

A microtubule bestiary: structural diversity in tubulin polymers

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ABSTRACT Microtubules are long, slender polymers of $\alpha\beta$ -tubulin found in all eukaryotic cells. Tubulins associate longitudinally to form protofilaments, and adjacent protofilaments associate laterally to form the microtubule. In the textbook view, microtubules are 1) composed of 13 protofilaments, 2) arranged in a radial array by the centrosome, and 3) built into the 9+2 axoneme. Although these canonical structures predominate in eukaryotes, microtubules with divergent protofilament numbers and higher-order microtubule assemblies have been discovered throughout the last century. Here we survey these noncanonical structures, from the 4-protofilament microtubules of *Prostheco bacter* to the 40-protofilament accessory microtubules of mantidfly sperm. We review the variety of protofilament numbers observed in different species, in different cells within the same species, and in different stages within the same cell. We describe the determinants of protofilament number, namely nucleation factors, tubulin isoforms, and posttranslational modifications. Finally, we speculate on the functional significance of these diverse polymers. Equipped with novel tubulin-purification tools, the field is now prepared to tackle the long-standing question of the evolutionary basis of microtubule structure.

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INTRODUCTION

Microtubules are polymers of $\alpha\beta$ -tubulin known for their canonical lattice structure. Describing microtubule structure has been a 50-year pursuit, from early examinations of negatively stained specimens by electron microscopy (EM; Ledbetter and Porter, 1963) to near-atomic resolution cryo-EM of purified microtubules (Zhang *et al.*, 2015). The canonical microtubule has 13 protofilaments (Figure 1A; Tilney *et al.*, 1973), and this lattice structure has been found in cells from every supergroup of eukaryotes (reviewed in Unger *et al.*, 1990). The 13-protofilament lattice is not, however, a uniform property of $\alpha\beta$ -tubulin polymers. Rather, tubulin polymers have an intrinsic flexibility; purified $\alpha\beta$ -tubulin nucleates spontaneously into microtubules ranging from 9 to 16 protofilaments, and 14-protofilament microtubules are the most abundant *in vitro* (Pierson *et al.*, 1978).

Nevertheless, the microtubules found in cells are uniform in their protofilament number. This uniformity *in vivo*, which contrasts with the variability observed *in vitro*, indicates that cells specify their protofilament number as 13 during nucleation, for example, with nucleation factors such as the γ -tubulin ring complex (γ -TuRC; Moritz *et al.*, 1995; Zheng *et al.*, 1995). The predominance of 13-protofilament microtubules in protists, fungi, plants, and animals indicates that the last eukaryotic common ancestor (LECA) specified 13-protofilament lattices and that this specification was conserved over 10^9 years of evolution. What are the pressures, if any, that select for the 13-protofilament lattice? The main explanation for the conservation of 13 protofilaments is what we call the “straight-protofilament hypothesis” (Amos and Schlieper, 2005; Kollman *et al.*, 2010). Because of the helical arrangement of subunits within the lattice, only the 13-protofilament geometry allows protofilaments to run straight relative to the long axis of the microtubule, whereas the protofilaments in other geometries (e.g., 14 protofilaments) supertwist around the microtubule (Figure 1B; Chrétien and Wade, 1991). The straight protofilaments of 13-protofilament microtubules may accommodate kinesin-1, a cargo-bearing motor protein that tracks along single protofilaments (Ray *et al.*, 1993). If kinesin-1 were to walk on a supertwisted microtubule, it would spiral around during long-range transport of organelles, perhaps problematically. According to the straight-protofilament hypothesis, 13-protofilament microtubules offer a selective advantage in the form of effective long-range transport.

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Abbreviations used: EM, electron microscopy; LECA, last eukaryotic common ancestor; PTM, posttranslational modification; TOG, tumor overexpressed gene; TRN, touch receptor neuron; γ -TuRC, γ -tubulin ring complex.

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Despite the prevalence of 13 protofilaments, microtubules with other protofilament numbers were discovered in many species during the early heyday of EM. These noncanonical lattices are known to microtubule aficionados primarily as curiosities. At the same time, microtubules in diverse species were found to be arranged in intricate bundles, spirals, rings, and cartwheels, in contrast to the radial arrays and noncentrosomal meshworks typical of tissue culture cells. Here we survey some of these fascinating lattices and morphologies and speculate on their function and evolution. We discuss how cells specify their protofilament numbers using nucleation factors, specific tubulin isoforms, and posttranslational modifications (PTMs) of tubulin. We have named our review after the ancient texts and medieval illuminated manuscripts that described real and mythical creatures. Unlike these historical texts (Bern, 830) and their modern equivalents (Borges, 1957; Gygas, 1977), none of the microtubule structures we describe below are imaginary. We argue that the structural diversity of tubulin polymers raises important questions about microtubule nucleation, about the relationship between microtubule structure and function, and, indeed, about the physiology of the cytoskeleton.

