Microtubule nucleation: γ-tubulin and beyond

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Summary
Centrosomes and their fungal equivalents, spindle pole bodies (SPBs), are the main microtubule (MT)-organizing centers in eukaryotic cells. Several proteins have been implicated in microtubule formation by centrosomes and SPBs, including microtubule-minus-end-binding proteins and proteins that bind along the length or stabilize the plus ends of microtubules. Recent work has improved our understanding of the molecular mechanisms of MT formation. In particular, it has shown that γ-tubulin and its associated proteins play key roles in microtubule nucleation and spindle assembly in evolutionarily distant species ranging from fungi to mammals. Other work indicates that γ-tubulin-mediated microtubule nucleation, although necessary, is not sufficient for mitotic spindle assembly but requires additional proteins that regulate microtubule nucleation independently of centrosomes.

Key words: Centrosome, γ-tubulin, Microtubule, Nucleation, XMAP215, TACC, TPX2, Aurora A

Introduction
Microtubules (MTs) are found in all eukaryotic cells and play essential roles in cell division (Fig. 1), directional transport of proteins and vesicles, cytoplasmic organization, cell shape, polarity, and motility. They are non-covalent polymers composed of α/β-tubulin heterodimers. The head-to-tail assembly of α/β-tubulin heterodimers into linear protofilaments confers intrinsic polarity on the MT, with α-tubulin at the slower-growing ‘minus’ end and β-tubulin at the faster-growing ‘plus’ end (Mitchison, 1993; Nogales et al., 1999) (Fig. 2). The MT lattice consists of ten to 15 protofilaments that associate sideways to form a hollow but relatively rigid cylinder that has a diameter of ~25 nm (Desai and Mitchison, 1998). To initiate MT formation, multiple α/β-heterodimers associate to form a seed upon which MTs can rapidly elongate. Cellular proteins that regulate MT formation presumably facilitate nucleation by either mimicking the seed or by stabilizing it. Alternatively, these proteins might influence MT stability at later stages during the assembly process.

MTs are inherently dynamic, undergoing repeated transitions from growth to shrinkage (Mitchison and Kirschner, 1984a; Mitchison and Kirschner, 1984b). MT dynamics and the organization of the MT network change dramatically during the cell cycle to generate different MT arrays. During interphase, MT arrays are typically composed of relatively long, stable MT polymers (Fig. 1). By contrast, MTs are short and highly dynamic in mitosis.

Although MTs can self-assemble in vitro from high concentrations of purified tubulin subunits, within the cell they form at much lower tubulin concentrations. At low tubulin concentrations, the nucleation of new MTs is kinetically limiting. To overcome this kinetic barrier, in vivo nucleation takes place primarily at morphologically distinct structures termed MT-organizing centers (MTOCs) (Pickett-Heaps, 1969). MTOCs vary greatly in shape, size and occurrence, both within a given cell and between species (Fig. 3). For example, the animal centrosome is a non-membrane-bound cytoplasmic organelle composed of a pair of centrioles surrounded by a pericentriolar matrix (PCM), whereas the spindle pole body (SPB) of Saccharomyces cerevisiae is a trilaminar plaque embedded in the nuclear envelope. The centrosome of the slime mold Dictyostelium discoideum shares features with both SPBs and metazoan centrosomes: it is a three-layered, box-shaped structure surrounded by an amorphous corona that, like the PCM of the metazoan centrosome, nucleates and anchors MTs (Gräf et al., 2000) (Fig. 3). In addition to centrosomes and SPBs, MTs are sometimes nucleated by transient, cell-cycle-regulated nucleation sites, such as interphase and equatorial MTOCs (iMTOC and eMTOC, respectively), that have been described in fission yeast (Hagan and Petersen, 2000). In animal cells, the midbody that forms between the daughter cells following cytokinesis also transiently nucleates MTs (Pielh et al., 2004).

