Tubulin and FtsZ Superfamily of Protein Assembly Machines

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Article Contents

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Eukaryotic $\alpha\beta$-tubulin and bacterial FtsZ self-assemble into dynamic cytoskeletal polymers, microtubules or filaments, which are essential for chromosome segregation or bacterial cell division, respectively. Both share homologous core structures with guanosine-5′-triphosphate (GTP)-binding and GTPase-activating domains joined by a central helix, and form similar protofilaments with 4 nm spaced subunits along them. During assembly, the GTPase-activating domain of one subunit associates with the GTP binding domain of the preceding subunit, completing the active site. GTP hydrolysis triggers disassembly, which is coupled to free subunits switching into inactive conformation. Microtubule dynamics is inhibited by anticancer drugs binding near tubulin assembly interfaces. FtsZ is a target for new antibiotics discovery; several bacterial division inhibitors bind between FtsZ domains or at its GTP site. Other proteins in this superfamily include: gamma-tubulin that is essential for microtubule organisation; bacterial tubulin, a primitive structural homologue; and recently discovered TubZ, distant homologue employed by plasmids and phages for deoxyribonucleic acid positioning.

Introduction: Distinct Families of Self-Assembling GTPases That Are Essential for Life

Proteins from the tubulin superfamily of guanosine-5′-triphosphatases (GTPases) are employed to segregate deoxyribonucleic acid (DNA), to divide cells or to perform cytoskeletal and motility functions. They spread into eukaryotes, archaea, bacteria, plasmids and viruses, and they include up-to-date $\alpha\beta$-tubulin, γ-tubulin, bacterial tubulin BtubA/B, FtsZ and TubZ. They show a low sequence identity, globally less than 10% in the case of TubZ, but have conserved signature motifs involved in nucleotide binding. Despite their divergent sequences, tubulin-like proteins share a common three-dimensional core structure, consisting of an *N*-terminal GTP binding domain with a modified Rossman fold and a GTPase activating domain, both connected by a central α-helix (Nogales *et al*., 1998). The *C*-terminal regions are very divergent between them: a two-helix bundle and an acidic short tail in tubulin; a two-stranded beta-sheet in FtsZ that extends into a flexible tail of variable length depending on the bacterial species; and a single long *C*-terminal helix followed by a flexible tail in TubZ (see **Figure 1**).

It has been hypothesised that each domain evolved from two independent proteins, which would be equivalents to a small GTPase (*N*-terminal domain) and its GTPase activating protein (GAP, the second domain). Both fused into a single common ancestor molecule that associated into a linear polymer based on the original protein–protein interactions and had a polymerisation-dependent GTPase activation mechanism (Oliva *et al*., 2004). This common ancestor protein could perform mechanical work in a GTP-dependent manner, applying force to nucleic acids or membranes in a primitive cell (Ludueña, 2013). This GTPase-based molecular machine later spread into the different types of cells, some plasmids and viruses (see **Figure 1**), where selection pressure worked in different ways inducing changes on the protein structure. Different types of lateral associations between protofilaments evolved giving structurally different polymers.

Current tubulin-like proteins typically associate head to tail into similar polar protofilaments with a 4 nm axial spacing between subunits (except γ-tubulin), which form different types of polymers and characteristic subcellular structures that perform different functions (see **Figure 2**).

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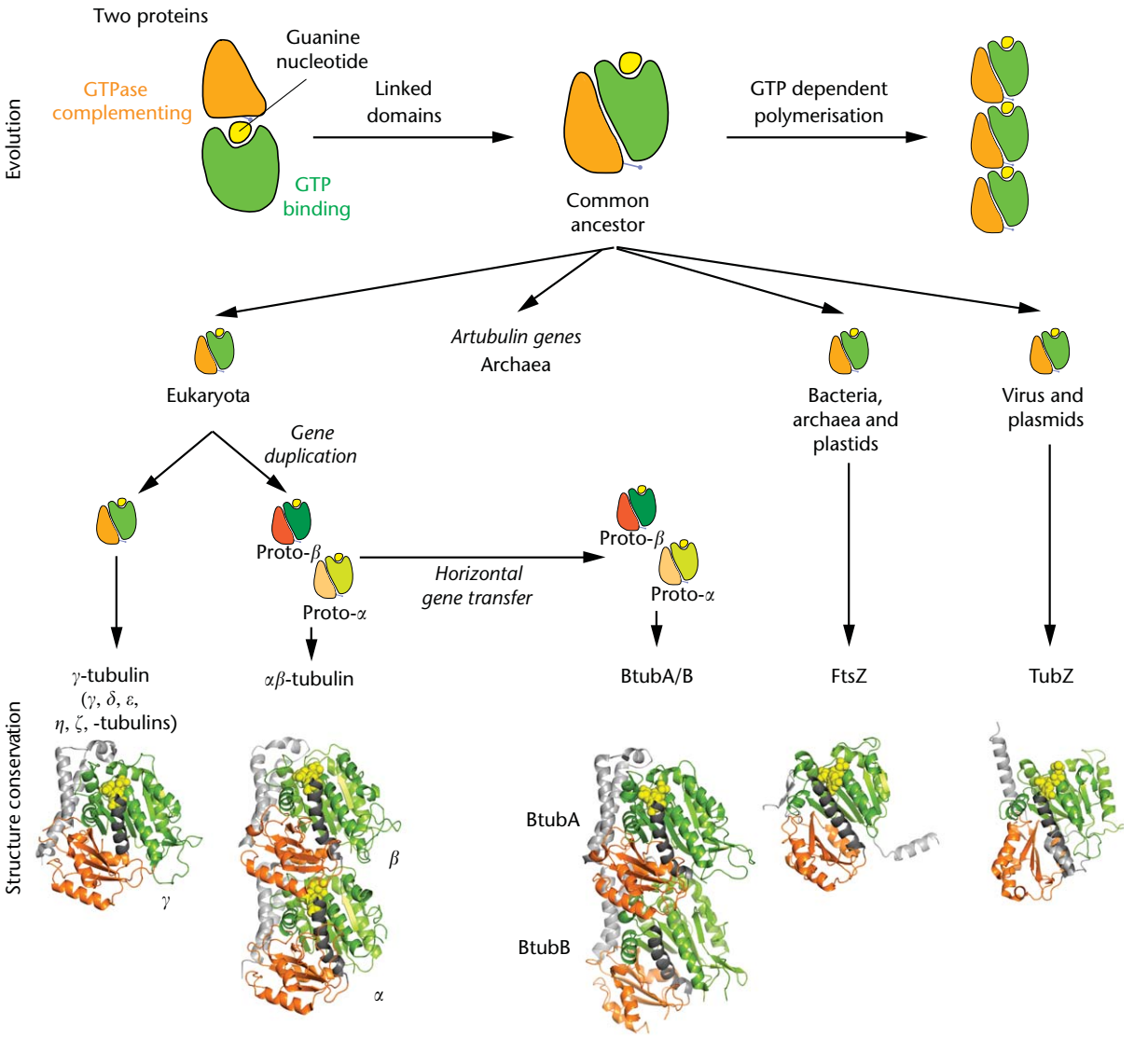