ANCESTRAL POLYMERS

We start our survey by considering the evolutionary origins of tubulin polymers. Tubulin-like proteins can be found in bacteria and archaea, indicating that ancestors to $\alpha\beta$ -tubulin arose in primitive,

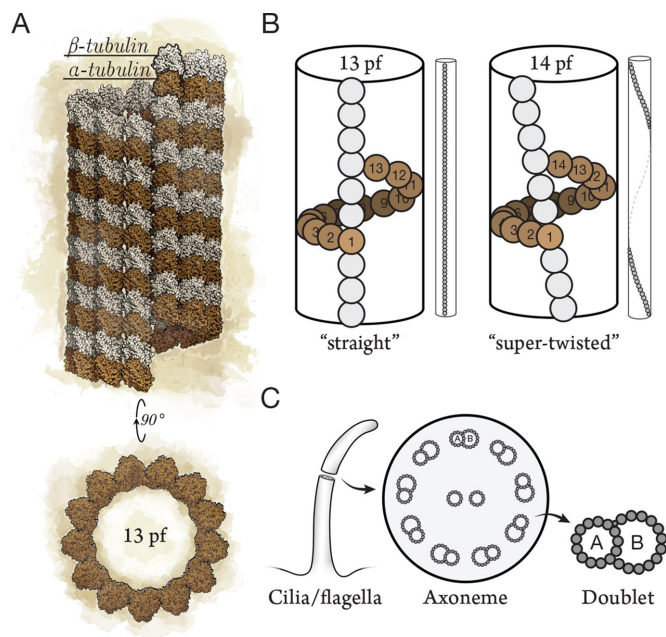


FIGURE 1: (A) Microtubules are polymers of α/β -tubulin dimers typically composed of 13 protofilaments. (B) Adjacent protofilaments have a longitudinal offset of 9.2 Å between tubulins such that 13-protofilament microtubules have straight protofilaments (left). The lattice of non-13-protofilament microtubules (14 protofilaments shown) must accommodate by imposing a protofilament supertwist (right; not to scale). (C) The functional unit of cilia and flagella (left) is the 9+2 axoneme, with 2 central pair singlets and 9 outer microtubule doublets (middle). Doublets (right) are composed of an incomplete microtubule (10-protofilament B-tubule) clutched to the side of a complete 13-protofilament microtubule (A-tubule). pf, protofilament.

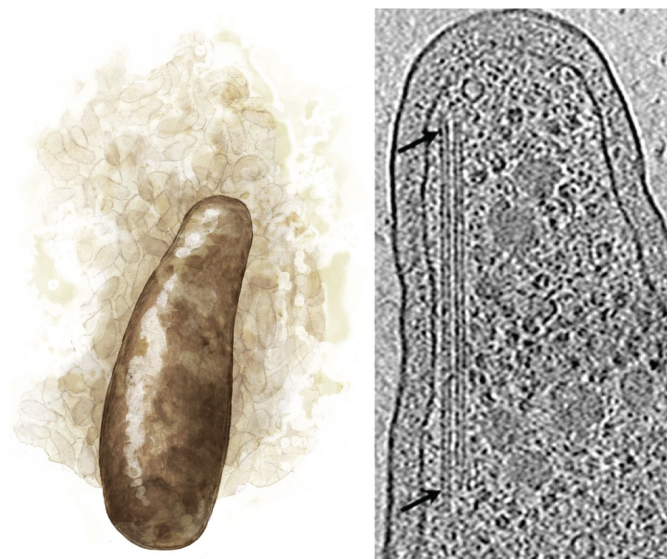


FIGURE 2: A genus of bacteria, *Prostheco bacter* (left), is unique in its acquisition of tubulin-like genes that form bacterial microtubules (right, arrows; adapted from Pilhofer *et al.*, 2011).

unicellular organisms. Like $\alpha\beta$ -tubulin, some prokaryotic homologues are part of the cell-division machinery. The most well-studied prokaryotic tubulin, FtsZ, forms the cytokinetic ring of bacteria and archaea (reviewed in Erickson *et al.*, 2010), whereas TubZ and RepX are essential for plasmid partitioning (Larsen *et al.*, 2007; Anand *et al.*, 2008). Unlike $\alpha\beta$ -tubulin, these homologues form single-stranded filaments or twisted filament pairs rather than hollow tubes. Lateral interactions between FtsZ filaments can occur, but they lack a defined lattice structure (Nogales *et al.*, 1998; Erickson *et al.*, 2010). The existence of single-stranded filaments in prokaryotes suggests that longitudinal bonds evolved first, whereas the defined lateral interactions that form the microtubule lattice evolved later. Interestingly, defined lateral interactions occur in the polymers of BtubA/BtubB, a tubulin-like heterodimer found in many *Prostheco bacter* species (Figure 2). BtubA/BtubB polymerizes into 4-protofilament “bacterial microtubules” in vitro (Deng *et al.*, 2017) and perhaps 5-protofilament polymers in cells (Pilhofer *et al.*, 2011). Because BtubA/BtubB appears in a single genus, the genes were probably acquired from a eukaryote by horizontal gene transfer (Martin-Galiano *et al.*, 2011). We can speculate that bacterial microtubules and FtsZ filaments are snapshots of early eukaryotic polymers, before 13 protofilaments became fixed in the LECA.