Here, we highlight recent progress in our understanding of the regulation of MT nucleation in animal cells and fungi, focusing on the roles of γ-tubulin and its associated proteins. Although orthologs of many of these proteins also exist in plants, we do not discuss MT nucleation and organization in plants here and refer readers to recent reviews elsewhere (Ehrhardt and Shaw, 2006; Lloyd and Chan, 2004).

γ-tubulin complexes
Discovered as the third member of the tubulin superfamily over 15 years ago (Oakley and Oakley, 1989; Stearns et al., 1991; Zheng et al., 1991), γ-tubulin is now recognized to be an indispensable component of MTOCs that contributes to MT nucleation and organization in all eukaryotes (Job et al., 2003). It is a ubiquitous and phylogenetically conserved tubulin isotype that does not incorporate into the MT wall but instead binds to the MT minus end. In all cell types examined, γ-tubulin associates with one or more proteins that are also highly conserved (see below and Table 1).


**Fig. 1.** Different types of microtubule array are organized by centrosomes during interphase and mitosis, as in these cultured *Xenopus laevis* kidney epithelial cells. Microtubules are shown in red, DNA in blue and the centrosomal γ-tubulin in green.

γ-tubulin complexes in budding and fission yeasts

Budding yeast Tub4p (γ-tubulin) (Marschall et al., 1996; Spang et al., 1996) is part of a protein complex whose size depends on the buffer conditions used to prepare the cell extracts (Vinh et al., 2002). The 9S Tub4p complex consists of two molecules of γ-tubulin and one each of two related and evolutionarily conserved proteins: Spc97p and Spc98p (Geissler et al., 1996; Knop et al., 1999; Knop and Schiebel, 1997; Vinh et al., 2002). The 22S complex is proposed to be an oligomer of this basic complex, but its composition and structure remain to be fully characterized (Vinh et al., 2002).

The fission yeast γ-tubulin complex (γ-TuC) migrates mainly as a 22S complex (Fujita et al., 2002; Vardy and Toda, 2000), and is composed of Twp1/Gtb1 (γ-tubulin) (Horio et al., 1991) and orthologs of Spc97p and Spc98p, named Alp4p and Alp6p (Vardy and Toda, 2000), respectively, together with at least two additional proteins, Gfh1p and Alp16p (Table 1) (Fujita et al., 2002; Sawin et al., 2004; Venkatram et al., 2004). Gfh1p and Alp16p share regions of sequence similarity with each other, with Alp4p and Alp6p, and with metazoan GCP4 and GCP6. All γ-TuC components localize to SPBs throughout the cell cycle and to cMTs during cytokinesis. Mutations in any of the γ-TuC genes cause defects in cytoplasmic MT organization, but conditional inactivation of Gtb1p, Alp4p or Alp6p results in more severe MT and mitotic defects than loss of gfh1 or alp16. Moreover, Gtb1p, Alp4p and Alp6p are required for the formation of all MTs in fission yeast, whereas the loss of Alp16p or Gfh1p function specifically affects cytoplasmic MTs and has little or no effect on the mitotic spindle (Fujita et al., 2002; Venkatram et al., 2004). This suggests that a core fission yeast γ-TuC, analogous to the budding yeast Tub4p complex is composed of γ-tubulin, Alp4p and Alp6p. This core is required for the formation of all MTs, whereas a larger complex that also contains Gfh1p and Alp16p regulates the formation and/or organization of cytoplasmic MTs.

