells and the inhibition of division (Van de Putte *et al.* 1964). These genes were involved in causing filamentous growth, a property that was temperature sensitive (Van de Putte *et al.* 1964). One of the genes in the 2-min region of *E. coli* genome disrupted the septum formation when mutated (Hirota *et al.* 1968). This gene was later given the name *ftsZ* to separate it from the other *fts* genes of the region (Lutkenhaus *et al.* 1980). It was found to be the target of cell division inhibitor during SOS response in *E. coli*, which shed light on its central role in the cell (Hirota *et al.* 1968, Lutkenhaus & Donachie 1979, Lutkenhaus *et al.* 1980, Lutkenhaus 1983).

FtsZ was first characterized as a cytoskeletal protein based on experiments that determined the location of the protein. It has been found to be uniformly distributed throughout the cell during the growth phase and to form a ring-like structure at the mid-position in the dividing cells (Bi & Lutkenhaus 1991). An overproduction of FtsZ in cells using multicopy plasmids induced the formation of additional septa (other than the medial septa for cell division), which resulted in the production of minicells (Ward & Lutkenhaus 1985). The ring structure formed by FtsZ was proposed to be involved in constricting the cell wall to form the septum (Erickson 1995). Even though the function of FtsZ was similar to actin (which forms the contractile ring and cleavage furrow in eukaryotes), the two proteins were not found to be homologous (Bi & Lutkenhaus 1991).

A 7-amino acid segment conserved in FtsZ called the G-box motif (SAG)GGTG(SAT)G was also found to be conserved across tubulin from different species (Mukherjee *et al.* 1993). Another segment (KR)GXXXXG is present at the inverted P-loop region of the two structures (Hesse *et al.* 1987, Sternlicht *et al.* 1987,

Mukherjee *et al.* 1993, De Pereda *et al.* 1996). These are related to the GTPase family sequence motif GXXXXGK(ST) (Bourne *et al.* 1991). The probable homology of FtsZ to tubulin triggered a series of experiments to test whether FtsZ showed similar biochemical properties as that of tubulin. FtsZ binds to GTP and hydrolyzes it to GDP, a property also observed with tubulin (de Boer *et al.* 1992, Erickson & O'Brien 1992, RayChaudhuri & Park 1992, Mukherjee & Lutkenhaus 1998). This categorized these two proteins as GTPases but as a distinct family in the group. They had the unique property where GTPase activation was coupled to self-association of the proteins (Nogales *et al.* 1998a).

Yet, the sequence homology between the two proteins was found to be low. Secondary structure prediction studies involving 200 tubulin sequences from different eukaryotic species and 12 FtsZ sequences demonstrated that tubulin is indeed homologous to FtsZ, having similar folds and percentage of helices and sheets (De Pereda *et al.* 1996). Multiple sequence alignment of the two proteins and proteolysis studies provided partial structural working models for tubulin and FtsZ structural regions at the GTP-binding site (De Pereda *et al.* 1996).

Studying the archaeal FtsZ bridged the gap between the prokaryotic and eukaryotic cytoskeleton, showing that FtsZ may have evolved to tubulin. In a study involving the isolation of a FtsZ homolog from an archaeal species *Halobacterium salinarum*, the FtsZ homolog was seen to be more related to tubulin of eukaryotes than to FtsZ of bacteria (Keeling & Doolittle 1995, Margolin *et al.* 1996). FtsZ was thus proposed to be the prokaryotic cytoskeletal protein homolog of tubulin (Erickson 1995). The low sequence identity may suggest a convergent evolution for FtsZ and tubulin, but the similarity in their three-dimensional structures strongly suggests a prokaryotic origin for the eukaryotic cytoskeleton (van den Ent 2001).

Confirming the homology of FtsZ and tubulin through X-ray crystal structures

Microtubules are polymers of tubulin heterodimers. They are composed of two classes of tubulin- α and β (Bryan & Wilson 1971, Amos & Klug 1974). Many additional classes of tubulin have been identified to date. γ tubulin is involved in the nucleation of the microtubules and plays a role in regulation at the minus end (Joshi 1994, Oakley 1995). Other types of tubulin δ , ϵ and ζ have been identified but little is known about their structure and function (Findeisen *et al.* 2014).

Researchers had been trying to obtain the crystal structure of tubulin for at least two decades before it was finally resolved at a resolution of 3.7 Å as an $\alpha\beta$ tubulin dimer using electron crystallography of zinc-induced tubulin sheets (Erickson 1998, Nogales *et al.* 1998b). As shown in Fig. 1A, both α and β tubulin showed identical structures with a core of β sheets surrounded by α -helices. The structure is divided into three domains: N-terminal domain bearing the GTP-binding region, an intermediate domain, which has the taxol-binding site and the C-terminal domain postulated to be the binding region of motor proteins (Nogales *et al.* 1998b). The C-terminal domain was suggested to be involved in the binding of MAPs and functioned as a regulatory region during self-assembly (Serrano *et al.* 1984).

Soon after, FtsZ structure also came into light. In prokaryotic systems, the problems of genetic heterogeneity and post-translational modifications were absent making the crystallization process relatively easier. FtsZ was crystallized from *Methanococcus jannaschii* at a resolution of 2.8 Å (Löwe & Amos 1998). The crystal structures of the two proteins depict the similarity of the folds and domains in the two proteins and give insights to their assembly orientation. FtsZ upon folding gives

Figure 1

Tubulin and FtsZ show structural similarity. (A) Tubulin dimer with alpha and beta tubulin is shown as cartoon model. GTP and GDP nucleotides are bound to alpha and beta tubulin, respectively. They are shown as rainbow colored ball and stick models. (B) FtsZ is shown as a monomer with GDP bound to the N-terminal. N-terminals (blue) and C-terminals (pink) are separated by H7 helices (yellow). N-terminal regions show the typical nucleotide-binding motif with parallel beta sheets connected by alpha helices known as the Rossmann fold. From these structures, the differences in C-terminal region between the two proteins are evident. The figures were rendered using PyMOL. PDB ID for tubulin: 1SAO. PDB ID for FtsZ: 1FSZ.

two independent domains (Fig. 1B). A linker domain (H7 helix) connects the two domains. The N-terminal region shows the typical nucleotide-binding motif with parallel beta sheets connected by alpha helices, known as the Rossmann fold, also found in tubulin. This region was also shown to be important for the localization and assembly of FtsZ (Ma *et al.* 1996). The C-terminal domain contains a small, highly conserved (across prokaryotes) terminal region whose function was initially unknown as it was dispensable for the polymerization (Ma *et al.* 1996, Yu & Margolin 1997). This C-terminal region is now found to be essential for the interactions with other membrane proteins like FtsA and ZipA (Ma & Margolin 1999). A flexible linker region present at the C-terminal acts as a membrane tether for the cytokinetic ring formation (Buske & Levin 2013). The active site is formed when the T7 loop in the C-terminal domain is in synergy with GTPase domain in the N-terminal of the next subunit. This enables the association dependant activation of the GTPase activity. Hydrolysis of GTP occurs by the hydrogen bonding of aspartate side chains in the T7 loop with water molecules, promoting the nucleophilic attack on the γ -phosphate. The N-terminal residue, glutamine (Gln75 in *Methanococcus jannaschii* FtsZ) helps in the polarization of the γ -phosphate in coordination with magnesium ion, water molecules and α, β -phosphates (Löwe & Amos 1998, Nogales *et al.* 1998a).