EUKARYOTIC MICROTUBULE ARCHITECTURES

Although both prokaryotes and eukaryotes require machinery for cell division, eukaryotes also require machinery for internal organization and long-range transport. This requirement is acute in neurons, where synaptic vesicle precursors and mitochondria are carried tens to hundreds of microns down the axon shaft by kinesins. We therefore speculate that eukaryotic microtubule structure evolved straight protofilaments to optimize long-range transport by kinesins, as mentioned above. Many kinesins do not follow a single protofilament during transport, however, but rather drift or wander (Brunnbauer *et al.*, 2012), making it unclear whether straight protofilaments would actually offer a selective advantage. Kinesin-1 may be the exception and not the rule. Furthermore, several eukaryotes, including the model organism *Caenorhabditis elegans*, do not have 13-protofilament microtubules at all. These examples indicate that

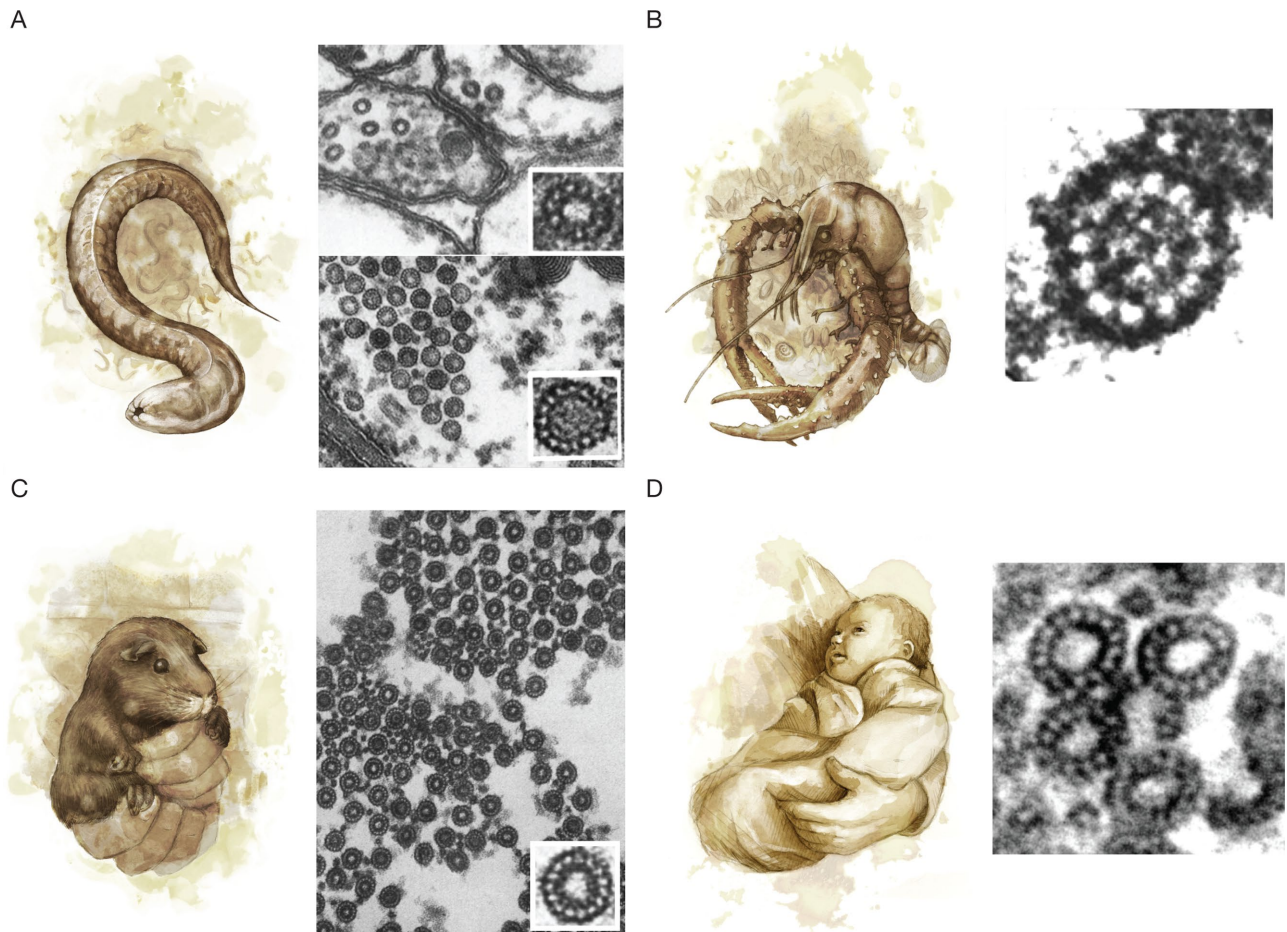


FIGURE 3: (A) The nematode *C. elegans* (left) has diverged from the 13-protofilament microtubules observed in other eukaryotes, with 11-protofilament microtubules in ventral cord neurons (top right) and 15-protofilament microtubules in TRNs (bottom right; adapted from Chalfie and Thomson [1982] and reprinted with permission from Rockefeller University Press). (B) The crayfish *Procambarus clarkii* (left) has 12-protofilament microtubules in the neurons of the nerve cord (right), whereas supporting glial cells have 13-protofilament microtubules (not shown; adapted from Burton *et al.* [1975] and reprinted with permission from Rockefeller University Press). (C) The guinea pig *Cavia porcellus* (left) has bundles of 15-protofilament microtubules in its inner pillar cells (right; adapted from Saito and Hama [1982] and reprinted with permission from Oxford University Press). (D) Divergent protofilament numbers are also found in humans (left). A cross-section through a human blood platelet after treatment with 10 μ M ADP (right) shows that microtubules sometimes have 14 protofilaments (top) as opposed to the standard 13 protofilaments (bottom; adapted from Xu and Afzelius [1988] and reprinted with permission from Elsevier).

13 protofilaments are not required for the viability of complex organisms. Indeed, in some cases, an atypical lattice may be necessary for complex animal behavior.

Non-13-protofilament microtubules in eukaryotes

Curious electron microscopists have discovered non-13-protofilament microtubules in plants, animals, and protists. Some of these microtubules have fewer protofilaments and thus smaller diameters. For example, in nematodes, species in both the *Rhabditina* clade (*C. elegans*) and the *Spirurina* clade (*Trichostrongylus colubriformis* and *Ascaridia galli*) have 11-protofilament microtubules in the nerve cord as well as in hypodermal, intestinal, and pharyngeal cells (Figure 3A, top right; Chalfie and Thomson, 1982; Davis and Gull, 1983). The occurrence of 11 protofilaments in multiple cell types from multiple clades suggests a shift to 11 protofilaments in the *Nematoda* phylum. Did the protofilament number in nematodes simply drift or were there pressures that selected for small-diameter

