When cytoskeletal worlds collide

Eva Nogales

The Howard Hughes Medical Institute, Molecular and Cell Biology Department, University of California, Berkeley, CA 94720-3220; and The Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Cytoskeletal aficionados and molecular evolutionists are in for a surprising treat in PNAS. Löwe and colleagues, who have for some years now brought to our attention the conservation of the actin and tubulin cytoskeletons across kingdoms through their structural studies (1, 2), are now giving us new striking images to think about (3). Just test your knowledge of cytoskeleton structure by looking at their figure 4. Do not think twice and say aloud what you think those filaments are. Now read the title of their article. Surprised? Read more.

TubZ was recently identified as a tubulin/FtsZ-like protein involved in plasmid segregation in bacteria (4). This process appears to require a minimal set of two plasmid-encoded genes, one corresponding to a centromere-binding protein, the other a self-assembling, filament-forming element that in many cases is either an actin-like (ParM) or a tubulin-like protein (TubZ) (4, 5). This minimalist segregation system parallels the complex kinetochore–microtubule spindle apparatus of eukaryotes, in a fit of efficient simplicity. Although some obvious parallels do exist, they break down once we start thinking about the molecular details. The dynamic character of the cytoskeletal polymer is in both cases essential for the movement of plasmids/chromosomes (6). But whereas microtubules move chromosomes to the poles by depolymerization during anaphase (7), the ParM and TubZ filaments seem to act mostly by pushing during polymer growth or relying on treadmilling (4, 8). The work of Aylett et al. now indicates that the distinct roles that TubZ and tubulin play in plasmid and chromosomal segregation, respectively, determine the very different architectures of the TubZ filament and the microtubule. Whereas tubulin forms hollow cylinders of laterally interacting, (almost) straight protofilaments, TubZ forms a two-stranded filament that resembles actin and ParM! Löwe and colleagues put forward several arguments about why these two-stranded TubZ and ParM filaments would be optimal for their role in plasmid segregation. Although this is a hard question to answer, it is interesting to think of how the distinctiveness of the TubZ and tubulin protofilaments could arise.

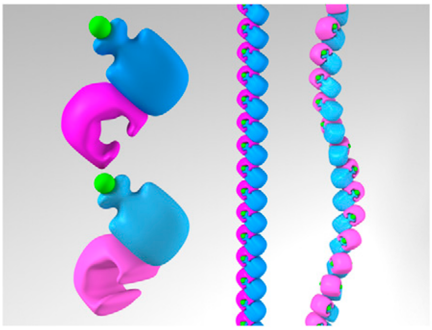

Fig. 1. Distinct filament structure with the same assembly interfaces. Tubulin (brighter colors) and TubZ (lighter colors) share a conserved interface along the filament that sandwiches the nucleotide (shown in green). The twist in the TubZ filament (filament on the right) originates from a rotation of the two domains within one subunit (compare relative orientation of the magenta and blue domains for bright and light subunits). Figure courtesy of Gabriel C. Lander.

As when comparing tubulin with FtsZ, the sequence conservation between tubulin and TubZ is very low overall but very significant when it concerns the residues involved in nucleotide binding. Importantly, and true for all members of the tubulin family thus far identified, the nucleotide site happens to sit at the monomer–monomer longitudinal interface. This arrangement is in strong contrast to what occurs for actin and its bacterial homologs, where the ATP binding site is not directly involved in the contact between monomers. Interestingly, ParM lacks the polymerization interfaces present in actin and uses an alternative assembly arrangement (filaments are left-handed rather than right-handed) (9), although ultimately actin and ParM filaments are clearly structurally related. So, how can it be that tubulin and TubZ, which are highly conserved at assembly interfaces, give rise to such distinct polymers? It is not the interface, but the protein structure, that has significantly changed!