Comparing the structures of FtsZ and tubulin (Fig. 1), it is evident that N-terminal domains of the two structures are, expectedly, similar. Even though the sequence similarity is completely absent in the C-terminal domains of FtsZ and tubulin, their three-dimensional structures are similar with some differences, as seen in Fig. 1. FtsZ has predominant β sheet terminus while tubulin ends with α helices, which suggests that the C-terminal regions in tubulin arose due to functional demands of binding to motor proteins such as kinesin (Erickson 1998). Based on the orientation of tubulin dimers, the orientation of FtsZ subunits during assembly could be predicted.

FtsZ and tubulin comprise a distinct family of GTPases

The structures of FtsZ and tubulin show similarity to other G-proteins even though they do not contain the signature GTP-binding sequence present in other G-proteins (Erickson 1998). They have their own unique GTP-binding sequence, which supported the hypothesis that they shared a common ancestry (Erickson 1998).

Studying the active site residues of the two structures, it was suggested that GTP binding and hydrolysis involved the association of two subunits (Wang & Lutkenhaus 1993, Erickson 1998, Mukherjee & Lutkenhaus 1998). This also led to the proposal that tubulin, perhaps FtsZ too, was its own GAP (GTPase-activating protein) (Wang & Lutkenhaus 1993, Erickson 1995, 1998). Binding studies and kinetics revealed that one mole of tubulin dimer binds two moles of the guanine nucleotide (Adelman *et al.* 1968). GTP bound to β tubulin is hydrolyzed to GDP, which remains in the polymer till it is exchanged with GTP following depolymerization. This triggers another round of polymerization. The α -tubulin does not exchange its bound GTP and this GTP is not hydrolyzed (David-Pfeuty *et al.* 1977, Spiegelman *et al.* 1977, Purich & MacNeal 1978, Desai & Mitchison 1997). However, one mole of FtsZ binds to one mole of GTP (Lu *et al.* 1998).

Structures of FtsZ and tubulin polymers

The surface lattice of microtubules is composed of 13 filaments in a 3-start helix (Ledbetter & Porter 1964, Tilney *et al.* 1973). A 3-start helix is described by starting 3 parallel helices of monomers, one above the other, to cover the entire surface of the microtubule lattice (Desai & Mitchison 1997). In a microtubule, one helical turn with one line of monomers will end 3 monomers above it (Desai & Mitchison 1997). A model was constructed with alternating α and β subunits to form a heterodimer of 8nm, which polymerized to give a longitudinal filament (Erickson 1974b). This arrangement makes the microtubules polar structures, with a plus end and a minus end. These linear protofilaments laterally interact with each other to give 25nm wide hollow cylindrical polymers (Desai & Mitchison 1997). To examine whether β tubulin was present at the plus ends of microtubules – labeling studies using GTP were done. Labeled GTP binds specifically to the plus ends showing that, at the plus end, an exchangeable GTP-binding site is available (Mitchison 1993). Another study used the motor protein kinesin, which binds to β tubulin, as the label and showed that it binds to the plus end (Hirose *et al.* 1995). Antibodies against α tubulin were seen to bind to the minus end (Erickson & Stoffer 1996, Fan *et al.* 1996). This gave the orientation of the dimer in the polymer with α at the minus end and β at the plus end.

Both FtsZ and $\alpha\beta$ tubulin were shown to assemble into straight filaments, which then form the protofilaments (Bramhill & Thompson 1994, Desai & Mitchison 1997).

The alternate conformation, which was observed in the tubulin family, was that of rings or spirals, where the subunits are all in curved conformation (Erickson & Stoffer 1996). Rings of tubulin were usually captured before the assembly and after the disassembly of microtubules (Borisy & Olmsted 1972). These were seen to be continuous with the protofilaments of the microtubule wall (Erickson & Stoffer 1996). This gives a typical fountain-like appearance to the microtubule ends, due to the curling of the protofilaments to form rings away from the microtubule walls (Warner & Satir 1973, Erickson 1974a, Kirschner *et al.* 1974, Mandelkow *et al.* 1991, Erickson & Stoffer 1996).

Like tubulin, rings formed by FtsZ *in vitro* are extensions of the protofilaments. There is a significant size difference between the tubulin rings and FtsZ rings. This is postulated to be due to the curved conformation occurring in all subunits in FtsZ, while in tubulin rings only β subunits were curved (Erickson & Stoffer 1996). In this regard, it was also proposed that the γ tubulin rings, which have a similar diameter to that of FtsZ rings were curved protofilaments (Erickson & Stoffer 1996). The structural evidence obtained from these studies pointed out that ring polymers are homologous across tubulin family (Erickson & Stoffer 1996). The functions of these rings were expected to be found in disassembly, in force generation due to shifting from one conformation to the other, and in the nucleation of the assembly when it comes to γ tubulin rings (Borisy & Olmsted 1972, Erickson 1974a, Kirschner *et al.* 1974, Erickson & Stoffer 1996).

Alternating conformations of tubulin and FtsZ

Tubulin, when bound to GTP favors the straight conformation while GDP-bound tubulin units adapt the curved conformation. The switch between the two conformations was postulated to be the driving force for assembly and disassembly (Melki *et al.* 1989, Erickson 1997). The force generated during the disassembly of microtubules could explain the mechanism for chromosome motion (Grishchuk *et al.* 2005, McIntosh *et al.* 2010). During polymerization, curved protofilaments change to the more favored straight conformation, and this transition is promoted as the monomers assemble. The transition from curved to straight state could be possible due to GTP binding (Nawrotek *et al.* 2011). However, experiments showed that unpolymerized tubulin in solution was

in the curved conformation irrespective of whether it was bound to GTP or GDP (Buey *et al.* 2006, Rice *et al.* 2008, Brouhard & Rice 2014). Thus, the transition of GTP-tubulin to straight conformation would take place as the subunits got incorporated into the microtubule (Kueh & Mitchison 2009). Depolymerases are proteins that control the length of microtubules by accelerating growth and shrinkage. Depolymerases identified in the recent years affect the conformations of tubulin at the microtubule ends (Howard & Hyman 2007, Brouhard & Rice 2014). Some microtubule-associated proteins were also found to be conformation selective in binding to tubulin monomers for promoting assembly (Ayaz *et al.* 2012, 2014, Brouhard & Rice 2014).