microtubules? Nematodes are not alone; 11-protofilament microtubules are also found in the ovary epidermal cells of the grass lily *Ornithogalum umbellatum* (Kwiatkowska *et al.*, 2006). In some animals, small-diameter microtubules are found in specific cells: lobsters (*Nephropidea*) and crayfish (*Astacoidea*) have 12-protofilament microtubules in their nerve cords (Figure 3B), even though neighboring glial cells have 13-protofilament microtubules (Burton *et al.*, 1975). In all the cells described above, the microtubules are uniform in their smaller diameters. We do not know how these cells have changed their nucleation pathways to make uniformly small-diameter microtubules and whether these changes were caused by selective pressures or by genetic drift.

A clear example of specialized function can be found in the large-diameter microtubules implicated in mechanotransduction. Fifteen-protofilament microtubule bundles are found in mechanosensory cells throughout the animal kingdom, for example, in the pillar cells of the inner ear of guinea pigs and mice (*Cavia porcellus*

and *Mus musculus*; Figure 3C; Saito and Hama, 1982; Tucker *et al.*, 1992) and the touch receptor neurons (TRNs) of nematodes (*C. elegans* and *T. colubriformis*; Figure 3A, bottom right; Chalfie and Thomson, 1982; Davis and Gull, 1983). One hypothesis is that 15-protofilament microtubules provide rigidity that might be necessary for efficient mechanotransduction (Tolomeo and Holley, 1997). Indeed, 15-protofilament microtubules are predicted to be 35% stiffer than those with 13 protofilaments (Gittes *et al.*, 1993). An alternative hypothesis is that 15 protofilaments are better at forming microtubule bundles (Cueva *et al.*, 2012; Topalidou *et al.*, 2012); the cross-links within microtubule bundles can increase rigidity fourfold (Tolomeo and Holley, 1997). Outside of mechanotransduction, the function of large-diameter microtubules is less clear. Fifteen-protofilament microtubules are found in the epidermal cells of insects (*Drosophila melanogaster* and *Blattella germanica*; Nagano and Suzuki, 1975; Tucker *et al.*, 1986), but there is no obvious reason to have stiffer microtubules in these cells. Interestingly, some cells increase their protofilament number in response to biochemical signals. For example, microtubules in the marginal band of human blood platelets switch from 13 protofilaments to 14 and 15 protofilaments upon platelet activation (Figure 3D; Xu and Afzelius, 1988). Another striking example occurs in *Ciliophora* protists (*Novisuccinea ovalis* and *Paramecium tetraurelia*), in which 13 protofilaments become 14–16 protofilaments during anaphase (Eichenlaub-Ritter, 1985; Tucker *et al.*, 1985). It may be that these cells express factors that increase protofilament number in response to their change in state. Alternatively, the state change may cause the cells to lose control of their protofilament number; in this case, tubulin's intrinsic flexibility begins to show through.