Figure 1 Evolution scheme and structural homology of the tubulin superfamily of proteins. Top, two hypothetical independent proteins, a GTP binding protein (green, nucleotide in yellow) and a GTPase complementing protein (orange) fused into a single protein with two domains, which had to assemble for GTP hydrolysis activation. Middle, the ancestor protein spread had independently evolved into the tubulin, FtsZ and TubZ families of proteins. The presence of tubulin genes in two *Nitrosoarchaeum* genomes (named *artubulin*) suggests an archaeal origin of eukaryotic tubulins (Yutin and Koonin, 2012). Bacterial tubulin BtubA/B encoded by several *Prosthecobacter* species is thought to have evolved following horizontal transfer of primitive tubulin genes to an ancestor of this group of bacteria (see main text). Bottom, the structures of the extant proteins in the tubulin superfamily share a conserved fold of two domains (green, GTP binding; orange, GTPase activating) joined by a core helix (dark grey). Significant differences include surface loops, the *C*-terminal structural elements at the left-back of each view and the *N*-termini (both marked light grey). The protein structural files were taken from the Protein Data Bank (PDB) and displayed as ribbons with PYMOL. The PDB entries used are: gamma-tubulin, 1Z5W; alphabeta-tubulin, 1JFF; bacterial tubulin, 2BTQ; FtsZ, 1FSZ; TubZ and 3M8K.

Protofilament assembly involves the formation of a subunit–subunit interface where the catalytic loop from the GTPase activating domain of the upper subunit in the row complements the GTP binding domain of the lower subunit. GTP hydrolysis triggers polymer disassembly and is followed by spontaneous subunit reloading with GTP. In contrast with classical GTPases, there is no need of an additional GAP or a nucleotide exchange factor (GEF). Polymer dynamics is thus based on assembly–disassembly events and shows different features depending on the precise mechanism involved, such as dynamic instability or treadmilling (see below). These dynamic cytoskeletal filaments that can produce motility without the assistance of motor proteins have also been named cytomotive filaments. In this article, we present a current perspective of the structures, mechanisms and evolution of these essential assembly machines, with examples of their important biomedical implications. **See also**: G Proteins