In a similar manner, FtsZ was seen to switch between the straight and the curved conformation, and it was proposed that this transition generates the necessary bending force to constrict the membrane (Erickson *et al.* 1996). Genetically modified FtsZ which could directly bind to the membrane, when introduced into liposomes *in vitro*, caused the membrane to constrict (Osawa *et al.* 2008, McIntosh *et al.* 2010). Further study in liposomes added evidence to the hypothesis that the membrane constriction was caused due to the force generated as a result of the change in the conformation of FtsZ filaments (Osawa *et al.* 2009). In addition, the C-terminal membrane tether of FtsZ is oriented toward the outside of the curved protofilament, which was opposite to what was observed in tubulin (Housman *et al.* 2016). This gave an insight into the evolutionary relationship between FtsZ and tubulin. Tubulin's precursor would have had similar features of that of FtsZ. As the evolution to tubulin took place, the straight conformation involved in forming the protofilaments was retained, but the inner bending or curvature was lost and replaced by the outer bending (Housman *et al.* 2016). This outer bending seems to be favorable due to its advantages during chromosome separation (Housman *et al.* 2016). A mechanical analogy of FtsZ to tubulin was established. Both proteins associated with larger cellular structures – kinetochore for microtubules and cell membrane for FtsZ – alter their conformations with respect to the environment of the cell. The ability of these polymers to locally interact and undergo structural changes at the cellular level has been called the 'hallmark of cytoskeletal polymers' (McIntosh *et al.* 2010). The tubulin bending forces and their coupling to motile functions in the cell are still unexplored concepts and FtsZ, given its similarity to tubulin with respect to its mechanics, provides a platform for this study.

Dynamics of assembly

GTP hydrolysis is tightly coupled to the assembly of microtubules while studies *in vitro* have demonstrated that the GTPase activity is not necessary for the assembly of FtsZ (Stewart *et al.* 1990, Mukherjee & Lutkenhaus 1998). Yet, the dynamics of FtsZ filaments like the tubulin polymers is considered to be regulated by GTP hydrolysis (Desai & Mitchison 1997, Mukherjee & Lutkenhaus 1998). One of the first concepts of microtubule dynamics was that of treadmilling – so called because of the continuous association of tubulin dimers at one end of a microtubule and the dissociation of tubulin dimers from the other end of the microtubule occurring at the steady state of assembly (Margolis & Wilson 1978). Dynamic instability was then introduced to describe the existence of a microtubule in prolonged states of polymerization and depolymerization without actually reaching the steady-state length (Mitchison & Kirschner 1984, Desai & Mitchison 1997). In an individual microtubule, both the ends exist in either of the two phases, the growing phase and the shortening phase. Using DIC microscopy, it was shown that microtubules alternate between an elongation phase and a rapid shortening phase (Walker *et al.* 1988). There is a continuous switch between the two phases in a stochastic manner. A study of the seeded assembly of pure tubulin using high-resolution microscopy, established elongation and shortening as two independent mechanisms (Housman *et al.* 2016). The active end (so called due to the rate of inter-conversion being more than the other end) shows more fluctuations in length and grows faster and shortens slower than the inactive end (Horio & Hotani 1986, Walker *et al.* 1988). It was suggested that the biological function of dynamic instability could be the recycling of microtubules that have missed their targets (Erickson & O'Brien 1992). Catastrophe is the switching of states from an elongation to a shortening state, while rescue represents the switching from a shortening to an elongation state (Walker *et al.* 1988). Although the polymerization reaction depends on tubulin concentration, depolymerization seems to occur independently of it (Desai & Mitchison 1997, Housman *et al.* 2016). The interplay between catastrophe and rescue, the transition frequencies, kinetics of microtubule assembly and the influence of tubulin concentration pose as a complex concept and is poorly understood (Panda *et al.* 2002, Akhmanova & Steinmetz 2015).

An important, now widely accepted theory of microtubule dynamics is that the growing microtubule

filaments are stabilized by a 'GTP cap' that prevents catastrophe (Carlier & Pantaloni 1981, Hill & Carlier 1983, Mitchison & Kirschner 1984, Melki *et al.* 1989, Panda *et al.* 2002). Loss of the GTP cap will expose the GDP-tubulin lattice leading to a catastrophe. 3D mechanochemical studies showed that the GTP-bound subunits interact strongly with each other while the GDP-bound subunits interact weakly. When the GTP cap is removed, the GDP-bound subunits will thus disassemble (Melki *et al.* 1989).

The growing microtubules have ends with relatively straight open sheets and the shrinking microtubules have ends with frayed, outward curling protofilaments (Mandelkow *et al.* 1991, Chrétien *et al.* 1995). The structural transitions at the polymer ends representing the structural plasticity of microtubules provide an alternative theory of microtubule dynamics beyond the GTP cap model (Kueh & Mitchison 2009). By exchange of nucleotides, GTP for GDP, the conformation of tubulin subunits undergoes a loss in its curvature, which enables their association to protofilaments (Nogales & Wang 2006). The GTP in the E-site is an allosteric effector, which drives the tubulin subunits to be in the straight conformation and forms a stable GTP cap at the microtubule ends (Melki *et al.* 1989). Hydrolysis of this GTP begins along with the assembly forming protofilaments with GDP-bound tubulin. As the GTP-tubulin subunits add to the growing end of microtubules, they straighten to give sheets. These sheets close to form the cylindrical tubes (Chrétien *et al.* 1995). The hydrolysis of GTP at the 'caps' will expose the GDP-tubulin subunits that tend to bend outwards, releasing the free energy stored during GTP hydrolysis before finally disassembling (Kueh & Mitchison 2009). The process has been represented as a model in Fig. 2.

Catastrophe was said to be linked to the 'aging' of the microtubule tips (Coombes *et al.* 2013). As the microtubule grows, the tips become tapered with time, and this change is independent of the hydrolysis of GTP. Proteins that modify the microtubule tip structures might promote the catastrophe (Coombes *et al.* 2013).

Comparing to the plus end of microtubules that binds GTP, the GTP-binding surface of the FtsZ protofilaments has been named as the plus end (Scheffers & Driessen 2001). Evidently, the variation in dynamics of FtsZ from microtubules arises from the fact that in FtsZ polymers, all subunits can exchange the nucleotide and that free FtsZ exists as a monomer rather than a heterodimer (Mukherjee *et al.* 1993, Sossong *et al.* 1999).

Figure 2

A model for microtubule assembly and disassembly is shown. (A) Free tubulin dimer bound to either GTP or GDP. (B) Tubulin dimers polymerize to form sheets of 13 protofilaments. (C) The sheets close to form a cylindrical tube. (D) Hydrolysis of GTP at the ends results in loss of GTP cap, exposing the GDP-tubulin lattice. GDP-tubulin units interact less strongly with each other, curling away from the tube and finally disassemble.

No evidence of FtsZ exhibiting dynamic instability has been found (Romberg & Levin 2003). However, a recent study indicated that the reorganization of FtsZ polymers could be due to treadmilling (Loose & Mitchison 2014). This study showed that FtsZ filaments in the presence of FtsA polymerized at one end and showed depolymerization at a faster rate at the opposite end emphasizing that large-scale dynamics of FtsZ is influenced by FtsA (Loose & Mitchison 2014). The filaments of FtsZ and FtsA are involved in positioning enzymes required for septal peptidoglycan (PG) synthesis for cell division process. To understand septal PG synthesis, the dynamic movements of FtsA and FtsZ filaments with respect to the enzymes recruited to the division site were examined using pulse-labeling and 3D illumination microscopy (Bisson-Filho *et al.* 2017). The septum was found to be constructed discontinuously around the division plane. The treadmilling of FtsZ and FtsA filaments around the division plane in a circular fashion led to the relative motions of the enzymes required for PG synthesis, thereby controlling the rate of PG synthesis and cell division (Bisson-Filho *et al.* 2017).