Fifteen- and 16-protofilament microtubules are quite large, but even thicker microtubules are found in the axonemes of insect sperm, where the 9 doublets are surrounded by 9 “accessory microtubules” with typically 13–20 protofilaments (Figure 4A; reviewed in Dallai *et al.*, 2006). In an extreme case, accessory microtubules in the mantidfly *Mantispa perla* have giant, 40-protofilament “macro-tubules” that are filled with polysaccharides (Figure 4B; Dallai *et al.*, 2005). The function of accessory microtubules is not completely understood, but this 9+9+2 configuration might contribute to the sperm's double-helical waveform (reviewed in Werner and Simmons, 2008). It would be interesting to know whether divergent accessory microtubule protofilament numbers alter the waveform of insect sperm or take on novel roles.

DETERMINANTS OF MICROTUBULE ARCHITECTURE

Whatever their roles in cell physiology, non-13-protofilament microtubules provide a series of case studies for the problem of microtubule nucleation. For example, the 11-protofilament microtubules in *C. elegans* are uniformly 11 protofilaments (Chalfie and Thomson, 1982), despite the fact that *C. elegans* tubulin forms a range of protofilament numbers when nucleated spontaneously *in vitro* (Aamodt and Culotti, 1986). How are the structures of noncanonical microtubules specified during nucleation? In a simple view, the protofilament number is established by the angle of the lateral interactions between tubulin subunits; the flexibility of this interprotofilament angle is what produces a range of protofilament numbers when microtubules are nucleated spontaneously. To specify a certain protofilament number, cells need to fix the interprotofilament angle. Cells do so in three ways: 1) with nucleation factors, 2) by expressing specific tubulin isoforms, and 3) through PTM of tubulin.

The simplest way to fix the interprotofilament angle is by providing a nucleation template, such as the 13 γ -tubulins of the γ -TuRC (Figure 5A) or the A-tubules of axonemes, which exclusively nucleate

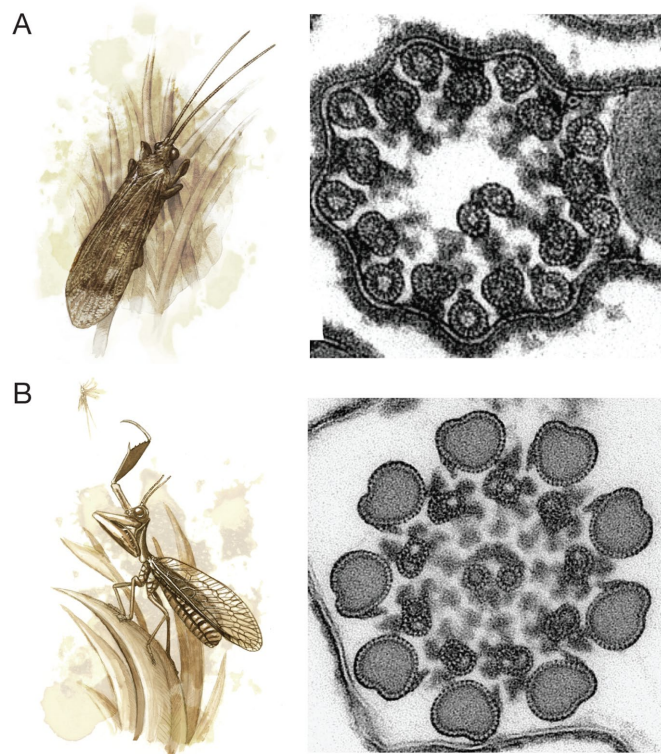


FIGURE 4: (A) Many insects, such as the caddisfly *Stenophylax permistus* (left), have unusual sperm axonemes with 9 accessory microtubules surrounding the 9 microtubule doublets in a 9+9+2 configuration (right; adapted from Dallai *et al.* [2016] and reprinted with permission from the *Annual Review of Entomology*). (B) The sperm axoneme accessory microtubules of the mantidfly *M. perla* have 40 protofilaments and are the largest microtubules observed in nature (adapted from Dallai *et al.* [2005] and reprinted with permission from Elsevier).