**Fig. 2.** Microtubules are assembled from α/β-tubulin dimers, which associate head-to-tail to form linear protofilaments (boxed area). 12-15 protofilaments associate laterally to form the walls of the microtubule cylinder, which has a diameter of ~25 nm. The GTP-binding pocket in α-tubulin is covered by β-tubulin at all times and is therefore not exchangeable (see text). By contrast, the nucleotide on β-tubulin can be exchanged while the dimer is not part of a microtubule. GTP hydrolysis is coupled to the incorporation of the dimer into the microtubule, and its energy facilitates rapid microtubule disassembly. What triggers the transition between growth and shrinkage of the microtubule, or between its shrinkage and growth, is not yet understood. Tubulin subunits can add to both ends of the cylinder, but the plus end grows about three times faster than the minus end in vitro. Within the cell, almost all microtubule minus ends are anchored at MTOCs, leaving the plus ends to extend into the cell periphery. During rapid microtubule disassembly, protofilaments peel back from the microtubule wall. Disassembly is facilitated by tension introduced into the protofilament by a conformational change in β-tubulin upon GTP hydrolysis, which occurs when the subunit is incorporated into the microtubule lattice.

**γ-tubulin complexes in animal cells**

The γ-TuCs of metazoan cells contain γ-tubulin and orthologs of Spc97p/Alp4p, Spc98p/Alp6p, Alp16p and Gfh1p, as well as at least two additional proteins (Table 1). γ-Tubulin complexes purified from *Xenopus* eggs appear ring-shaped when viewed by electron microscopy and were therefore named γ-tubulin ring complexes (γTuRCs) (Zheng et al., 1995). γTuRCs have subsequently also been purified from *Drosophila* early embryos (Oegema et al., 1999) and human tissue culture cells (Murphy et al., 2001). Two predominant nomenclatures are used in the literature to designate γTuRC subunits: Grips (γ-tubulin ring proteins) and GCPs (γ-tubulin complex proteins) (see Table 1). For simplicity, we use mainly the Grip nomenclature here, which is based on the first letter of the organism (e.g. Dgrip for *Drosophila* and Xgrip for *Xenopus*) and the approximate molecular mass of the protein.

Depending on the lysis conditions, *Drosophila* γ-tubulin can also be found in a smaller complex named the γ-tubulin small complex (γTuSC). The γTuSC appears to be analogous to the Tub4p complex in budding yeast: it migrates as an ~10S complex and is composed of two molecules of γ-tubulin and one molecule each of Dgrip84 (the Spc97p ortholog) and Dgrip91 (the Spc98p ortholog) (Oegema et al., 1999). Analyses of γTuSC and γTuRC purified from early *Drosophila* embryos...
suggest that each γTuRC consists of multiple γTuSCs plus one or more of each of the remaining subunits (Dgrip75, Dgrip128, Dgrip163 and Dgp71-WD; Fig. 4) (Oegema et al., 1999; Gunawardane et al., 2003). However, the currently favored model for the arrangement of the Grips within the γTuRC, which postulates that the γTuSCs are held together by a cap complex made up of the non-γTuSC subunits (Fig. 4), has recently been called into question by reconstitution experiments in baculovirus-infected Sf9 cells. *Drosophila* γTuSCs reconstituted from coexpressed Drosophila γTuRC components resemble purified endogenous γTuSCs in size, subunit stoichiometry, nucleotide binding and MT-nucleating activity (Gunawardane et al., 2000). Pair-wise expression showed that Drosophila γ-tubulin can directly interact with either Dgrip84 or Dgrip91 (Gunawardane et al., 2000). Moreover, *Drosophila* γ-tubulin also directly interacts with grip-motif-containing proteins Dgrip163 and Dgrip128 in reconstitution experiments (Gunawardane et al., 2003). The direct interaction between γ-tubulin and individual cap subunits suggests that the grip motifs could potentially participate in the formation of the ring of the γTuRC. For example, dimers of γ-tubulin and one of the non-γTuSC Dgrips might co-assemble with several γTuSCs to form one γTuRC. The interactions between the Dgrips could then stabilize the ring.

With the exception of the WD-motif-containing Dgp71WD/GCP-WD/Nedd1 (Gunawardane et al., 2003; Haren et al., 2006; Lüders et al., 2005), γTuRC subunits are not only highly conserved across species (see Table 1) but also contain at least one of two conserved gamma ring protein (grip) sequence motifs (Fig. 5) (Gunawardane et al., 2000). The high level of sequence conservation among the Grips indicates that the γTuRC is an evolutionarily conserved structure in animal cells (Gunawardane et al., 2000; Murphy et al., 2001; Gunawardane et al., 2003).