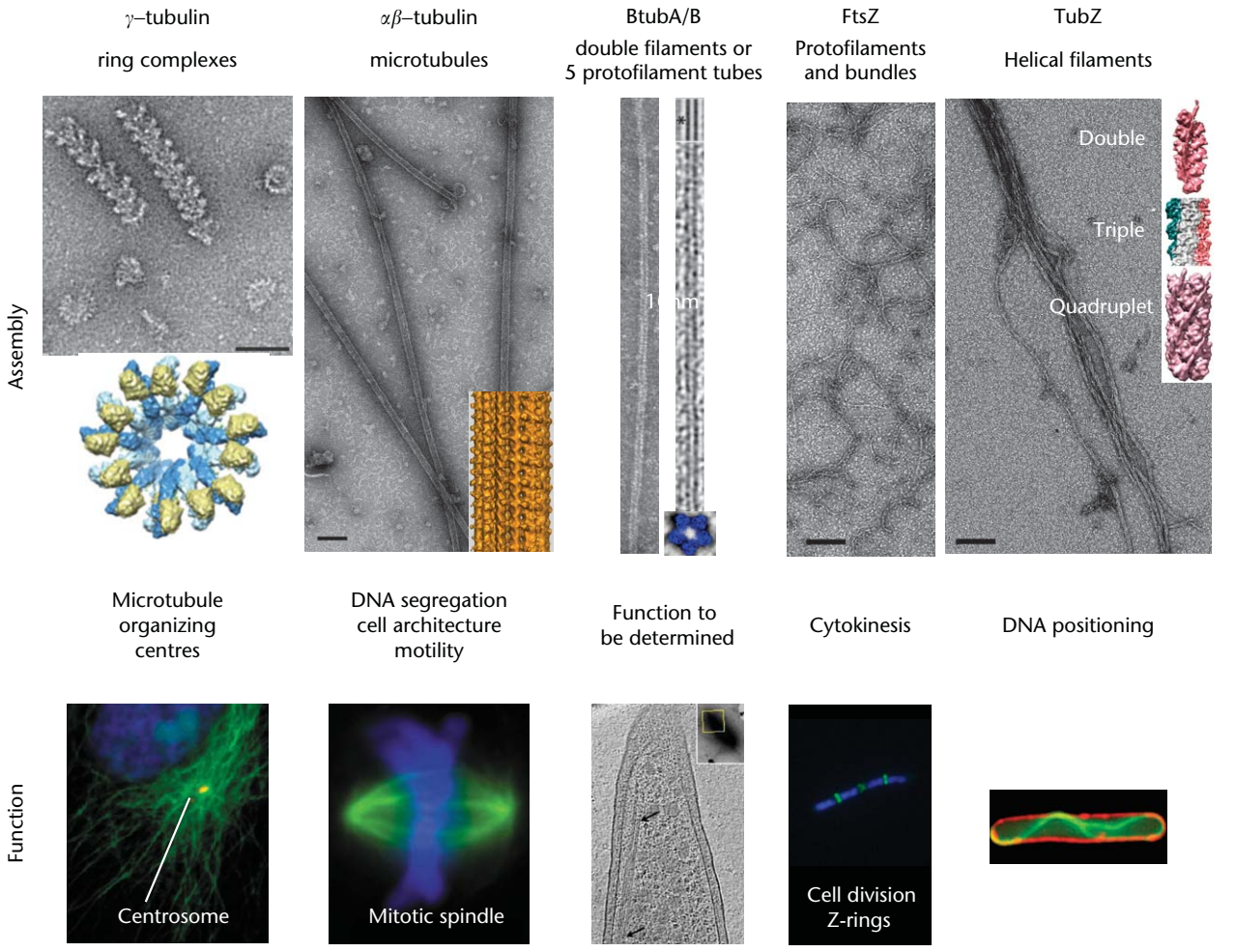

Figure 2 Assemblies and subcellular structures formed by the tubulin superfamily proteins. Top, negative stain electron microscopy images of the polymers assembled from these proteins. Magnification bars indicate 100 nm. Medium resolution structural models shown coloured are: gamma-tubulin ring complexes (reproduced from Kollman *et al.*, 2011, 2010. © Nature Publishing Group); microtubules (reproduced from Electron Microscopy Data Bank, EMD-2004, Maurer SP *et al.*, 2012) and TubZ 2, 3, and 4-stranded filaments (reproduced from the Electron Microscopy Data Bank, EMD- 5762, 5763 and 5783; Montabana and Agard, 2014; Zehr *et al.*, 2014). BtubA/B polymers shown are filaments (reproduced from Schlieper *et al.*, 2005. © The National Academy of Sciences) and tubes (electron cryo-tomography and model; reproduced from Pilhofer *et al.*, 2011). Bottom, functions and characteristic sucellular structures formed by each protein, using light microscopy or cryo-tomography (BtubA/B, reproduced from Pilhofer *et al.*, 2011). Centrosomes are stained with antibodies to gamma-tubulin (yellow), cytoplasmic and spindle microtubules are directly imaged here with a green fluorescent derivative of the antitumor drug taxol (see method in Barasoain *et al.*, 2010). DNA is stained blue . Bacterial Z-rings are visualized green with FtsZ fused to green fluorescent protein (GFP). Filaments formed by TubZ-GFP expressed in *Bacillus* cells (reproduced with permission from Larsen *et al.*, 2007. © Cold Spring Harbor Laboratory Press).

Eukaryotic αβ-Tubulin, Microtubule Assembly and Anticancer Drugs

Tubulin was first purified as the colchicine-binding protein proposed to be the subunit of microtubules (Weisenberg *et al.*, 1968), and later, on reconstitution, experiments proved that microtubules could be formed by self-assembly of αβ-tubulin heterodimers, when supplied with GTP and magnesium under favourable solution conditions (Lee and Timasheff, 1975). Microtubules are 25 nm wide, long hollow cylinders typically made of 13 protofilaments. They form the spindle that segregates chromosomes during eukaryotic cell division and are components of the cytoskeleton essential for intracellular trafficking, shape, resistance and polarity of non-dividing cells. Cytoplasmic microtubules are found in most types of eukaryotic cells, and also forming centrioles, cilia, flagella and basal bodies (for microtubule structures and functions). **See also**: Tubulin and Microtubules

Both α- and β-tubulin subunits are approximately 35% identical in sequence and have very similar atomic structures (Nogales *et al.*, 1998). Their *C*-terminal α-helices (H11 and H12) and the disordered acidic tail protrude at the microtubule outer surface, where they participate in the binding of numerous microtubule-associated proteins

(MAPS) and motor proteins. Compared to FtsZ and TubZ, tubulin has larger surface loops, several of which form contacts with neighbouring subunits in microtubules. Tubulin monomers only reach a folded functional structure with the assistance of several cytosolic chaperones (like CCT) binding through these loops. Then, the formation and dissociation of the high-affinity $\alpha\beta$-tubulin heterodimers require the participation of other protein cofactors. Considering that microtubules contain approximately 1500 tubulin heterodimers per micron of length and that correct assembly is critical for their cellular functions, such elaborate regulation system prevents tubulin aggregation and presumably carries out a strict quality control of subunits. In fact, several mutations in the gene encoding tubulin chaperone cofactor E (TBCE), implied in tubulin dimer homoeostasis, cause heterozygous HRD syndrome, in which functional TBCE protein and a limited tubulin dimer formation are rescued by cryptic out-of-frame translational initiation (Tian *et al*., 2006).

One of the main differences between α- and β-tubulin is the co-catalytic residue involved in GTP hydrolysis that is a conserved Glu254 in α-tubulin but a Lys254 in β-tubulin. This sequence variation has a crucial role in microtubule formation because Lys254 does not activate GTP hydrolysis in the contacting α-tubulin below within the $\alpha\beta$-heterodimer (Nogales *et al*., 1998). GTP binding by β-tubulin facilitates microtubule assembly and subsequent hydrolysis at the association interface with α-tubulin from the contacting heterodimer in the protofilament induced disassembly. The majority of tubulin molecules inside the microtubule lattice are GDP-bound and poised for disassembly, whereas the microtubule end is held together by a cap of GTP-bound dimers. When the cap is lost, the microtubule rapidly depolymerises from the end, but it can regain the GTP cap by incorporating surrounding GTP-bound dimers and then grow again. Thus the polar nature of microtubule protofilaments together with GTP binding and hydrolysis originate their characteristic dynamic instability (Mitchison and Kirschner, 1984) (see **Figure 3a**). However, steady state microtubules can undergo treadmilling, consisting of opposite end assembly and disassembly while maintaining a constant polymer length, similarly to actin filaments (Margolis and Wilson, 1978). Depolymerising microtubule ends show curved protofilaments peeling off, whereas growing ends are straight and regular (**Figure 3a**). Continuously growing and shrinking microtubules explore space for binding cellular components (e.g. capturing chromosome kinetochores during mitosis) and also generate pushing and pulling forces.

There are three main coupled processes involved in the tubulin assembly and disassembly mechanism: (1) the longitudinal association between tubulin dimers that is modulated by GTP/GDP, (2) a tubulin monomer conformation switch form curved to straight including displacements of the central helix and the GTPase activating domain, and (3) the lateral association between monomers in microtubules. Whereas the GTP gamma phosphate permanently bound to α-tubulin and its coordinated Mg^{2+} ion stabilise the tight $\alpha\beta$-tubulin heterodimer, the β-tubulin bound GTP and Mg^{2+} are thought to stabilise the axial association between tubulin dimers in microtubule protofilaments. GTP hydrolysis within microtubules has been inferred to remodel the binding site compacting the longitudinal association interface between dimers and generating an internal monomer strain that can be released by bending (Alushin *et al*., 2014), thus triggering depolymerisation. In fact, structural studies have shown tubulin in two clearly different conformations: straight within associated protofilaments and always curved in unassembled tubulin dimers in complexes with several inhibitor proteins, irrespectively of the nucleotide state (Pecqueur *et al*., 2012; see **Figure 3b**). Unassembled tubulin dimers are thus in a naturally curved conformation that is incompatible with microtubule assembly but switches into the straight conformation upon incorporation into the microtubule lattice, whereas depolymerising tubulin goes back into the curved conformation. This is quite different from classical GTPases, where the activating structural change is induced by GTP binding rather than by the associating partners. Finally, lateral interactions in a microtubule keep tubulin straight, and include the H1-S2 and H2-S3 loops of one subunit with the M-loop (S7-H9 loop) of the neighbour subunit (Alushin *et al*., 2014).

Lower eukaryotes have one or two genes for each α- and β-tubulin, but vertebrates have multiple tubulin genes encoding seven α- and six β-tubulin isotypes, whose sequence differences cluster mainly at their acidic *C*-terminal tails. Tubulin isotypes can modify microtubule dynamicity and some are specifically expressed in different tissues. For instance, βIII neuronal isotype enhances microtubule dynamics and is abundant in a variety of embryonic tissues (Ludueña, 2013). Interestingly, its overexpression is associated to cancer cells resistance to the antitumor drug taxol (paclitaxel). Also, mutations in the genes encoding several α- and β-tubulin isotypes impair neural migration and cause brain malformations and neurological disorders (Tischfield *et al*., 2011).

