

The microtubule cytoskeleton is a dynamic structure that can participate in a variety of cellular processes owing to its remarkable ability to organize itself into diverse structures. The organization of microtubules involves microtubule motor proteins, microtubule-binding proteins and, perhaps most importantly, the microtubule-organizing centre (MTOC), a small organelle from which most microtubules grow. MTOCs of different cell types take many forms, but they all share the common properties of nucleating the polymerization of microtubules from free tubulin subunits and of anchoring the nucleated microtubules. Because the major functions of microtubules involve the generation of force by the unidirectional microtubule motors dynein and kinesin, it is essential that the microtubule arrays on which these motors move be oriented with the appropriate polarity and geometry. The MTOC controls polarity by nucleating microtubules with their minus-ends (defined *in vitro* as the more slowly growing end) at the organizing centre and their plus-ends radiating into the cytoplasm; it controls the geometry of the microtubule cytoskeleton by providing a single focus of microtubule growth that typically is located adjacent to the nucleus.

The discovery of γ -tubulin marked a major advance in understanding microtubule nucleation. γ -Tubulin is a conserved member of the tubulin superfamily. Two other members of the tubulin family, α - and β -tubulin, form the heterodimeric subunit of microtubules. γ -Tubulin, however, does not form a stable dimer with α - or β -tubulin and is not incorporated into microtubules. Instead, γ -tubulin is a component of MTOCs¹⁻³. Its similarity to the other tubulins and its unique localization have led to the idea that γ -tubulin is a key component of the microtubule-nucleating machinery. Interestingly, γ -tubulin can form filamentous structures when overexpressed in vertebrate cells⁴. However, these structures do not form when γ -tubulin is overexpressed in yeast⁵, and the biological significance of this observation remains unclear.

Immunofluorescence experiments show that the most obvious concentration of γ -tubulin is at the centrosome. However γ -tubulin has also been found to be in the mitotic spindle⁶, at the midbody⁷, the poles of *Schizosaccharomyces pombe* cells⁸ and along the microtubules in plant cells⁹. Most importantly, cell-fractionation experiments reveal that more than half of the γ -tubulin in a typical somatic animal cell is actually soluble in the cytoplasm^{10,11}. In oocytes, which lack a centrosome prior to fertilization, all of the γ -tubulin is soluble. Thus, the cytoplasm-rich eggs of frog, clam and *Drosophila* have proved to be excellent sources of material for the characterization of γ -tubulin. Studies in these systems have revealed that the cytoplasmic form of γ -tubulin is part of a very large protein complex with a sedimentation coefficient of approximately 30S (Ref. 10). This large complex is able to nucleate microtubule polymerization, and electron microscopy shows that it is a ring-shaped structure with a diameter similar to that of a microtubule

γ -Tubulin complexes: size does matter

Robert Jeng and Tim Stearns

γ -Tubulin is a conserved component of all microtubule-organizing centres and is required for these organelles to nucleate microtubule polymerization. However, the mechanism of nucleation is not known. In addition to its localization to organizing centres, a large pool of γ -tubulin exists in the cytoplasm in a complex with other proteins. The size of the γ -tubulin complex and number of associated proteins vary among organisms, and the functional significance of these differences is unknown. Recently, the nature of these γ -tubulin complexes has been explored in different organisms, and this has led us closer to a molecular understanding of microtubule nucleation.

(25 nm)¹², suggesting that it might act to nucleate microtubules by a direct templating mechanism, although other models have been proposed (see below)^{12,13}. γ -Tubulin-containing structures similar in size and shape to the cytoplasmic ring complex have also been observed in the pericentriolar material of centrosomes^{14,15}. Although these findings have provided insight into possible mechanisms of nucleation, the function of the γ -tubulin complex, and the relationship between the cytoplasmic γ -tubulin complex and the centrosomal form of γ -tubulin, remain unclear.