RNA interference (RNAi) studies showed that each of the grip-motif-containing cap subunits (Dgrip75, Dgrip128 and Dgrip163) is required for the assembly of the *Drosophila* γTuRC (Vérollet et al., 2006), whereas downregulating Dgp71WD or its human ortholog GCP-WD/Nedd1 has little effect on the assembly of a discrete complex that has γTuRC-like biophysical properties. This suggests that the grip-containing subunits are integral components, whereas the WD-containing subunit might be a peripheral component of the γTuRC (Haren et al., 2006; Lüders et al., 2005; Vérollet et al., 2006).

Four observations led to the notion that the γTuRC rather than the γTuSC is the MT nucleator at the animal centrosome: (1) electron tomography of purified *Drosophila* embryo centrosomes and γTuRC revealed γ-tubulin-containing rings that have γTuRC-like dimensions embedded in the PCM, and are often associated with MT ends (Moritz et al., 1995a; Moritz et al., 2000); (2) purified γTuRC is more than 30 times more active than purified γTuSC at nucleating MTs from purified tubulin in vitro (Oegema et al., 1999); (3) purified γTuRC remains attached to the minus end of the MT it nucleates
### Table 1. Summary of γ- tubulin complex components in various species

| Genus                  | γTuRC (9.8 S) | γTuRC (36 S) | Antrodiapecten thaliana γ-TuC | S. cerevisiae Tub4p complex (6-11 S) | S. pombe γ-TuC (22 S) | A. nidulans γ-TuC (8-20 S) | Dictyostelium discoidal γ-tubulin small complex (S-value not determined) | Caenorhabditis elegans (γ-tubulin complex not yet characterized) |
|------------------------|---------------|--------------|-------------------------------|--------------------------------------|-----------------------|---------------------------|--------------------------------------------------------------------------|------------------------------------------------------------------|
| Drosophila melanogaster| γTuRC         | γTuRC        |                               |                                       |                       |                           | DdSp-97p                                                                 | Ce-Grip-1                                                        |
|                        | (25 S)†,‡      | (25 S)†,‡     |                               |                                       |                       |                           |                                                                          |                                                                  |
| Xenopus                |               |              |                               |                                       |                       |                           |                                                                          |                                                                  |
| Human                  | γTuRC         | γTuRC        |                               |                                       |                       |                           |                                                                          |                                                                  |
| Arabidopsis thaliana   |               |              |                               |                                       |                       |                           |                                                                          |                                                                  |
| S. cerevisiae          |               |              |                               |                                       |                       |                           |                                                                          |                                                                  |
| S. pombe               |               |              |                               |                                       |                       |                           |                                                                          |                                                                  |
| A. nidulans            |               |              |                               |                                       |                       |                           |                                                                          |                                                                  |
| Dgrip84                | Xgrip10       | hGCP2        | AtSp97p (836 aa, 677 aa)       |                                       |                       |                           |                                                                          |                                                                  |
|                        | (three splice variants) |       |                               |                                       |                       |                           |                                                                          |                                                                  |
| Dgrip91                | Xgrip109      | hGCP3        | AtSp98p (196268, 838 aa)       |                                       |                       |                           |                                                                          |                                                                  |
|                        | (three splice variants) |       |                               |                                       |                       |                           |                                                                          |                                                                  |
| Dgrip75                | Xgrip76       | hGPC4        | AtGC4 (190944, 745 aa)         |                                       |                       |                           |                                                                          |                                                                  |
|                        | (three splice variants) |       |                               |                                       |                       |                           |                                                                          |                                                                  |
| Dgrip28                | Xgrip133      | hGCP5        | AtGC5 (five isoforms)          |                                       |                       |                           |                                                                          |                                                                  |
|                        | (three splice variants) |       |                               |                                       |                       |                           |                                                                          |                                                                  |
| Dgrip163               | Xgrip210      | hGCP6        | AtGC6 (189947, 1120 aa)        |                                       |                       |                           |                                                                          |                                                                  |
|                        | (three splice variants) |       |                               |                                       |                       |                           |                                                                          |                                                                  |
| Dgrip71WD              | X-Nedd1       | GCP-WD/Nedd1 | At-Nedd1 (BABI0802, 787 aa)    |                                       |                       |                           |                                                                          |                                                                  |