Following the nomenclature used by Aylett et al., the structure of the tubulin family members consists of an N-terminal, nucleotide-binding domain and a C-terminal domain (for tubulin, the term "C-terminal domain" has traditionally been reserved for the tubulin-unique C-terminal helices that define the crest of protofilaments on the microtubule—and which are lacking in FtsZ and TubZ—whereas the rest of the protein up to the nucleotide-binding domain has been referred to as the "intermediate domain"). For all family members, the N-terminal domain of one subunit interacts with the C-terminal domain of the next along the filament, burying the nucleotide between them (the C-terminal domain contributing essential residues for nucleotide hydrolysis, and thus coupling hydrolysis with polymerization) (10, 11). Surprisingly, TubZ maintains the same orientation of the N-terminal and C-terminal domains across an interface as that used by tubulin along protofilaments, but these two domains, within one subunit, have dramatically rotated with respect to each other compared with the tubulin and FtsZ cases. The result is that whereas the latter form linear arrays where one subunit is simply translated along the filament axis, in TubZ there is a twist reminiscent of that in a single F-actin strand. TubZ has produced a radically different polymer structure while maintaining the main longitudinal interface, which is under very strong evolutionary pressure to preserve nucleotide binding. Instead, TubZ has significantly modified its overall tertiary structure via domain rearrangements (Fig. 1). The change is driven by a new structural element at the N-terminal end of TubZ (helix H0) that wedges between the N-terminal and C-terminal domains. This same helix appears to play a second role in the interaction between strands in the double filament (3).

It is interesting to compare the actin and tubulin assemblies with those of the third cytoskeletal system involving a nucleotide-binding protein: septin filaments (thus far identified only in eukaryotes—but see ref. 6 for an interesting hypothesis). Interestingly, these three cytoskeletal systems play intermingled roles in cell division across kingdoms: in eukaryotes, tubulin segregates chromosomes in mitosis, whereas septins assemble abut to the membrane in cytokinesis to define the site of septation and recruit an actomyosin ring for constriction (12). In bacterial cell division, FtsZ defines the site of septation and carries out the constriction itself, whereas plasmids are segregated by ParM or TubZ filaments.

Whereas F-actin is polymerized from actin monomers, and microtubules are made of αβ-tubulin heterodimers, the assembly units for septin filaments are nonpolar multimers, of different order from

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E-mail: enogales@lbl.gov.

organism to organism, that elongate into a nonpolar filament by end-on interaction (13–15). The septin filament has no twist, just like the tubulin protofilaments and unlike the TubZ or actin filaments. Interestingly, each septin protein along this arrangement makes two different types of interactions. One involves septin-unique regions that extend N- and C-terminal of the Ras-like GTP-binding domain. The other involves the nucleotide-binding domains of two consecutive septins, where the nucleotides themselves constitute a significant part of the interface (13). Thus, along the septin filaments, interfaces that involve a highly conserved nucleotide-binding domain (like tubulin and TubZ) alternate with interfaces away from the nucleotide (like in actin and ParM). Although both interfaces are relatively well conserved across septins, that involving the nucleotide is markedly more so, in agreement with the concept that retaining nucleotide binding is a stronger evolutionary constraint than maintaining self-assembly contacts.

Septins lack the polarity along the filament (up–down polarity) present in all actin-like and tubulin-like systems. On the other hand, they do have lateral polarity (left–right polarity) that is present in the FtsZ and tubulin protofilaments (but not in microtubules!) and is lacking in filaments that twist, such as actin, ParM, and TubZ. This septin and FtsZ property seems to make sense functionally, as filaments must interact with the plasma membrane (or a membrane-bound protein) along their length using one side, while the other, facing the cytosol, is available for binding to other cellular factors. Finally, septin filaments appear to lack the "cytomotive" property of actin and tubulin families (6), or at least lack the nucleotide-dependent dynamic behavior of actin and tubulin.

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Each tubulin-like protein characterized thus far appears to form a distinct type of polymer that has been optimized for its function: microtubules for eukaryotic tubulin, straight filaments for FtsZ, and twisted, double-stranded filaments for TubZ. It is interesting that eukaryotic tubulin itself is capable of assembly into different polymer forms, involving different curvatures and lateral arrangements, although always via protofilaments with little or no twist. There is an innate capacity of the tubulin subunits to use alternative lateral interfaces, which may be unique to eukaryotic tubulins, as well as to change conformation at the monomer level (16). The latter occurs via rotations of the N-terminal versus C-terminal domain that are slightly reminiscent of, but qualitatively distinct from, those seen when comparing tubulin with TubZ. Importantly, some of these conformational changes give rise to alternative polymer forms that correspond to intermediates in the microtubule assembly and disassembly processes (17, 18). Such intermediates are likely to play important cellular roles (19).

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