In addition to tubulin isotype diversity, microtubules are subjected to several posttranslational modifications (PTMs), such as detyrosination/tyrosylation, acetylation, phosphorylation, polyglutamylation and polyglycylation, which generate further microtubule diversity associated to their stability and specific cellular functions. Much of the chemical diversity is located at the flexible *C*-terminal tubulin tails on the microtubule surface. Altered levels of tubulin PMTs are involved in ciliopathies and neurodegeneration, and have also been observed in cancer cells (Janke and Bulinski, 2011).

Microtubules interact with numerous regulatory and adaptor proteins, including microtubule associated proteins (MAPs), microtubule motors (kinesin, dynein), microtubule severing (katanin), tubulin sequestering proteins (stathmin) and microtubule plus end tracking proteins (+TIPs) (Akhmanova and Steinmetz, 2008). The microtubule inhibitor stathmin recognises naturally curved tubulin (Barbier *et al*., 2010) as do microtubule

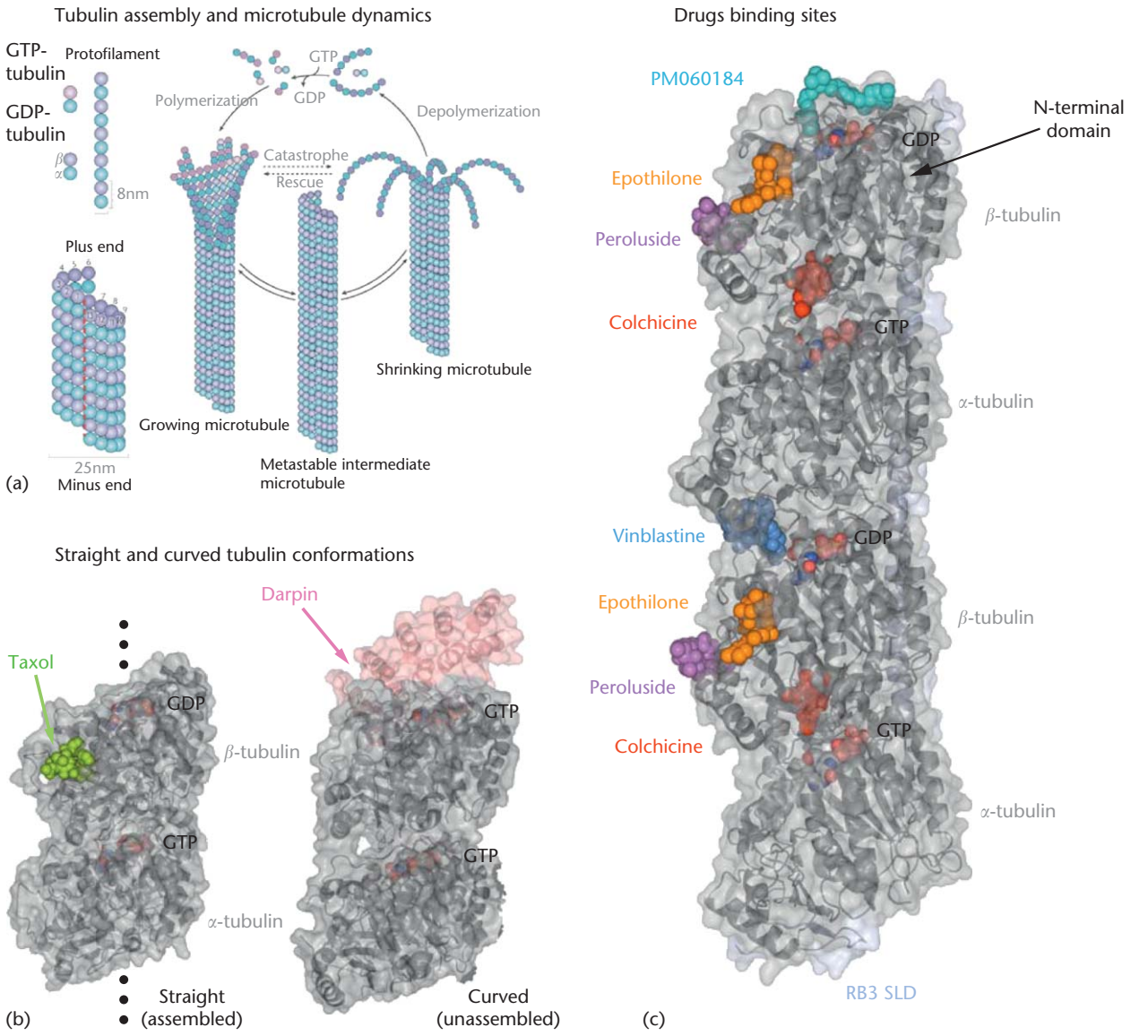

Figure 3 (a) Scheme of $\alpha\beta$-tubulin assembly and microtubule dynamic instability. Reproduced with permission from Akhmanova and Steinmetz (2008) © Nature Publishing Group. (b) Straight and curved conformations of the $\alpha\beta$-tubulin heterodimer; the straight structure on the left was obtained by electron crystallography of tubulin sheets with bound taxol (from PDB entry 1JFF). The curved structure shown was determined by x-ray crystallography of $\alpha\beta$-tubulin in complex with a plus end capping DARPIN domain (from PDB 4DRX). (c) Localisation of drug binding sites, shown on the complex of two curved tubulin heterodimers with the stathmin-like domain of protein RB3 [RB3-SLD, light blue]. We only show one ligand for each characterised binding pocket: epotilone (orange) and peroluside (purple) (from PDB 404L), colchicine (red) and vinblastine (blue) (from PDB 1Z2B) and PM060184 (green blue) (from PDB 4TV9), which shares a new binding site with maytansine (Prota *et al.*, 2014b). As a reference for orientation we highlight the location of the nucleotide at the *N*-domain.

polymerases that catalyse tubulin addition to microtubule ends. In contrast, doublecortin, a stabilizing MAP (Bechstedt and Brouhard, 2012) and end-binding proteins (Maurer *et al.*, 2012) recognise the 13-protofilament microtubule lattice and bind at growing microtubule ends. Similarly to altered tubulin isotypes and levels of PMTs, there are several microtubule associated proteins whose modifications are associated with human diseases. For instance, the soluble stabilizing MAP Tau is hyperphosphorylated in cytotoxic oligomers and insoluble neurofibrillary tangles that are characteristic of Alzheimer's and other neurodegerative diseases. Tau mutations cause inherited frontotemporal dementia and Parkinsonism linked to chromosome 17 (Spillantini and Goedert, 2013). Also, doublecortin mutations affect neural migration causing human X-linked lissencephaly and double cortex syndrome (Bechstedt and Brouhard, 2012); and lacking or defective axonemal dynein arms are the main causes of primary ciliary dyskinesia and Kartagener's syndrome (Afzelius, 1976).