The *Drosophila* γTuRC is currently the most well-characterized complex and is listed first to serve as a reference point. γ-tubulin complexes isolated from *Drosophila* embryos (Oegema et al., 1999), *Xenopus* eggs (Zheng et al., 1995), or human tissue culture cells (Murphy et al., 2001) have been shown to appear as rings and are called γTuRCs. Others are referred to as γ-tubulin complexes (γ-TuCs). Although orthologs of γTuRC subunits exist in plants (Arabidopsis, shown here, and rice, not shown) and fungi (A. nidulans, shown here, and A. fumigatus, not shown), it is currently not known whether they associate with γ-tubulin (with the exception of AtSp98p) (Erhardt et al., 2002).

—, Orthologs not present; ?, insufficient information available to establish the presence or absence of an ortholog. *, essential genes; †Oegema et al., 1999; ‡Moritz et al., 1995b; §Zheng et al., 1995; ¶Stearns and Kirschner, 1994; **Murphy et al., 2001; ††Erhardt et al., 2002; ††Geissler et al., 1996; Knop et al., 1997; Knop and Schiebel, 1997; †††Vin et al., 2002; ‡‡Venkatram et al., 2004; ‡‡‡Akashi et al., 1997.

††Three splice isoforms of Dgrip84 are reported in the NCBI database and differ by the presence or absence of a C-terminal 33 amino acid insertion or an N-terminal 74 amino acid extension (C.W., unpublished). †‡Subunits identified in BLAST searches for *Xenopus* homologs of γTuRC subunits (L. Liu and C.W., unpublished). ‡‡‡Subunits identified as homologs to the *S. pombe* γ-TuC subunits in a BLAST search (number of amino acids, predicted molecular mass, GenBank accession numbers are indicated, respectively). Their functions or membership in γ-tubulin complexes has not been tested experimentally. ***Subunits identified by crosslinking to γ-tubulin; the molecular identity of these proteins is not known. ****Dauberer and Gräf, 2002; †††Hannak et al., 2001.
(Keating and Borsy, 2000; Moritz et al., 2000; Wiese and Zheng, 2000; Zhang et al., 2000; Zheng et al., 1995); and (4) all of the subunits of the γTuRC are found at the centrosome. Since the complexes purified from cell lysates represent a soluble pool, γTuRC might assemble in the cytosol and be recruited to the centrosome as a ring complex in animal cells (Wiese and Zheng, 1999). This view has recently been called into question following the finding that the Drosophila γTuSC can associate with the centrosome in the absence of the cap subunits, albeit at reduced levels (Vérollet et al., 2006). Additional studies are therefore necessary to define the roles of the γTuSC and the γTuRC in animal cells. However, it is tempting to speculate that distinct γ-tubulin complexes might be responsible for MT nucleation from distinct nucleation sites in Drosophila. This hypothesis is supported by the finding that certain mutations in γ-tubulin or Dgrip75 affect some but not all MTs in Drosophila oocytes (Schnorrer et al., 2002).

Although γ-tubulin subcomplexes consisting of γ-tubulin and two grip-motif-containing proteins (Spc98p-Spc97p or Dgrip91-Dgrip84) occur in fungi and Drosophila, they are not ubiquitous. For example, Dictostelium γ-tubulin exists in a complex with DdSpc98 that does not contain DdSpc97 (Dauneder and Gräf, 2002). Similarly, the nematode genome encodes only one Grip-motif-containing protein, Ce-Grip-1, which is most closely related to Spc97p/GCP2 (Hannak et al., 2002). Ce-Grip-1 localizes to centrosomes and its disruption leads to MT disorganization. We do not yet know whether the C. elegans γ-tubulin exists in a complex and, if so, whether this complex includes Ce-Grip-1.