The γ -tubulin complex: structure and function

The yeast *Saccharomyces cerevisiae* has been instrumental in identifying components of the γ -tubulin complex. The yeast spindle pole body is morphologically quite different from the centrosome but is functionally equivalent. The spindle pole body is a layered structure that remains embedded in the nuclear envelope throughout the cell cycle. It has three major layers: the inner, outer and central plaques. The inner plaque faces the nucleoplasm, the outer plaque faces the cytoplasm, and the central plaque is embedded in the nuclear envelope. The nuclear membrane remains intact throughout mitosis in yeast and separates two distinct classes of microtubules. Nuclear microtubules grow from the inner plaque of the spindle pole body, whereas cytoplasmic

The authors are in the Dept of Biological Sciences, Stanford University, Stanford, CA 94305-5020, USA.
E-mail: stearns@stanford.edu

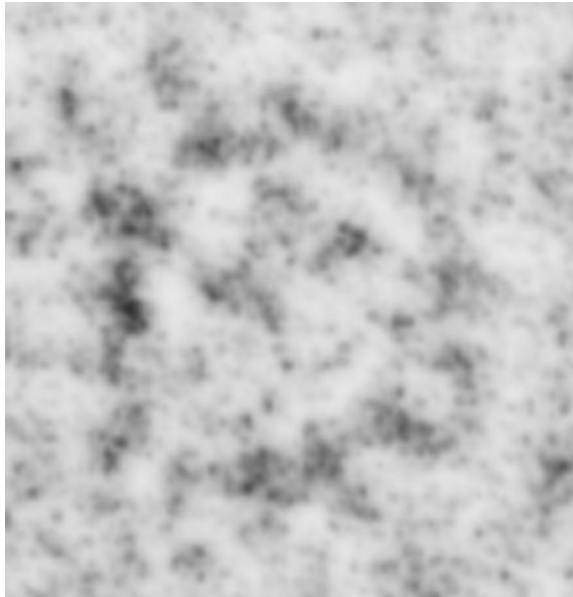


FIGURE 1

The ring-shape of γ -tubulin complexes. Electron microscopic image of *Drosophila* ring-shaped γ -tubulin complexes. The 13 subunits can be seen, although the composition of each subunit is unknown. (Figure courtesy of Yixian Zheng.)

microtubules grow from the outer plaque. As would be expected if it has a direct involvement in microtubule nucleation, γ -tubulin is concentrated at both the inner and outer plaques¹⁶. Furthermore, phenotypic analysis of mutations in the yeast γ -tubulin gene, *TUB4*, reveal defects consistent with a failure of microtubule nucleation^{5,16}.

As in animal cells, yeast γ -tubulin is found in the cytoplasm as well as at the spindle pole body. Genetic screens have identified components of the yeast cytoplasmic γ -tubulin complex. Spc98p (for: 'spindle pole body component 98 kDa') was identified as a suppressor of a γ -tubulin mutant¹⁷, and a similar screen identified Spc97p as a suppressor of an *spc98* mutant¹⁸. Spc98p was identified previously by generation of monoclonal antibodies against purified spindle pole body components¹⁹. Like Tub4p, both Spc97p and Spc98p localize to the spindle pole body at the outer and inner plaques, and they are also present in the cytoplasm in association with γ -tubulin. Estimates of the size and stoichiometry of proteins in the yeast cytoplasmic γ -tubulin complex suggest that it is much smaller than that found in animal cells, consisting of one molecule each of Spc97p and Spc98p, and two molecules of γ -tubulin¹⁸.

In contrast to yeast, the cytoplasmic animal cell γ -tubulin complex contains six or more proteins in addition to γ -tubulin, and this composition appears to be conserved in mammals, frogs and flies. The pioneering work in yeast led to the identification of two of these proteins as homologues of Spc97p and Spc98p, both by protein sequencing^{20,21} and by DNA sequence database comparisons^{22,23}. Because these proteins go by several different names in the literature, Murphy *et al.*²³ proposed the unifying nomenclature of GCP (for: 'gamma-tubulin

complex protein') – with gamma-tubulin as GCP1, Spc97p homologues as GCP2, and Spc98p homologues as GCP3 – which we will follow in this review.