While tubulin always forms multi-stranded protofilaments, FtsZ protofilaments can exist in the single-stranded state (Romberg & Levin 2003). Like proposed for GDP-tubulin subunit interaction (Melki *et al.* 1989), FtsZ subunits bound to GDP interact less strongly with each other (Bramhill & Thompson 1994, Mukherjee & Lutkenhaus 1998, Sossong *et al.* 1999, Rivas *et al.* 2000, Romberg & Levin 2003). In tubulin association, GTP hydrolysis is closely coupled to microtubule elongation,

which is prevalent at the initial stages of assembly also (Vandecandelaere *et al.* 1999, Caplow & Fee 2003). Therefore, most of the polymer consists of the GDP-tubulin. The crystal structure of the tubulin protofilaments explained this view by showing that the occluded region of GTP binding would not allow the nucleotide to be released even after it is hydrolyzed (Oliva *et al.* 2004, Romberg & Mitchison 2004). This also was in conflict with the idea that the stabilization of growing microtubules was due to an extended GTP cap (Vandecandelaere *et al.* 1999, Caplow & Fee 2003). During FtsZ polymerization, however, the rate-limiting step is the nucleotide hydrolysis and not nucleotide exchange. The filament structure of FtsZ has exposed GTP sites, which allow for easy exchange of nucleotides (Mingorance *et al.* 2001, Oliva *et al.* 2004). Polymerization studies that measured the content of nucleotides in FtsZ protofilaments using filter-binding assays concluded that the FtsZ polymer is mostly GTP bound (Mingorance *et al.* 2001, Huecas & Andreu 2003).

Residues that establish the longitudinal contacts in microtubule filaments are conserved in FtsZ, while those which are involved in lateral contacts are not present in FtsZ (Nogales *et al.* 1998a). Lateral contacts cause bundling induced by magnesium, calcium and ruthenium red, *in vitro* (Yu & Margolin 1997, Rivas *et al.* 2000, Santra *et al.* 2004, Erickson *et al.* 2010). However, the physiological relevance of these *in vitro* studies on lateral contacts is still poorly understood.

Recent *in vivo* cryoelectron tomography studies done in *Caulobacter crescentus* revealed that the Z-ring is made

up of loosely arranged irregular patches of protofilaments, which gives it an overall discontinuous structure (Li *et al.* 2007, Holden *et al.* 2014). Following studies using high-resolution fluorescence microscopy techniques revealed that such was the case in many other bacterial species (Strauss *et al.* 2012, Rowlett & Margolin 2014, Jacq *et al.* 2015). It was also hypothesized that GTP-bound FtsZ assemble into straight protofilaments at many nucleation points at the mid-cell and attach themselves to the membrane (Lu *et al.* 2000). Unlike tubulin, GTP hydrolysis does not take place immediately. They establish lateral contacts to form a discontinuous Z-ring at the mid-cell region. GTP hydrolysis will induce a conformational change from straight to curved form of FtsZ subunits. This causes the overlapping filaments of FtsZ polymers to slide along each other and generate a contractile force that helps in membrane constriction (Lu *et al.* 2000, Lan *et al.* 2009, Szwedziak *et al.* 2014). However, recent studies have suggested that Z-ring remodeling for membrane constriction is due to polymerization dynamics and not because of previously proposed sliding mechanism (Li *et al.* 2007, Niu & Yu 2008, Lan *et al.* 2009, Loose & Mitchison 2014). The GDP-bound subunits are unstable and will finally result in depolymerization (Fig. 3).

However, it was shown that, even though the Z-ring displays a patchy network of FtsZ filaments, the fine-structural organization varies among different bacterial species. This variation is attributed to the variation in the modes of cytokinesis and array of divisome proteins present, differing from species to species (Haeusser & Margolin 2016). FtsZ does not only exist as a part of the cytokinetic Z-ring, but also forms highly dynamic helical cytoskeleton in the rest of the cell (Thanedar & Margolin 2004) indicating a strong analogy with the cytoplasmic microtubules that organize into multiple structures inside the eukaryotic cells.

The cooperative model shows assembly that starts with one dimer, which favors the attachment of the next subunit, which further favors the attachment of another and so on. This model is also called the lateral cooperative assembly model, which involves the stabilization of a dimer by a third subunit at a different interface. There is a slow nucleation phase and a rapid growth phase. For multi-stranded polymers such as microtubules, this model of assembly seems to fit well while for single-stranded polymers such as FtsZ filaments, there was much debate over whether the assembly was isodesmic or cooperative. Isodesmic assembly of FtsZ was proposed based on characteristics such as the single-subunit thickness of the protofilaments and rapid polymerization of FtsZ without

a clear nucleation phase (Romberg *et al.* 2001). However, more evidence for the cooperative model was collected over the years. It was shown that during assembly, pairs and bundles of protofilaments were formed, which indicated the presence of more than one kind of bond between the subunits (Mingorance *et al.* 2001, Huecas & Andreu 2003, Oliva *et al.* 2003). The absence of hydrolysis below a certain concentration of the protein provided evidence for the cooperative behavior of FtsZ assembly and suggested that the assembly requires a critical concentration of FtsZ. An increase in concentration showed higher activity and greater extent of polymerization (Wang & Lutkenhaus 1993, Oliva *et al.* 2003, Romberg & Mitchison 2004). The kinetics of FtsZ assembly as monitored by fluorescence fit the cooperative mechanism well. The length of the polymers remained uniformly independent of the total protein concentration (Chen *et al.* 2005). Thus, the cooperative mode of assembly has been accepted as the model for FtsZ assembly.

It was also derived that the N-domain of FtsZ is analogous to β -subunits of tubulin, which have the ability to form polymers-sheets, rings (Oxberry *et al.* 2001). A recent study on the N-terminal and C-terminal regions of FtsZ showed that truncated N-domains polymerized to form filaments while truncated C-domains did not show polymerization. This supported the hypothesis of the directional assembly of FtsZ (Jindal & Panda 2013).

Microtubule and FtsZ dynamics have constantly influenced each other in the study of assembly dynamics

Figure 3

A model for Z-ring assembly and disassembly. (A) Free FtsZ (monomer) exists predominantly as GTP-bound state. (B) GTP-FtsZ associates to form longitudinal filaments. (C) Protofilaments laterally contact to form a discontinuous Z-ring at the mid-cell region. (D) GTP hydrolysis leads to change in conformation as GDP-FtsZ is in curved state, which helps in membrane constriction. Finally Z-ring disassembles completely and cytokinesis occurs.

of cytoskeletal polymers. Even though the overall mechanism of tubulin dynamics has been understood, the understanding of the dynamics within the GTP cap is minimal. Understanding FtsZ dynamics is a key to unveiling ideas about microtubule core dynamics.