Tubulin is the target of numerous small molecules that bind to the unassembled or assembled protein, respectively

inhibiting or stabilizing microtubules. These ligands frequently bind at association interfaces between tubulin molecules, either at microtubule ends or along the microtubule lattice. Binding of a small per cent of tubulin molecules to an inhibitor, such as colchicine, can thus prevent microtubule assembly. Actually, both microtubule destabilising agents (MDA) and microtubule stabilizing agents (MSA) can inhibit microtubule dynamics just by binding to a few tubulin subunits per microtubule. The substoichiometric inhibition of cellular microtubule assembly and dynamics underlies the effects of widely employed cytotoxic antitumor drugs, such as vinblastine and taxol, and their potent anti-proliferative effects. Their effects on tumour cells can include (1) mitotic arrest or impairment, leading to death of actively dividing cancer cells, (2) interference with cytoplasmic microtubule dynamics and intracellular trafficking, leading to death of quiescent cancer cells and (3) inhibiting microtubules and motility of noncancer endothelial cells in the tumour, leading to antiangiogenic and vascular-disrupting effects shutting down the blood supply to the tumour.

The antitumor drug binding sites that have been crystallographycally documented at the time of writing this article are all located in β-tubulin (see **Figure 3c**): (1) the taxol MSA site (**Figure 3b**) shared by epothilone and zampanolide (Prota *et al*., 2013), which is located between core helix H7 and the M-loop; (2) the peloruside-laulimalide MSA site that is adjacent to epothilone and interacts with helix H9 following the M-loop (Prota *et al*., 2014a); (3) the colchicine-podophyllotoxin MDA site near the association interface with the α-tubulin subunit within the tubulin heterodimer (ligand binding at this site only takes place when tubulin is in the curved conformation that is incompatible with microtubule assembly (Ravelli *et al*., 2004)); (4) the vinblastine-phomopsin-soblidotin MDA site at the association interface between consecutive curved tubulin dimers, where these ligands introduce a wedge impairing proper assembly (Cormier *et al*., 2008). Finally, a new MDA-binding site at the very plus end of β-tubulin has been proposed for the powerful phase I antitumor drug PM060184 (Pera *et al*., 2013; see **Figure 3c**).

The structural determination of tubulin-drug complexes at high resolution and the finding, nearly half a century after its isolation, of a new antitumor drug binding site in tubulin will likely facilitate understanding the mechanism of other clinically relevant microtubule drugs, as well as the development of better tubulin-targeting anticancer drugs able to overcome the appearance of tumour resistance during the course of chemotherapy.

γ-Tubulin, Microtubule Organising Centres and Other Eukaryotic Tubulins

Screening for revertants of β-tubulin mutations in the genetically amenable fungus *Aspergillus nidulans* lead to the discovery of the γ-tubulin gene coding for a protein closely related to α- and β-tubulin (Oakley and Oakley, 1989), which was subsequently shown to localise in complexes with other proteins at microtubule organising centres (MTOCs): centrosomes in animal cells, spindle pole bodies in fungi and acentrosomal microtubule arrays in plant cells.

Structural studies of the γ-tubulin containing complexes have shown that the core of the microtubule nucleating machinery is the γ-tubulin small complex (γTuSC), which can further assemble into a larger 'lock washer' shape structure, the γ-tubulin ring complex (γTuRC). In the γTuSC, two monomers of γ-tubulin together with associated proteins build a Y shape structure (**Figure 4a**). The γTuRC is formed by six and a half γTuSCs per helical turn (**Figure 4b**) matching the microtubule 13-fold symmetry. In this complex, the γ-tubulin is fully exposed for interaction via longitudinal contacts with the minus-end of α-tubulin, functioning as a microtubule template thus preventing the microtubule growth in this direction (**Figure 4c**). Despite the electron microscopy structures of γTuSC and γTuRC show minor contacts between γ-tubulins within the ring, just small movements could realign those molecules to perfectly

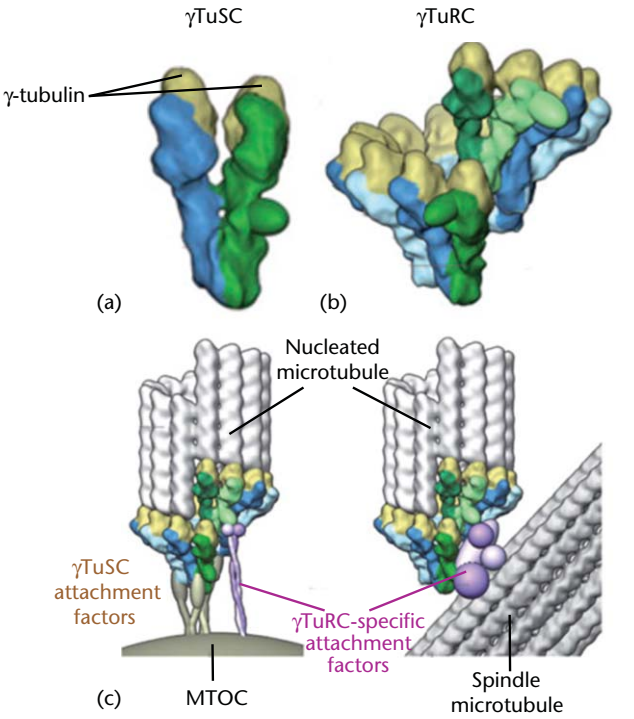

Figure 4 Model of γ-tubulin complexes and microtubule attachment. (a) In γTuSC small complexes, associated proteins (green, blue) act as scaffolds for two γ-tubulin molecules (yellow). (b) The 'lock washer' structure of γTubRC ring complexes made of several laterally interacting γTuSC. In this complex, γ-tubulin molecules are exposed for interaction with microtubules. (c) The γTuRC ring complex functions as a template nucleating the microtubule minus-end. Specific protein attachment factors are required for the attachment of these complexes to the MTOC or to other microtubules. Reproduced with permission from Kollman *et al.* (2011) © Nature Publishing Group.

fit the microtubule-like lateral contacts (Kollman *et al*., 2011). In fact, the crystal structures of γ-tubulin monomers both in the GTP- and the GDP-bound states had revealed lateral contacts through the same region that $\alpha\beta$-tubulin dimers use in microtubules to form lateral interactions between protofilaments. Both structures also showed a curved conformation, similar to non-polymerized $\alpha\beta$-tubulin dimers. Therefore, the role of GTP binding and possible hydrolysis by γ-tubulin upon forming the axial contact with α-tubulin remains to be understood (Kollman *et al*., 2011).