Sequence comparisons revealed that GCP2 and GCP3 are related proteins²³, although they are not functionally redundant as both are required for viability in yeast^{17,18}. As in yeast, animal GCP2 and GCP3 colocalize with γ -tubulin at the centrosome^{20,22,23}. Estimates of the stoichiometry of proteins in the animal γ -tubulin complex indicate that it contains multiple copies of γ -tubulin, GCP2 and GCP3¹². The other components of the complex, which have not yet been identified, are present in lower amounts, suggesting that the large ring-shaped complex might be an oligomer of γ -tubulin, GCP2 and GCP3, held together by the other proteins.

The conservation of γ -tubulin, GCP2 and GCP3 suggests that there is a common mechanism for microtubule nucleation in yeast and animals, but there are several perplexing differences between the two systems. Most striking is the difference in size and complexity of the yeast and animal γ -tubulin complexes. This difference raises the question of which components are required for nucleation and which are specific to the different organizing centres. In particular, do the remaining unidentified vertebrate components have homologues in yeast, or are they species-specific components that are not a part of the essential nucleation mechanism? Recent studies on the *Drosophila* γ -tubulin complex provide a link between the complexes and suggest a possible explanation for their differences.

The *Drosophila* γ -tubulin complex

Using size-fractionation techniques, Moritz *et al.*²⁴ and Oegema *et al.*²¹ found that, in *Drosophila* embryos, γ -tubulin is found in both a small complex and a large complex. The *Drosophila* small complex is similar in size to the yeast complex and, like the yeast complex, is estimated to consist of one molecule each of GCP2 and GCP3, and two molecules of γ -tubulin²¹. The large complex, on the other hand, is similar in size to vertebrate γ -tubulin complexes. The protein profile of the large complex resembles animal complexes, with approximately five to six unidentified proteins in addition to γ -tubulin, GCP2 and GCP3. Electron microscopy showed that the large complex forms a circular ring structure similar to the *Xenopus* complex (Fig. 1).

To understand microtubule nucleation, the basic unit of nucleation must be identified. Both *Drosophila* studies showed that the small complex is likely to be a core subunit of the large complex: in low-salt conditions, both complexes are present in cell extracts, but, after high-salt treatment, only the small complex remains. As the large γ -tubulin complex is able to nucleate microtubules, Oegema *et al.*²¹ compared the *in vitro* nucleation activities of small and large complexes to determine whether the small complex is sufficient for microtubule nucleation. Although the small complex had weak nucleating activity in some *in vitro* assays, it was a poor

microtubule nucleator when compared with the large complex. This suggests either that the other components of the large complex are necessary for nucleation or that a specific geometry imposed by them is required (Fig. 2).

Given the dependence of microtubule nucleation on a higher-order oligomer of the small γ -tubulin complex, it seems likely that yeast cells have a mechanism for organizing their small γ -tubulin complex. A simple model to explain the lack of a full-sized γ -tubulin complex in yeast cytoplasm is that components required from the large complex might only be found at the spindle pole body, restricting formation of the active complex to that location. Indeed, two proteins that interact with the γ -tubulin complex have been identified in yeast, Spc72p and Spc110p^{19,25–28}. These proteins are not part of the cytoplasmic yeast complex but are localized to the spindle pole body and appear to act as receptors for the γ -tubulin complex^{26,27,29}. It is possible that, in addition to anchoring the γ -tubulin complex, they also help assemble the subunits into a higher-order structure.