Associating proteins of the cytoskeleton

The dynamics of microtubules and FtsZ play important roles during cell division. Microtubule organization and dynamics are regulated by several proteins that alter the behavior of microtubules known as microtubule-associated proteins (MAPs). MAP2, MAP4 and tau have similar repetitive microtubule-binding regions, which interact with the C-terminal region of tubulin subunits and stabilize the microtubules (Maccioni & Cambiazio 1995). Several other proteins such as stathmin are known to destabilize microtubules (Belletti & Baldassarre 2011, Biao xue *et al.* 2016). MAPs energized the search for similar associating array of proteins that interact with FtsZ and regulate its assembly. Similar to tubulin, the C-terminal domain region of FtsZ bears a C-terminal linker that participates in interaction with FtsZ-associated proteins (Vaughan *et al.* 2004, Singh *et al.* 2007, 2008a, Kuchibhatla *et al.* 2011, Buske & Levin 2013, Ortiz *et al.* 2016). Recently, many proteins of the divisome of bacteria involved in cytokinesis have been identified and characterized (Massidda *et al.* 2013). Initiation of the assembly of the Z-ring is preceded by the spatial regulation of FtsZ at the mid-cell by negative spatial regulators such as Min proteins, which restrict the Z-ring assembly to mid-cell and inhibit their assembly at the cell poles (Raskin & de Boer 1999); positive spatial regulators like MapZ in *S. pneumoniae* lead to the localization of FtsZ at the mid-cell directly and stimulate assembly (Fleurie *et al.* 2014). FtsZ is now tethered to the membrane forming the Z-ring. Membrane-associated proteins such as ZipA and FtsA in *E. coli* mediate the attachment of the Z-ring to the cell envelope (Haeusser & Margolin 2016). ZipA is proposed to be the ancient prototype of MAP2/Tau family members (RayChaudhuri 1999, Dehmelt & Halpain 2005). It prevents the ClpXP-directed degradation of Z-ring (Pazos *et al.* 2013). SepF, a protein that enhances FtsZ assembly (Singh *et al.* 2008a), is thought to complement FtsA in early stages of formation of the Z-ring (Ishikawa *et al.* 2006). MciZ, an inhibitor of Z-ring formation in bacteria, binds to the minus end of the FtsZ filaments and produces shorter filaments, which are unable to form the Z-ring (Bisson-Filho *et al.* 2015).

Bridges between FtsZ and tubulin

FtsZ/tubulin superfamily of proteins consists of major cytoskeletal proteins found in all three domains of life – archaea, bacteria and eukaryotes. Some of these proteins are paralogs and orthologs of FtsZ found in eukaryotes and of tubulin found in archaea and bacteria. These proteins open new doors to study the cytoskeletal evolution from prokaryotes to eukaryotes. Gain and loss of functions as the proteins evolved represent new adaptations to incorporate the complexities of eukaryotic living. It is believed that during evolution, archaea diverged from its bacterial ancestors and further archaea and eukaryota diverged from each other. So it was likely to find a cytoskeletal protein similar to its prokaryotic ancestor, yet showing new properties similar to the eukaryotic successor. CetZ is a family of proteins present in archaea, which bears resemblance to both prokaryotic FtsZ and eukaryotic tubulin. Sequence alignments of CetZ with tubulins and FtsZ show that the active site residues are identical for tubulin and CetZ, while other regions of CetZ show greater similarity to FtsZ (Vaughan *et al.* 2004, Yutin & Koonin 2012, Duggin *et al.* 2015). FtsZ and CetZ/tubulin diverged during the evolution and further, and CetZ and tubulin diverged as cell architecture evolved (Yutin & Koonin 2012). This protein is found to co-exist with FtsZ indicating dynamic structural and cytoskeletal behavior of the protein inside the cell (Duggin *et al.* 2015).

Apart from archaea, proteins similar to tubulin are found in other species of bacteria as well. FtsZ is one of the most conserved proteins present in almost all bacteria, with the exception of a few families of Verrucomicrobia and genus *Chlamydia* (Mahendran *et al.* 2011). BtubA and BtubB were discovered in a bacterial species of Verrucomicrobia phylum, *Prostheco bacter de jonegi*, which lacked FtsZ. These proteins bear more resemblance to eukaryotic tubulin than to FtsZ, in terms of their sequence (Jenkins *et al.* 2002). Structural analysis suggested that the presence of these genes in bacteria may be due to the horizontal gene transfer from a eukaryotic organism (Schlieper *et al.* 2005). *In vitro* assembly studies revealed that BtubA did not polymerize on its own. However, BtubB assembled to form rings whose diameter was very close to that of microtubule single rings (Sontag *et al.* 2005). BtubA and BtubB together formed bundles of protofilaments similar to the calcium-induced bundles of FtsZ protofilaments, unlike the microtubule wall with regular lateral interactions (Lu *et al.* 2000, Sontag *et al.* 2005). These proteins provide a new platform for mutation analysis studies of tubulin *in vivo*.

(Sontag *et al.* 2005). *In vivo* electron microscopy studies revealed that BtubA/B form microtubule-like lattice forming 5-filament cylinders rather than the 13-filament of typical eukaryotic microtubules. It was proposed that these should be called 'bacterial microtubules' (Pilhofer *et al.* 2011).

Cytoskeletal proteins across prokaryotes and eukaryotes are involved not only in cell division, but also in the division of other structures and organelles inside the cell. One such protein of the FtsZ/tubulin superfamily is TubZ, involved in the division of low copy number plasmids (Larsen *et al.* 2007). TubZ, isolated from *Bacillus thurigiensis*, was considered to be homologous to FtsZ and tubulin based on the similarity of its X-ray crystal structure to that of tubulin and FtsZ (Ni *et al.* 2010). Characteristics of TubZ such as high critical concentration, rapid GTP hydrolysis rate and filaments made of subunits mostly bound to GDP are similar to those of tubulin (Anand *et al.* 2008, Chen & Erickson 2008, Akhtar *et al.* 2009, Aylett *et al.* 2010). A GTP cap model for this protein assembly was suggested based on these observations (Chen & Erickson 2008). Polymerization studies revealed that TubZ undergoes treadmilling and bundling, unlike FtsZ (Larsen *et al.* 2007, Anand *et al.* 2008). Electron microscopic observations indicated that TubZ forms parallel double helical filaments *in vitro* and *in vivo*, which show rotational symmetry, unlike tubulin (Aylett *et al.* 2010). The unique dynamics may explain the function of TubZ to be of a partitioning protein for plasmid segregation (Aylett *et al.* 2010). The unique sequences may be responsible for the ability of these proteins to twist and writhe, showing the adaptation of FtsZ/tubulin family cytoskeletal protein for cytomotive functions such as plasmid segregation (Aylett *et al.* 2010).