Microtubule nucleation complexes are associated through attachment factors to MTOCs for microtubule organisation and also through augmin to the surface of pre-existing microtubules. Given its key role nucleating microtubules with a defined geometry, pharmacologically targeting γ-tubulin would be an attractive possibility, but to our knowledge this has been very limitedly accomplished. There are other monomeric tubulins that are known to be involved in the formation of centrosomes and basal bodies. Thus δ- and ε-tubulin have been observed in the basal bodies and centrosomes of protists and vertebrates, whereas η-tubulin has been identified in the basal bodies of protists and some animals, and ξ-tubulin in protist basal bodies (Ludueña, 2013).

Bacterial Tubulin BtubA/B, A Close Structural Homologue of Eukaryotic Tubulin

The genes coding for bacterial tubulin (Btub) A and B were discovered when sequencing the genome of the bacterium *Prosthecobacter dejongeii* (Jenkins *et al*., 2002). Remarkably, BtubA and BtubB are much closer to eukaryotic tubulin (sharing 32–36% sequence identity with α and β-tubulins) than to prokaryotic FtsZ (approximately 17%) or TubZ ($<10\%$). Both proteins showed a high structural similarity with α and β-tubulin, with a root-mean square deviation (RMSD) of only 1.3 Å to 1.5 Å respectively, whereas when compared to FtsZ the RMSD is 2.7 Å (Schlieper *et al*., 2005). Thus, they are the closest prokaryotic homologues of eukaryotic tubulin. However, they are not widely spread and have only been described in *Prosthecobacter* species, where they coexist with genuine bacterial cell division protein FtsZ, therefore it is unlikely they are involved in division.

The BtubA/B proteins co-polymerise in the presence of GTP and magnesium forming tubulin-like protofilaments (Schlieper *et al*., 2005). Despite the high sequence identity and structural similarity with eukaryotic tubulin, there are several remarkable differences between them: (1) it is impossible to assign BtubA and BtubB to α or β-tubulin because both contain an activating synergy loop (with a conserved Glu like α-tubulin) and none contains the typical β-tubulin M-loop but instead, both have a short S9-S10 loop in the taxol-binding pocket similarly to α-tubulin (Schlieper *et al*., 2005); (2) bacterial tubulins include the typical *C*-terminal H11 and H12 helices, but BtubB lacks the highly acidic flexible tail that is involved in the interaction with other proteins in eukaryotes; (3) the most divergent zones in BtubA/B are the surface loops, where BtubA and BtubB have indeed mosaic sequences with intertwining features from both α- and β-tubulin (Martin-Galiano *et al*., 2011); (4) BtubA/B exhibits ancient properties of folding without chaperone requirement and form a weak dimer that does not require cofactors (Schlieper *et al*., 2005); (5) BtubA/B are homogeneous bacterial polypeptides, meaning there is no isotype diversity as in eukaryotic tubulin; (6) BtubA/B has more primitive assembly properties than eukaryotic tubulin and polymerises over a wider range of solution conditions (Martin-Galiano *et al*., 2011).

BtubA/B protofilaments laterally associate into filament pairs and bundles (Schlieper *et al*., 2005; Martin-Galiano *et al*., 2011) or five-protofilaments tubules (Pilhofer *et al*., 2011) (**Figure 2**), which have been suggested to be a primitive cytoskeletal architecture that later evolved into 13-protofilament microtubules. The combination of all these features (low spread in bacteria, distinct intertwined loop sequences and primitive assembly properties) supports the origin of BtubA/B from spontaneously folding primitive tubulins. Shortly after gene duplication of the tubulin ancestor into proto-α- and proto-β subunits, a bacterial *Prosthecobacter* ancestor probably acquired them from a primitive eukaryotic cell by horizontal gene transfer. Afterwards, divergent hetero-polymer evolution (**Figure 1**) and co-evolution of the tubulin folding machinery gave rise to the more complex eukaryotic microtubules.

Given the close structural similarity, easy expression and good stability of bacterial tubulin it seems feasible to humanise different sections of these proteins to engineer recombinant binding sites of interest. Thus BtubA/B could be used as a framework to study binding of anti-tumour drugs, to obtain well-defined substrates for the enzymes responsible for tubulin posttranslational modifications or as simplified trails for motor proteins.

FtsZ, the Organiser of Bacterial Cell Division

The essential bacterial cell division gene *ftsZ* (*f*ilamentous *t*emperature *s*ensitive *Z*) was found to encode a protein that localises to the division site. FtsZ assembles into a ring structure called the Z-ring (Bi and Lutkenhaus, 1991), which functions as a scaffold for the other cell division proteins involved in membrane attachment, cell wall synthesis and DNA segregation, and constricts during division (see **Figure 5**). FtsZ proteins are widely distributed in bacteria, archaea, chloroplast and the mitochondria of some simple eukaryotes, where they share 40–50% of sequence identity. However, the Planctomycetes-Chlamydiae phyla, *Mycoplasma*, bacillar L-forms and the archeal division Crenarchaeota divide without FtsZ.

Bacterial FtsZs share $\leq 20\%$ sequence identity with eukaryotic tubulin. FtsZ was related to tubulin in the early 1990s when different groups found that it binds and hydrolyses GTP, and assembles into tubulin-like filamentous structures. Comparing the three-dimensional structures of FtsZ and tubulin showed that both share the same fold and form a distinct family of GTPases different from classical ones such as ras or EF-Tu (Nogales *et al.*, 1998). FtsZ has shorter surface loops than tubulin, it folds spontaneously and the *C*-terminal flexible tail is important for assembly and partner proteins binding although it is not observed in the crystal structures.