The mechanism of microtubule nucleation

A major unanswered question is how the large γ -tubulin complex nucleates microtubule polymerization. The observation that the complex has the shape of a ring of the approximate diameter of a microtubule strongly suggests that it directly templates protofilament formation by addition of tubulin subunits to γ -tubulin in the same manner that the tubulin subunits themselves interact in an elongating microtubule (Fig. 3a). The new results from Oegema *et al.*²¹ support this model; they used electron microscopy to show that the *Drosophila* ring complex appears to have 13 subunits making up the ring, the same as the number of protofilaments in the typical microtubule. But, what are those 13 subunits? If they are individual γ -tubulin molecules, then how can the odd number be reconciled with the minimal unit of two γ -tubulins per small complex? If they are individual small complexes, how could the 26 γ -tubulin molecules present be involved in templating microtubules with 13 protofilaments? One possibility is that there are actually seven small complexes (14 γ -tubulins) per ring, and that there is an overlap of one γ -tubulin at the open end of the ring. This would explain the lock-washer appearance reported for the *Xenopus* ring complex and fit with the idea that the end of a microtubule is unlikely to be flush, based on the three-start helix structure of the microtubule polymer¹².

Another proposed model for nucleation by the γ -tubulin complex views the ring as simply a curled-up protofilament (Fig. 3b)¹³. When extended, this protofilament could interact laterally with tubulin subunits to nucleate their assembly into the microtubule polymer. This model is based on electron microscope images of curved protofilaments at microtubule ends. Although this model has some attractive points, it seems less likely given the excellent match between the size and subunit number of the γ -tubulin complex and the microtubule structure.

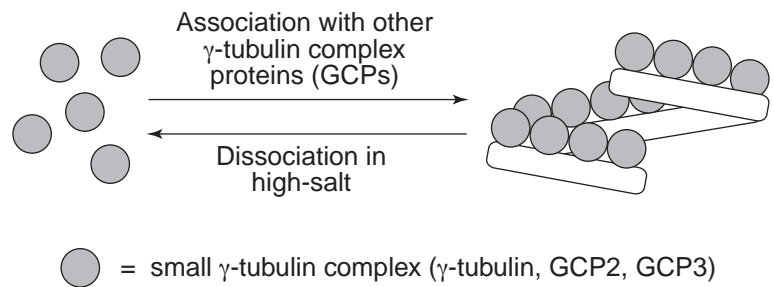


FIGURE 2

Relationship between small and large γ -tubulin complexes. *Drosophila* embryos contain both small and large γ -tubulin complexes. Salt-treatment of *Drosophila* extract dissociates proteins from the large γ -tubulin complex, resulting in only small complexes that contain γ -tubulin, GCP2 and GCP3. One model is that the small complexes (shaded circles) can be assembled into the ring-shaped larger complex by association with other proteins (clear ring). Salt-treatment of the large complex removes the clear ring proteins from the core proteins of the small complex. The actual number of subunits and their orientation in the ring-complex is unknown.

Both of these nucleation models invoke a direct interaction between γ -tubulin and the α - β -tubulin heterodimer, either end-on for the ring-templating model or laterally for the protofilament model. It is remarkable that no such direct interaction has yet been detected, despite several attempts using different approaches. Although a definitive experiment is still lacking, it is useful to consider other models in which γ -tubulin does not interact directly with α - β -tubulin. For example, one possible model is that the conserved GCP2 and GCP3 proteins actually nucleate microtubule polymerization by direct interaction with α - β -tubulin and that γ -tubulin is only required to hold GCP2 and GCP3 in the appropriate configuration. This model is identical to the ones presented in Fig. 3 but emphasizes that the orientation of the γ -tubulin small complex does not necessarily position γ -tubulin adjacent to α - and β -tubulin. Only further experiments with purified components will answer the question of which of the theoretically possible models for microtubule nucleation are correct.