FtsZ from eukaryotic organelles

Chloroplasts and mitochondria are derived from prokaryotic organisms called endosymbionts (Gray 1999, Cavalier-Smith 2000, McFadden 2001). FtsZ in chloroplasts and other non-synthetic plastids was derived from the cyanobacterial ancestor of these organelles (Vitha *et al.* 2001, TerBush *et al.* 2013). Unlike in bacteria, FtsZ found in plastids have two phylogenetically distinct families of FtsZ, which co-localize at the division site to form the Z-ring (Osteryoung *et al.* 1998). However, the biochemical properties and dynamic behaviour of the two are different (Osteryoung *et al.* 1998). The two FtsZs represent gene duplication during chloroplast evolution from cyanobacteria to algae and further, to

higher plants (Osteryoung *et al.* 1998, Osteryoung & McAndrew 2001, Miyagishima *et al.* 2004). Recent studies on *Arabidopsis* chloroplast FtsZ families-FtsZ1 and FtsZ2 showed that FtsZ1 could co-assemble with FtsZ2 to form heteropolymers *in vitro*, showing extensive bundling with lateral interactions (Olson *et al.* 2010). The functional significance of such assembly *in vivo* is still elusive. The study of chloroplast FtsZs will give more insight into chloroplast evolution and the gene duplication of FtsZ in eukaryotic forms (TerBush *et al.* 2013).

Mitochondrial FtsZ isolated from chromophytealgal species (Beech *et al.* 2000, Takahara *et al.* 2000) bears resemblance to α -proteobacterial FtsZ (Beech *et al.* 2000). The division of mitochondria was also seen to be similar to that of plastid, involving two families of FtsZ proteins forming the ring at the division site (Takahara *et al.* 2001, Nishida *et al.* 2003). However, in higher eukaryotes, the gene encoding FtsZ is absent, indicating the loss of mitochondrial FtsZ during evolution (Miyagishima *et al.* 2004). Investigation of the mitochondrial division machinery of higher plants will thus provide insights into the evolutionary selection against FtsZ proteins and significance of the proteins that replaced FtsZ to carry out the function.

Targeting tubulin and FtsZ

FtsZ and tubulin form essential polymers for the growth and division of the prokaryotic and eukaryotic cells, respectively. Therefore, targeting FtsZ and tubulin presents an interesting strategy for antibacterial and anticancer therapy. Several compounds have been discovered over the years that target tubulin and disrupt microtubule dynamics. These agents stall mitosis and finally induce apoptosis of the cells. Clinical use of these compounds as anticancer drugs has been popular since the late 90s. In addition, many of the microtubule-targeting agents are presently in clinical trials for the treatment of various cancers such as pancreatic, breast and prostate cancer. Microtubule stabilizing agents promote microtubule assembly and prevent its depolymerization. Paclitaxel is one of the first microtubule-stabilizing agents identified (Schiff *et al.* 1979) and since then, many derivatives and analogs of it have been discovered (Singh *et al.* 2008b, Field *et al.* 2014). Vinblastine, combretastatin, estramustine and colchicine are among the popular microtubule destabilizing agents. Vinblastine, one of the most successful microtubule-targeting agents, has been in use for the treatment of cancer since 1960 (Field *et al.* 2014). Estramustine, a valuable drug used to treat hormone refractory prostate

cancer, was shown to dampen the dynamic instability of individual microtubules by suppressing the rate of growth and shortening and increasing the time duration of the pause state of the microtubules (Panda *et al.* 1997, Mohan & Panda 2008).

Combretastatin A4 phosphate (CA-4P), a member of the combretastatin family, inhibits tubulin polymerization and blocks cells at mitosis (Jordan & Wilson 2004). The drug binds to tubulin at the colchicine-binding site. CA-4P is a disodium phosphate derivative of CA-4 drug and exhibits enhanced water solubility. It underwent phase I clinical trials for anticancer therapy (Stevenson *et al.* 2003, He *et al.* 2011). Neurotoxicity and cardiovascular toxicity effects of CA-4P present major limitations (Rustin *et al.* 2003). Several analogs of CA-4 have been synthesized to minimize these effects. This led to the identification of a new combretastatin-2-aminoimidazole analog having better efficacy than CA-4 (Fig. 4) (Chaudhary *et al.* 2016). The compound exerts much stronger effects on microtubules than CA-4.

Other than antitumor therapy, microtubule-targeting agents have also been studied as drug targets in neurodegenerative diseases. TP-287 has the ability to cross the blood–brain barrier, target tau protein and is currently under clinical trials for the treatment of brain cancer and Alzheimer's disease (Field *et al.* 2014).

Although microtubule-targeting agents have been investigated extensively, the studies on FtsZ inhibitors are limited. FtsZ being the master coordinator of cytokinesis, when inhibited, would lead to the prevention of Z-ring formation. The failure of septation and division would thereby lead to cell death. Being one of the most highly conserved proteins in bacteria, inhibitors of FtsZ can be used as broad-spectrum antibiotics to target a wide range of pathogens (Rothfield *et al.* 1999, Margolin 2000,

Kapoor & Panda 2009, Singh *et al.* 2012, Ojima *et al.* 2014, Panda *et al.* 2016). The structural and functional homology of tubulin and FtsZ provides a platform to explore the possibility of studying agents that target tubulin as FtsZ inhibitors or at least as potential leads for them. The differences in some of the sequence regions and polymerization properties will allow the discovery of anti-FtsZ agents specific to prokaryotic cells, which have minimum effects on eukaryotic cells.

In an attempt to identify FtsZ-targeting agents based on anti-tubulin agents, *Mycobacterium tuberculosis* FtsZ was used to screen for taxane derivatives that showed anti-FtsZ activity. Some of them showed significant cytotoxicity against bacteria and were categorized as potential antituberculosis agents (Huang *et al.* 2006, Kumar *et al.* 2010). A taxane derivative, SB-RA-2001, was recently identified to have minimum host cytotoxicity and MIC₉₉ value (Huang *et al.* 2006). The compound promotes the assembly of purified FtsZ and stabilizes FtsZ filaments and unlike paclitaxel, SB-RA-2001 did not promote and stabilize the assembly of tubulin (Singh *et al.* 2014). Without affecting karyokinesis, SB-RA-2001 seems to inhibit the localization of FtsZ to form the Z-ring (Fig. 5). It inhibited the proliferation of *Bacillus subtilis* and *Mycobacterium smegmatis* cells and induced filamentation in these cells. It is proposed that the mechanism of action of SB-RA-2001 on FtsZ is similar to that of paclitaxel on microtubules (Singh *et al.* 2014).

PC190723, a potent taxane-related compound was shown to have inhibitory effects on *Bacillus subtilis*, *Staphylococcus* species and a methicillin-resistant *Staphylococcus aureus* strain (Haydon *et al.* 2008). Clinical studies on mice infected with a lethal dose of staphylococcal infection were treated with PC190723. Mice treated with the drug showed 100% survival while

Figure 4

Effects of CA-4 and compound 12 on microtubules. Compound 12 (panel A) depolymerizes microtubules (green) more strongly than CA-4 (panel B). Reproduced with permission from Chaudhary V, Venghateri JB, Dhaked HPS, Bhoyar AS, Guchhait SK & Panda D 2016 Novel combretastatin-2-aminoimidazole analogues as potent tubulin assembly inhibitors: exploration of unique pharmacophoric impact of bridging skeleton and aryl moiety. *Journal of Medicinal Chemistry* **59** 3439–3451. Copyright 2016 American Chemical Society.