Depending on the conditions, FtsZ protofilaments laterally associate into polymorphic bundles and sheets, or form rings with different diameters or toroids, but no microtubules. FtsZ filaments typically curve upon GTP hydrolysis probably due to a direct effect of missing the nucleotide γ-phosphate and coordinated Mg^{2+} ion at the association interface between subunits. In addition, there is evidence indicating a structural switch of FtsZ monomers upon assembly. The cooperative assembly of single stranded FtsZ filaments requires FtsZ monomer self-switching between low affinity inactive and active associating conformations, possibly entailing an opening movement of the *C*-terminal domain (Martin-Galiano *et al.*, 2010). Structural studies of unassembled FtsZs from different bacterial and archaeal species all showed very similar conformations irrespective of the nucleotide bound (Oliva *et al.*, 2007), in which the cleft between the *C*-terminal domain and central helix H7 is closed. More recently, the structure of one FtsZ from *Staphylococcus aureus* (Sa-FtsZ) has shown monomers with an open cleft and a downward translation of helix H7, which form crystalline filaments (Matsui *et al.*, 2012; Elsen *et al.*, 2012) (see **Figure 5**), offering a potential

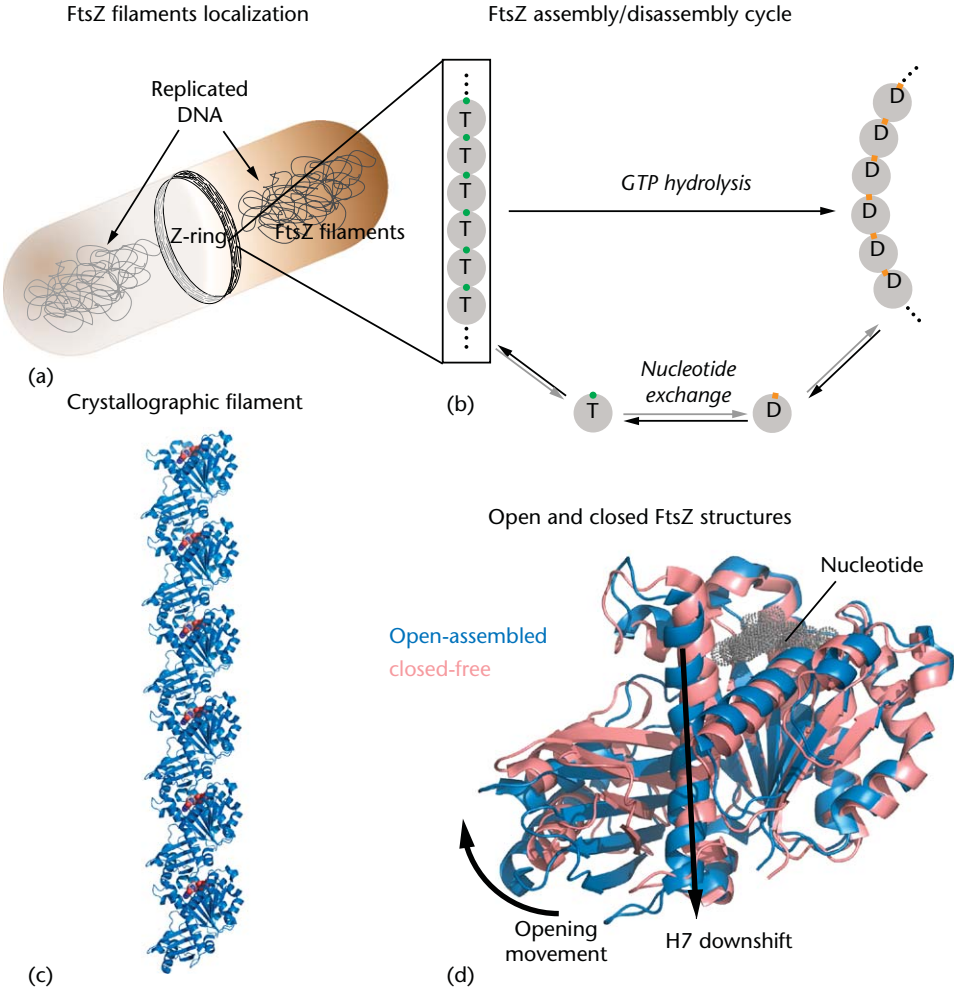

Figure 5 FtsZ filaments localisation, dynamics and structural assembly switch. (a) The Z-ring localises at cell division site after the replication and segregation of the bacterial chromosome and it is made up of multiple FtsZ protofilaments. (b) Scheme of the assembly-disassembly cycle of purified FtsZ. FtsZ protofilaments remain relatively straight when GTP (T) is bound and curve upon hydrolysis to GDP (D). (c) A straight filament (blue) observed in the crystal packing of *S. aureus* FtsZ (PDB 3VO8). (d) The putative FtsZ assembly switch from a close structure when unassembled (salmon, PDB 2VAM) to an open structure in the protofilament (blue, PDB 3VO8) involves an opening movement of the *C*-terminal domain and a downshift movement of the central helix H7 (see main text).

structural mechanism for the FtsZ switch. However, Sa-FtsZ has not been crystallised as unassembled monomers and further structural studies are required to fully understand the mechanism of FtsZ filament assembly and dynamics.

Bacterial division is essential for propagation, and so the Z-ring has to assemble at a correct position and in a precise time during cell cycle, and keep dynamic until its disassembly shortly before membrane closure upon cytokinesis. FtsA and ZipA anchor FtsZ filaments to the membrane and there are different FtsZ-interacting proteins that regulate its assembly/disassembly dynamics (positively and negatively) in different bacteria. Several protein systems help positioning the ring, such as the Min proteins, DivIVA or MipZ that avoid FtsZ assembly at the cell poles. Noc/Slma systems prevent the formation of a closing ring over the nucleoid, which occupies the central part of the cell before segregation. The FtsZ inhibitor SulA is part of the SOS system involved in avoiding division during DNA reparation after damage. Nutrient dependent regulators Opgh/Ugtp also inhibit FtsZ assembly to control cell size. Some proteins contribute to the dynamicity of the FtsZ filaments in the ring, including EzrA and ZapA, for the proper execution of the septum synthesis during cell division. How all these factors collectively control FtsZ polymer dynamics *in vivo* remains to be understood (for specific information on bacterial cell division and the Z-ring regulatory proteins). **See also**: Bacterial Cell Division

Fluorescence recovery after photo-bleaching (FRAP) experiments showed that FtsZ polymers in bacterial cells have a strong dynamic behaviour with continuous subunit exchange between the assembled subunits in the Z-ring and non-assembled FtsZ, but how FtsZ filaments organise into the contractile Z-ring (see scheme in **Figure 5**) is still a matter of debate and physical modelling. Groundbreaking results have come from electron cryo-tomography (Li *et al*., 2007) and super-resolution fluorescence microscopy of bacterial cells (Holden *et al*., 2014). Owing to the complexity of this work, considerable effort has been put in more simple reconstituted systems. Different studies using membranes (in vesicles or lipid bilayers) have shown that FtsZ can assemble into rings and generate constriction force but its membrane-anchoring partner FtsA is required for the organisation of FtsZ filaments into large dynamic structures (Loose and Mitchison, 2014).