13-protofilament microtubules (Scheele *et al.*, 1982). The budding yeast (*Saccharomyces cerevisiae*) γ -TuRC is a conical polymer composed of 7 γ -tubulin small complexes (γ -TuSC), each of which binds 2 γ -tubulins; a partial overlap between one set of γ -TuSCs creates a 13-protofilament template (Zheng *et al.*, 1995; Moritz *et al.*, 2000; Kollman *et al.*, 2010). If the γ -TuRC gained or lost one γ -TuSC, the protofilament number might shift from 13 protofilaments to 15 or 11 protofilaments, respectively, although there is no evidence for such a gain or loss. This idea may explain odd protofilament numbers (e.g., 11 protofilaments in *C. elegans*), but it cannot explain even protofilament numbers (e.g., 12 protofilaments in lobsters). An important caveat is that the template may not be sufficient to specify the protofilament number. For example, a 14-protofilament GMPCPP microtubule template nucleates a 13-protofilament microtubule lattice (Bechstedt and Brouhard, 2012).

To further confound the template story, some microtubules—for example, the accessory microtubules in insect sperm—are nucleated without a template. They instead nucleate as outgrowths from a specific location on the B-tubule, one protofilament at a time, before detaching and closing up (Dallai and Afzelius, 1993). It is not known what determines the protofilament number at which the outgrowing microtubule detaches from the B-tubule, but microtubule inner proteins (MIPs) may fix the interprotofilament angles of the outgrowths, as they do in the case of B-tubule outgrowths from

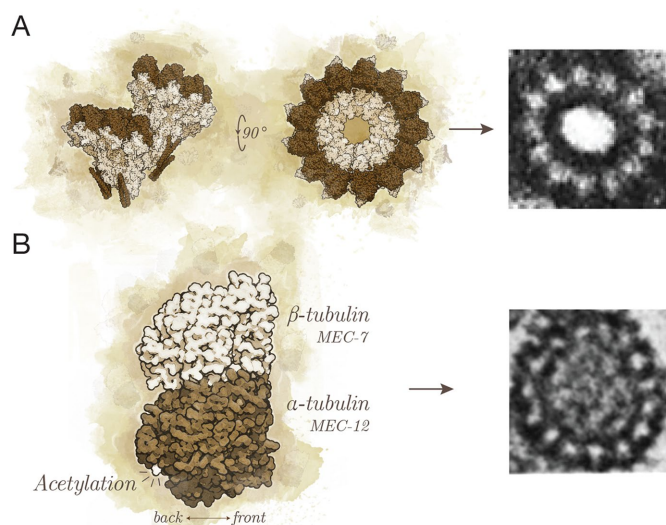


FIGURE 5: (A) The γ -TuRC provides a template for nucleation with its 13 exposed γ -tubulins (Kollman *et al.*, 2010; left). Shown is a 13-protofilament microtubule nucleated from the centrosome (right; adapted from Evans *et al.* [1985] and reprinted with permission from Rockefeller University Press). (B) The tubulin dimer (left) can be composed of different isoforms, such as the *C. elegans* MEC-12/MEC-7 (Savage *et al.*, 1989; Fukushige *et al.*, 1999), and can acquire PTMs, such as acetylation (Cueva *et al.*, 2012; Topalidou *et al.*, 2012). These modifications are able to specify the 15-protofilament microtubules of TRNs in *C. elegans* (right; adapted from Chalfie and Thomson [1982] and reprinted with permission from Rockefeller University Press).

the A-tubule (Ichikawa *et al.*, 2017). Template-free microtubule nucleation may also occur in neuronal growth cones, where local nucleation takes place in the absence of γ -TuRCs (Baas and Joshi, 1992; Ma *et al.*, 2004). The neuronal microtubule-associated protein (MAP) doublecortin is sufficient to nucleate 13-protofilament microtubules *in vitro* (Moores *et al.*, 2004), and we and others have speculated that it might function as a nucleation factor in growth cones (Moores *et al.*, 2004; Bechstedt and Brouhard, 2012). Doublecortin binds the microtubule lattice at the vertex of four tubulin dimers and shares this site with end-binding protein 1 (EB1), which also nucleates 13-protofilament microtubules *in vitro* (Vitre *et al.*, 2008; Maurer *et al.*, 2012). MAPs, MIPs, and templates are therefore able to specify protofilament numbers by binding different surfaces of the microtubule.

Another way to fix the interprotofilament angle is to change the preferred angle of lateral interactions between tubulins. Cells can change the preferred angle by expressing specific tubulin isoforms or by PTMs, both of which have been shown to modify protofilament numbers. Evidence that the isoform alone can specify protofilament numbers can be found in an experiment in which an endogenous β -tubulin in *D. melanogaster* was replaced with a testis-specific β -tubulin from the tobacco budworm (*Heliothis virescens*; Raff *et al.*, 1997). As a result, the 13-protofilament accessory microtubules of the *D. melanogaster* sperm were transformed to 16 protofilaments. Similarly, the 15-protofilament microtubules in *C. elegans* TRNs depend on specific α - and β -tubulin isoforms, namely MEC-12 and MEC-7 (Figure 5B; Fukushige *et al.*, 1999; Savage *et al.*, 1989). More recently, acetylation of α -tubulin by acetyltransferases was shown to specify the 15-protofilament microtubules in the TRNs as well (Figure 5B; Cueva *et al.*, 2012;

Topalidou *et al.*, 2012). In summary, cells possess numerous tools to specify the protofilament number of their microtubules. Indeed, these tools may have evolved to enable cells to tune the structure of their microtubules in response to selective pressures on complex animal behavior.