Figure 5

SB-RA-2001 perturbed the Z-ring formation without affecting nucleoid segregation. The immunostaining of *Bacillus subtilis* 168 cells showing the localization of FtsZ (red) and DNA (blue) in the absence and presence of compound. This compound adversely affects the localization of FtsZ at mid-site of cell. Reproduced with permission from Singh D, Bhattacharya A, Rai A, Dhaked HPS, Awasthi D, Ojima I & Panda D 2014 SB-RA-2001 inhibits bacterial proliferation by targeting FtsZ assembly. *Biochemistry* **53** 2979–2992; Copyright 2014 American Chemical Society. Figures available under the terms of the ACS AuthorChoice License.

those which were not treated with the drug did not survive. The efficacy and selectivity of the drug showed its ability to be a potential candidate for anti-staphylococcal therapy (Haydon *et al.* 2008).

Benzimidazole derivatives, used in chemotherapy of cancer, fungal and neuroleptic diseases, have also been shown to target FtsZ by affecting the polymerization and GTPase activity. One of the benzimidazoles, BT-benzo-29, was found to inhibit the formation of Z-ring and to perturb the localization of FtsZ-interacting proteins in bacteria as seen in Fig. 6 (Ray *et al.* 2015). The localization of GFP-tagged proteins – FtsZ, FtsA, SepF and ZapA in *Bacillus subtilis* cells are disturbed in the presence of the drug (Ray *et al.* 2015). It had a minimal effect on the assembly of tubulin. Another compound, CXI-benzo-84, showed similar effects on tubulin by inhibiting the assembly and GTPase activity of tubulin *in vitro* (Rai *et al.* 2013).

OTBA (3-[5-[4-oxo-2-thioxo-3-(3-trifluoromethyl-phenyl)-thiazolidin-5-ylidenemethyl]-furan-2-yl]-benzoic acid) was identified among 81 compounds for targeting FtsZ (Beuria *et al.* 2009). OTBA was shown to inhibit the proliferation of bacterial cell more strongly than the mammalian cells. The compound promoted the assembly of FtsZ and enhanced the bundling of FtsZ polymers while it had no discernible effect on tubulin assembly. Similar to the action of FtsZ-associated stabilizing proteins such

as SepF and ZipA, OTBA inhibited the dilution-induced disassembly of preformed FtsZ polymers indicating that OTBA stabilizes FtsZ polymers. Further, OTBA was found to reduce the GTPase activity of FtsZ (Beuria *et al.* 2009).

Curcumin, a dietary polyphenolic compound, is found to have both antibacterial and anti-microtubule properties (Rai *et al.* 2008, Banerjee *et al.* 2010). Recently, a potent carbocyclic curcumin analog (2d) was discovered, which inhibited *Bacillus subtilis* proliferation by targeting the assembly of FtsZ (Groundwater *et al.* 2017). 2d inhibited the assembly of FtsZ *in vitro*, whereas it had minimal effects on the assembly of tubulin. Interestingly, 2d strongly enhanced the GTPase activity of FtsZ while it reduced the GTPase activity of tubulin. The differential effect of this compound on bacteria and mammalian cells makes the compound a selective, potential drug for treating bacterial infections (Groundwater *et al.* 2017).

Comparison of the binding sites of the inhibitors of tubulin and FtsZ

The structural similarity between FtsZ and tubulin does not necessarily imply that the active sites and the binding pockets formed by these proteins are similar. Differences arise from the extents of solvent accessibility of the nucleotide-binding pockets in these proteins. The

Figure 6

Effects of BT-benzo-29 on the localization of FtsZ and its associated proteins. (Panel A) The cell division proteins GFP-FtsZ, GFP-FtsA, GFP-ZapA and GFP-SepF localize at the center in the control cells. These proteins do not localize at mid-cell and diffuse throughout the length in the treated cells. Panel B shows the disrupting effect of BT-benzo-29 on the Z-ring without perturbation of the nuclear material. Reproduced with permission from Ray S, Jindal B, Kunal K, Surolia A & Panda D 2015 BT-benzo-29 inhibits bacterial cell proliferation by perturbing FtsZ assembly. *FEBS Journal* **282** 4015–4033. Copyright 2015 FEBS.

nucleotide is exchangeable in FtsZ protofilaments while it is not exchangeable in tubulin protofilaments, which have occluded binding pockets, making the active site of FtsZ more open and partially water-filled. The tubulin GTP-binding site is more hydrophobic than that of FtsZ (Oliva *et al.* 2004, Läppchen *et al.* 2008). Agents that target microtubules and FtsZ could interact with the proteins by binding to the nucleotide site or by binding to other regions of the structure.

Over the years, many small-molecule inhibitors of tubulin have been identified and are found to bind to one of the three well-characterized ligand sites – taxol, vinca and colchicine sites (Jordan & Wilson 2004). High-resolution X-ray crystal structure revealed the residues participating in the binding of colchicine (Ravelli *et al.* 2004). The steric hindrance between the residues of α subunit and colchicine bound to β subunit is responsible for the prevention of establishment of lateral contacts during the polymerization of tubulin, which explains the destabilizing effect of this compound (Ravelli *et al.* 2004). There is a switching of the T7 loop from its normal position (when unbound to ligand) to accommodate the ligand at this site (Dorleans *et al.* 2009). Many compounds bind at this interface and are called colchicine-binding site inhibitors (Wang *et al.* 2016). Vinblastine, also induces such a conformational change in microtubules, but at the inter-dimer interface (Gigant *et al.* 2005). While vinblastine binds to both α and β subunits, taxol interacts

with the β subunit residues. The M-loop of the subunit is especially important in binding to the taxane ring (Löwe *et al.* 2001). The structural insights derived from these ligand–tubulin interactions can help us not only in identifying new drugs that target microtubules, but also to study the mechanisms of ordered assembly, disassembly, conformational switching along with the structural changes occurring with respect to these compounds and the role GTP plays in it.

Several compounds have been identified that target the GTP-binding site of FtsZ and thereby inhibit polymerization (Läppchen *et al.* 2008, Chan *et al.* 2013, Ruiz-Avila *et al.* 2013, Panda *et al.* 2016). A study using GTP analogs established several molecules to have potent anti-FtsZ activity, while some among them showed differential effects on tubulin activity (Läppchen *et al.* 2008). The difference in the nucleotide-binding sites, will allow the specific targeting of bacteria inside a mammalian host. These studies also establish the nature of interaction of GTP and the residues of the nucleotide-binding site of FtsZ. Another study used structure-based virtual screening to identify natural pyrimidine-substituted quinuclidines as FtsZ inhibitors (Chan *et al.* 2013). Using induced fit docking analysis, it was predicted that some of these compounds mimicked GTP and were able to establish key interactions within the triphosphate, sugar and nucleotide-binding regions of FtsZ (Chan *et al.* 2013).