Future questions

Although our knowledge of γ -tubulin has progressed rapidly in the past few years, many questions remain about the function and regulation of γ -tubulin complexes. While it is clear that γ -tubulin complexes are found both in the cytoplasm and centrosome, the role of γ -tubulin complexes in the cytoplasm is unclear. The *in vitro* studies showing the ability of the complex to nucleate microtubules have almost exclusively used purified cytoplasmic complex. Yet, most microtubule nucleation in cells occurs at the MTOC, suggesting that the cytoplasmic γ -tubulin complex is not active for nucleation *in vivo*. One simple explanation is that an inhibitor of the complex does not copurify with the other proteins, leaving only active cytoplasmic complex. However, it is unclear why cells would maintain a large pool of inactive material. Another possible explanation is that the cytoplasmic complex does have activity away from the centrosome. Indeed, many animal cells, and all plant cells, have

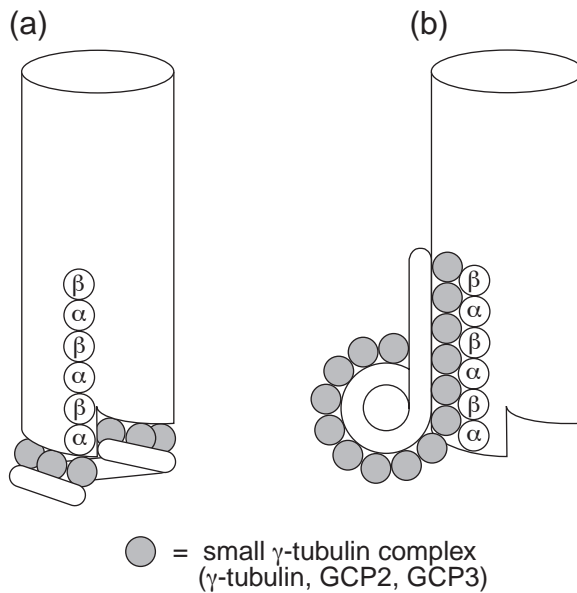


FIGURE 3

Two models of microtubule nucleation. (a) The γ -tubulin complex directly templates protofilament formation from the end of microtubules. A single γ -tubulin molecule or a small complex unit interact with tubulin heterodimers to start a protofilament. Although α - and β -tubulin heterodimers form the entire microtubule cylinder, only a few molecules are shown for clarity. (b) In this model, the ring complex ends with a short, linear segment that initiates protofilament formation by interacting with the α - and β -tubulin heterodimers laterally rather than end-on. A sheet of microtubule protofilaments would then close to form the cylindrical microtubule. This model is based on the observation of curled protofilament-like structures extending from the ends of microtubules. However, the details of the drawing, such as the inclusion of accessory proteins (clear rod shape) along the protofilament-like extension, and the placement of the extension along the side of the microtubule rather than in the microtubule, are completely hypothetical.

microtubules in their cytoplasm that are not attached to an organizing centre. In animal cells, these are likely to result from spontaneous polymerization³⁰ or from release from the centrosome^{31,32}. Perhaps the cytoplasmic γ -tubulin complex plays a role in nucleating or stabilizing these free microtubules; indeed, in cases where the dynamics of free microtubules have been examined, some of the minus-ends are stable^{32,33}.

Another major question is how the animal cell γ -tubulin complex is attached to the pericentriolar matrix of the centrosome. Moritz *et al.*²⁴ and Schnackenberg *et al.*³⁴ showed that centrosomes could be reduced to a central core by treatment with high salt. This core did not nucleate microtubules, but nucleation could be restored by adding extract containing the γ -tubulin complex. However, pure γ -tubulin complex alone was not sufficient, suggesting that there are attachment factors that link it to the matrix. Although there are no known animal cell homologues of the yeast proteins that link γ -tubulin to the spindle pole body, recent experiments suggest that pericentrin, a large coiled-coil component of the pericentriolar material that is associated with the γ -tubulin complex, might be important³⁵.

Lastly, there is evidence that a new member of the tubulin superfamily, δ -tubulin, is important in the functioning of the MTOC in *Chlamydomonas*³⁶. It is not yet clear whether δ -tubulin functions in conjunction with or separately from γ -tubulin or whether other tubulins remain to be found in higher eukaryotes. With the progress of the various genome projects, this question will be answered definitively soon.

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