Given its key role in division of the majority of bacteria, FtsZ has become a popular target for seeking new antibiotics to fight the widespread emergence of pathogens resistant to current antibiotics. A growing number of small molecules have been reported to interact with FtsZ, to impair the Z-ring or to inhibit bacterial cell division, although the hit frequency in FtsZ inhibitor screens is typically much lower than for tubulin. So far, the mechanism of action of a few FtsZ inhibitors has been studied in detail. Owing to the structural homology, the first challenge was to inhibit FtsZ while not affecting eukaryotic tubulin, which has been shown feasible. Several GTP analogues substituted at guanine C8 inhibit FtsZ polymerisation, by remodelling the association interface between FtsZ monomers (Marcelo *et al*., 2013), but support tubulin assembly (Lappchen *et al*., 2008). The FtsZ-bound nucleotide can actually be replaced by the new natural compound crysophaentin (Plaza *et al*., 2010) and by synthetic inhibitors (Ruiz-Avila *et al*., 2013), both with antibacterial activity against methicillin-resistant *S. aureus* (MRSA) and antibiotic-resistant *Enterococcus faecium*. The benzamide derivative PC190723 effectively protects mice from lethal doses of MRSA, impairs the Z-ring formation and selectively inhibits cell division in Gram-positive bacteria (Haydon *et al*., 2008). Biochemical and structural studies have shown the mechanism of action, where this compound binds into the cleft between domains of Sa-FtsZ filaments, thus blocking the protein in the open filament-forming conformation (Elsen *et al*., 2012), stabilizing FtsZ filaments and avoiding disassembly (Andreu *et al*., 2010). Other strategies have focused on FtsZ-interacting partners. For instance, the interaction of ZipA with FtsZ can be inhibited by small molecules and protease ClpP can be activated to degrade FtsZ by antibiotic acyldepsipeptides (Sass *et al*., 2011). These and other inhibitor studies are useful to better understand the mechanism and regulation of FtsZ assembly and hold promise towards the development of FtsZ-based antibiotics.

TubZ Assembly into Helical Filaments for Plasmid and Viral DNA Positioning

TubZ was identified as a divergent protein member of the tubulin superfamily, which is essential for the maintenance of virulence plasmid pBtoxis from *Bacillus thuringiensis* (encoding an insecticidal toxin) and pXO1 of *Bacillus anthracis* (encoding anthrax toxin). TubZ filaments form polar helical assemblies that self-organise and follow a treadmilling dynamics similarly to actin filaments (Larsen *et al*., 2007). TubZ homologues are relatively distant from each other (reaching less than 10% sequence identity) and are encoded by other plasmids, by chromosomal genes and even by bacteriophages. TubZ was described as the motor protein of the type III plasmid segregation systems that binds to centromere-like sequences through the adaptor protein (Ni *et al*., 2010). It works in the correct positioning of DNA after replication to evenly distribute plasmids between daughter bacterial cells (**Figure 6a**), a process analogous to the segregation of eukaryotic chromosomes by the mitotic spindle. In *Clostridrium botulinum* lysogenic phage cs-t (carrying botulism neurotoxin), TubZ is thought to segregate the plasmid prophage and there is an added regulatory protein TubY (Oliva *et al*., 2012). However, a TubZ homologue in lytic phage 201ϕ2-1 from *Pseudomonas chlororaphis* (also referred to as PhuZ) has been reported to centre, rather than segregate, viral DNA within the host cell before encapsidation, in a process analogous to metaphase chromosome centring (Kraemer *et al*., 2012; **Figure 6b**).

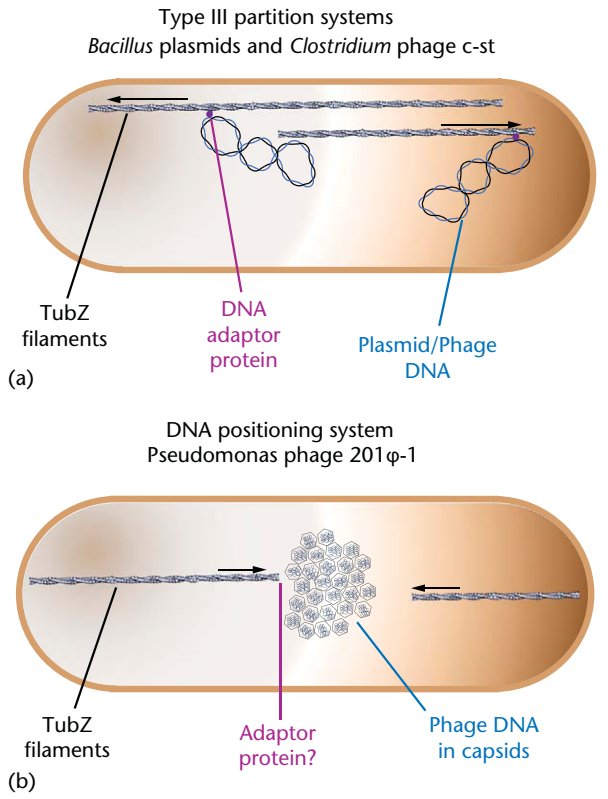

Figure 6 (a) Scheme of segregation of newly replicated extrachromosomal DNAs that are attached to mobile TubZ filaments through a DNA adaptor protein in type III segregation systems (data from Ni *et al.*, 2010). (b) Scheme of DNA positioning DNA by TubZ homologue filaments at the middle of the cell during encapsidation of phage 201ϕ2-1 (data from Kraemer *et al.*, 2012).

Despite the very low sequence identity with tubulin and FtsZ, the TubZ core structure is that of the superfamily, but showing a clear trend toward a reduction of several secondary structure elements. An important difference with other tubulin-like proteins is a rotation between the *N*- and GTPase activating domains that is intrinsic to TubZ and gives rise to a twist in filaments. Plasmids TubZs include a *N*-terminal α-helix (H0) that is tightly anchored between the GTPase domain and the central helix (H7) and a long basic flexible *C*-terminal tail (Aylett *et al.*, 2010; Ni *et al.*, 2010). Phage TubZs are smaller, lacking some secondary structure elements in the GTP binding (helices H0 and H6) and GTPase activating domains (helix H10), and the *C*-terminal tail is shorter and acidic (Oliva *et al.*, 2012; Kraemer *et al.*, 2012; Aylett *et al.*, 2013).

Structural studies show TubZ double, triple, or four-stranded helical filaments (Aylett *et al.*, 2010; Montabana and Agard, 2014; Zehr *et al.*, 2014; **Figure 2**). The *C*-terminal tail of TubZ is important during assembly making extensive polar interactions with the next molecule along the protofilament (Kraemer *et al.*, 2012). The active site and longitudinal association interfaces between TubZ monomers are well conserved and on GTP hydrolysis there are movements within the monomer due to the release of Mg^{2+} and phosphate (Aylett *et al.*, 2010, 2013). These small movements propagate inducing an opening of the protofilament twist that allows the accommodation of additional strands within the helical filament, which eventually disassembles (Montabana and Agard, 2014).

TubZ proteins are the more recently discovered members of the superfamily and they are still less well-understood than tubulin or FtsZ. Considering their key role on the spread of pathogenic bacterial virulence factors, it is conceivable they might become targets for new antibacterial drugs.

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