Many other compounds that interfere with FtsZ polymerization have also been identified, which bind to FtsZ at different sites other than the nucleotide-binding sites (Kapoor & Panda 2009, Panda *et al.* 2016). It is highly useful in terms of specificity, to identify anti-FtsZ agents that target unique sites in the protein, and not the nucleotide-binding site, which is similar to that in mammalian tubulin.

Crystal structure of *Staphylococcus aureus* FtsZ bound to PC190723 showed that the compound is present at a narrow pocket deep within a cleft formed by the C-terminal half of the H7 helix, the T7 loop and the four-stranded C-terminal sheet (Tan *et al.* 2012). This binding site has been compared to the taxol-binding site of tubulin. The mechanism of action of these compounds on their respective proteins is also similar. This lead to reporting PC as a new class of FtsZ polymer-stabilizing agents (Andreu *et al.* 2010). Docking of SB-RA-2001 showed the compound to bind at the site of PC190723 binding, near the cleft at the C-terminal (Singh *et al.* 2014). The putative binding site for BT-benzo-29 was identified using site-directed mutagenesis and molecular docking studies (Ray *et al.* 2015). The compound binds to the C-terminal region of the protein involving hydrophobic residues leucine, glycine, proline and valine (Ray *et al.* 2015). STD NMR and docking studies suggested the binding site for cinnamaldehyde, an antibacterial agent that targets FtsZ, to be around the T7 loop (Domadia *et al.* 2007) similar to Sula, a known cell division inhibitor that interacts with FtsZ (Cordell *et al.* 2003). It was also suggested that the binding of the compound to this region induces a conformational change in the protein, which could interfere in its polymerization and GTPase activity (Domadia *et al.* 2007). CRAMP (16–33), an inhibitor of FtsZ assembly, is predicted to interact with the T7 loop of FtsZ (Ray *et al.* 2014).

Though several studies examined the binding of FtsZ inhibitors, their binding sites on FtsZ are not well characterized. It has been suggested that FtsZ has a smaller number of regions that the small molecules can bind to, while tubulin has many such regions, making it a relatively easier protein to target (Hurley *et al.* 2016). The putative binding sites of several FtsZ-targeting agents seem to be around the T7 loop, also present in host tubulin which raises questions about the specificity of such compounds. Compounds targeting the GTP-binding domain also raise similar queries. Identification of alternative target-binding sites would be useful in accelerated development of drugs against FtsZ.

Targeting FtsZ and tubulin follows the same principle and the studies done on the two have influenced each other in several ways. Be it with respect to the binding site interactions, mode of action and the development of derivatives of the compounds. However, agents targeting tubulin such as colchicine, paclitaxel, vinblastine and benomyl have no discernable effect on FtsZ assembly (Yu & Margolin 1998, White *et al.* 2000, Beuria *et al.* 2005). Most of the FtsZ inhibitors currently under study such as PC190723, BT-benzo-29 and OTBA also show minimal effects on tubulin polymerization (Haydon *et al.* 2008, Beuria *et al.* 2009, Ray *et al.* 2015). The differential effect of these compounds raises the possibilities of finding inhibitors that specifically target FtsZ without harming the eukaryotic host.

Conclusion

Since tubulin and FtsZ share ancestry, the understanding of primitive mechanism of their dynamics will be helpful in the fundamental study of the dynamics of similar structures in higher organisms. The evolution of cytoskeleton from FtsZ to microtubules has been discussed previously (Erickson 2007). Being extremely divergent from their precursor protein FtsZ, tubulins show a high level of conservation across modern eukaryotic species while bacterial FtsZ shows 40–50% sequence identity across species. FtsZ is considered to be an ancient protein and the division mechanism carried out in the first cellular forms was suggested to be carried out by FtsZ (Davis 2002, Erickson 2007). The process was successful enough to be retained in the modern species of bacteria and archaea (Davis 2002, Erickson 2007). FtsZ is suggested to be the cell division protein in early eukaryotes as well (Doolittle 1995, Doolittle & York 2002). As evolution from FtsZ to microtubules took place, the functional role of the protein was transformed from cytokinesis to chromosome segregation. The complexities of this evolution might be the reason for the conservation of tubulin across modern eukaryotic species, as it would be difficult for these well-developed proteins to undergo further changes (Erickson 2007). This evolution of FtsZ to microtubules involves mutations that allow for binding of microtubule-associated proteins and help to form additional lateral contacts. FtsZ gene duplication in chloroplast and mitochondria of lower eukaryotes further bridges the gap between FtsZ formed by the association of a single subunit and microtubules formed by the association of two classes of subunits. New changes in the curvature advantageous for

DNA segregation were developed (Housman *et al.* 2016). However, the dynamic properties were conserved. New functions were associated to these dynamic cytoskeletal structures giving the modern day microtubules.

Microtubule dynamics have been studied for much longer than FtsZ dynamics; yet, the mechanism for neither has been fully established. New information regarding FtsZ dynamics can enhance our understanding of microtubule dynamics.

Though evidence for the existence of a more dynamic plus end of FtsZ and a less dynamic minus end has surfaced recently (Loose & Mitchison 2014, Bisson-Filho *et al.* 2015), a long-standing question whether FtsZ can exhibit dynamic instability and treadmilling like microtubules remains unanswered. In a recent work, FtsZ treadmilling has been suggested as a mechanism to explain the recruitment of enzymes at the division site (Bisson-Filho *et al.* 2017). A large body of evidence points toward the similarity of the dynamics of FtsZ and tubulin, thereby providing a platform to study the basic mechanism of the assembly of microtubules through FtsZ.

One such concept is that of the force generation by disassembling filaments. Many studies have tried to determine whether the disassembling FtsZ filaments can generate enough force to cause bending and constriction of the cell membrane. In eukaryotes, the question remains – is the force generated by the disassembling microtubules of the spindle apparatus enough to pull the chromosomes apart? The presence of molecular motors associated with the microtubules argues against it. Many other ‘passive’ forces such as viscous drag, frictional resistance and force generated by bending of the microtubules are proposed to also contribute (Forth & Kapoor 2017). Without the interference of the associated proteins like in microtubules, FtsZ dynamics can be studied to explain such force generation.

During anaphase, the chromosomes are pulled toward the poles by the disassembling microtubules. Different mechanisms are proposed for the disassembly occurring at only one end like in yeasts (plus end), or both ends like in human mitotic cells, or at the minus end coupled with assembly at the plus end (Asbury 2017). Studies to investigate how the ends of the microtubules switch from assembly states to disassembly state and what drives these changes in correlation to GTP binding and hydrolysis can be based on studies with FtsZ and its disassembly properties, and if present, the ability to treadmill.

The correlation between the processes of nucleotide-binding and filament assembly has been explained differently for FtsZ and tubulin, which further explains

their mechanical and functional roles in the cells. Conformational changes also form the basis for explaining the assembly and disassembly of the polymers. However, what drives this change in conformation is little understood and different models are proposed for FtsZ and microtubules. Understanding the dynamics of FtsZ may explain the GTP cap-GDP core model of microtubules.

Overall, these studies provide a platform to discover new drugs and agents that target the two proteins and disrupt their assembly dynamics. Such compounds have a great scope in anticancer and antibacterial therapy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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