HIGHER-ORDER MICROTUBULE ASSEMBLIES

As we have seen, early EM turned up an intriguing diversity at the level of individual tubulin polymers. At the same time, EM also discovered microtubules arranged into a wide range of higher-order assemblies. These assemblies far exceed the commonplace radial arrays and bundles found in most cells. In keeping with the spirit of this review, below we survey a selection of these extraordinary microtubule assemblies.

Specialized assemblies in protists

The greatest diversity of microtubule assemblies is found in the nearly mythical organelles, appendages, and machineries of protists. These unusual microtubule-based organelles are composed of large microtubule bundles with intricate geometries. In ciliates, for example, the order *Gymnostomatida* (class *Nassophorea*) feeds through a cytopharyngeal basket that is supported by massive rod structures crammed with hexagonally linked microtubules (Tucker, 1968). Hexagonal packing is a relatively simple geometry, but the bundle geometry can also be complex. Another group of ciliates, the order *Suctorida* (class *Phyllopharyngea*), feeds with tentacles that contain a ring of microtubules internally lined with “ribbons” of 7 to 10 microtubules; repeated sliding of the ribbons facilitates ingestion of a prey’s cytoplasm (Bardele, 1972). The number of microtubules within protist organelles can also be remarkably high. Metamonads of the order *Oxymonada* and *Trichomonadida* produce an axostyle: a contractile organelle made of thousands of microtubules arranged in spiraling parallel sheets. The undulating axostyle of *Saccinobaculus* propels the protists through the intestines of termites (McIntosh, 1973). Other metamonads also have microtubule assemblies specialized for invasion. *Giardia* uses a colossal microtubule-based structure known as the ventral disk to suction onto and colonize human intestinal cells (Elmendorf *et al.*, 2003; Brown *et al.*, 2016). The apicomplexans, on the other hand, invade other cells using the apical complex, a machinery built from numerous highly organized microtubule arrays. One such array in the class *Conoidasida* is the tightly wound microtubules of the conoid, where each microtubule is not a closed tube but rather a curved sheet of 9 protofilaments (Hu *et al.*, 2002). The intricate microtubule-based machineries of unicellular eukaryotes demonstrate the adaptability of tubulin polymers to the demands of each protist’s niche.

The axonemes of insect sperm and protists

Although multicellular eukaryotes may lack the fantastical microtubule assemblies found in protists, many have elaborations on the familiar axoneme. Insect sperm and protist axonemes frequently deviate from the textbook 9+2 doublet structure (Figure 1C). As few as 3 doublets can suffice, as in the flagella of the apicomplexan *Gregarinasina* (Prensier *et al.*, 1980). In insect sperm, anywhere from 6 to 16 doublets have been observed (Dallai *et al.*, 1996). Axonemes in the *Cecidomyiidea* family of insects no longer resemble the canonical form at all and instead have up to 2500 doublets arranged into spirals, rings, and cartwheels (Figure 6A; Dallai *et al.*, 2006). Going further, the *Coccoidea* superfamily lacks doublets altogether; concentric rings of singlets propel the sperm instead (Baccetti *et al.*, 1982).

Captivating singlet-based axonemes are also found in *Heliozoa* protists. Their numerous axopods are thin extensions into the

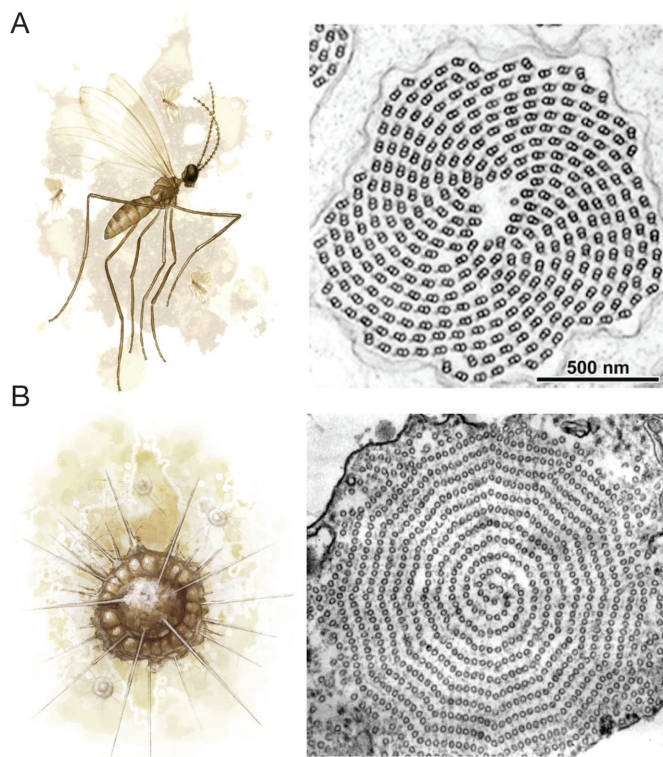


FIGURE 6: (A) A gall midge (left) has cartwheel arrangements of microtubule doublets in the axonemes of its sperm (right; adapted from Dallai *et al.* [1997] and reprinted with permission from John Wiley & Sons). (B) Heliozoans, such as *A. nucleofilum* (left), have thin extensions into the environment that contain two interlocking spiral sheets of microtubules that are subdivided into twelve sectors (right; adapted from Tilney and Byers [1969] and reprinted with permission from Rockefeller University Press).

environment that contain two interlocking spiral sheets of microtubules subdivided into 12 sectors (Figure 6B; Tilney and Byers, 1969). Some heliozoans have triangular or hexagonal microtubule arrangements in their axopods (Febvre-Chevalier and Febvre, 1984). These axonemes emanate from a point known as the centrioplast or from trilaminar plaques on the nuclear membrane (Tilney, 1971; Cachon *et al.*, 1977). The nucleation factors and cross-linking proteins responsible for these complex arrangements remain a mystery.

DISCUSSION

It is clear that cells specify the protofilament number of their microtubules during nucleation using nucleation factors, tubulin isoforms, and PTMs. Because cells care about their protofilament numbers, so should we. Although 13 protofilaments are far from universal, their prevalence in every eukaryotic supergroup suggests a persistent selective pressure. But the straight-protofilament hypothesis is hard to reconcile with the 11-protofilament microtubules in *C. elegans* and the irregular paths of many kinesins. We therefore wondered about other selective pressures for 13 protofilaments. While curating the microtubule structures for this review, we noticed that every axoneme we found had 13-protofilament A-tubules, even in the ciliated neurons of *C. elegans* (Chalfie and Thomson, 1982). Perhaps the straight protofilaments of the 13-protofilament lattice are the result of selective pressures on cilia and flagella. More specifically, the straight protofilaments of a 13-protofilament A-tubule may be necessary for the B-tubule to attach with-

out twisting around the A-tubule. Similarly, the beating of motile cilia and flagella relies on adjacent doublets sliding parallel to one another; straight protofilaments may therefore be essential for ciliary beating. Indeed, the LECA is thought to have been ciliated, and this ancestral cilium may have originated from cytoplasmic microtubules (reviewed in Carvalho-Santos *et al.*, 2011). If the 13-protofilament lattice arose because of selective pressures on cilia and flagella, it is intriguing that 13 protofilaments nevertheless persist in fungi that lack cilia and flagella. Of course, we cannot rule out the hypothesis that the 13-protofilament lattice was an evolutionary accident. Furthermore, cells may not specify their microtubules in every context; for example, the variable protofilament numbers in human platelets and mitotic ciliates may represent a loss of control rather than a regulated transition. Nevertheless, it is our hypothesis that selective pressures have tuned protofilament numbers in different cell types.

Although microtubule structure is diverse, an overwhelming majority of in vitro studies are performed with tubulin purified from the brains of ungulates, primarily cows and pigs. Although ungulate tubulin has enabled extraordinary progress, recent advances in tubulin expression and purification have allowed us to begin experimenting with other tubulins. For example, yeast tubulin is now obtainable using an inducible expression system (Johnson *et al.*, 2011). Tubulins from other eukaryotes, including *C. elegans* and *Xenopus laevis*, have been purified by taking advantage of tubulin's strong affinity to the TOG (tumor overexpressed gene) domains of XMAP215 family proteins (Widlund *et al.*, 2012). Moreover, human tubulin can be recombinantly expressed and purified in a lepidopteran cell line (Minoura *et al.*, 2013). These techniques have opened the door to comparative studies of tubulin from different species, of tubulin isoforms within the same species, and of tubulin mutations. Indeed, the initial comparisons of different human tubulins or yeast tubulin have already uncovered changes in the parameters of dynamic instability (Geyer *et al.*, 2015; Ti *et al.*, 2016; Vemu *et al.*, 2016).

Solving the structure of microtubules from different species will allow us to determine the structural basis of divergences in lattice structure and dynamic behavior. Advances in EM and three-dimensional reconstruction methods have made near-atomic-resolution models of microtubules possible. These models have revealed the tertiary and quaternary structure of tubulins with extraordinary precision, from residues at the laterally interacting M-loops to the long-range conformational changes that accompany GTP hydrolysis (Zhang *et al.*, 2015; Howes *et al.*, 2017). When near-atomic-resolution EM is combined with novel tubulin-purification methods, we will be able to answer questions about the basis of microtubule quaternary structure and, importantly, about the relationship of microtubule structure to cell physiology.

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