Two additional, uncharacterized grip-motif-containing proteins (Dgrip79 and Dgrip225) are present in the Drosophila genome (Gunawardane, 2003; Murphy et al., 2001). Sequence alignments of the Drosophila Grips show that Dgrip79 is a member of this family and that it is most closely related to Dgrip84/GCP2 (Fig. 5). Whether Dgrip79 or Dgrip225 are subunits of the γTuRC has not been established. However, database searching reveals that all of the 11 expressed sequence tag (EST) entries currently available in the NCBI databases for Dgrip79 are from testis cDNA libraries (C.W., unpublished data); Dgrip79 might therefore serve a tissue-specific role. It remains to be seen whether this role also involves other components of the γTuRC.

The recruitment of γ-tubulin complexes to MT-nucleating sites

γ-tubulin is recruited to MT-nucleating sites from a soluble pool

Fig. 5. Sequence alignments of five known grip-motif-containing Drosophila γTuRC subunits (Dgrip75, Dgrip84, Dgrip91, Dgrip128 and Dgrip163) with Dgrip79, a protein identified in database searches on the basis of the presence of the grip motifs; it is not yet known whether Dgrip79 is a component of the γTuRC. (A) An overall sequence alignment shows two regions of higher sequence similarity (orange), labeled ‘grip motif 1’ and ‘grip motif 2’, shared among the six grip-domain proteins. (Please note that the sequence of Dgrip163 was truncated except in regions of homology to the other grip proteins.) The number of amino acids for each of the proteins is indicated to the right of the alignments. (B,C) Sequence alignments of (B) grip motif 1 or (C) grip motif 2. Starting and ending amino acid numbers, respectively, are listed at the left and the right of the alignment for each protein. Shading indicates the degree of sequence conservation (see key). Sequence alignments were generated using the ‘T-coffee’ algorithm (Notredame et al., 2000; Poirot et al., 2004).
cytoplasmic pool (Fig. 6). Time-lapse fluorescence microscopy with green fluorescent protein (GFP)-labeled γ-tubulin showed that a fraction of the γ-tubulin at the centrosome exchanges freely with the cytoplasmic pool during interphase (the non-exchangeable fraction of γ-tubulin is presumed to be associated with the centrioles) (Khodjakov and Rieder, 1999). γ-tubulin recruitment also takes place during centrosome maturation, a process in which both the size and the nucleation capacity of the centrosome increase several-fold at the onset of mitosis (Ducat and Zheng, 2004). In addition, γ-tubulin associates with spindle MTs in animal cells (Khodjakov and Rieder, 1999; Lajoie-Mazenc et al., 1994). This suggests that γ-tubulin is bound to the ends of those spindle MTs that are not anchored in the centrosome (Lajoie-Mazenc et al., 1994). Alternatively, this could indicate that γ-tubulin can bind to the sides of preexisting MTs during mitosis in animal cells, which would be analogous to the situation in plants and fungi (Drykova et al., 2003; Janson et al., 2005). Mitotic changes in MT nucleation also take place in yeasts, which undergo closed mitosis. γ-tubulin localizes to the cytoplasmic face of the SPB throughout the cell cycle, but needs to be recruited to the nuclear face of the SPB during mitosis to mediate formation of spindle MTs inside the nuclear envelope (Fig. 3) (Knop and Schiebel, 1997; Knop and Schiebel, 1998).

The recruitment of γ-tubulin complexes in yeasts
Two budding yeast SPB proteins, Spc110p and Spc72p, tether γ-tubulin complexes to the inner and outer plaques of the SPB to mediate the nucleation of nuclear and cytoplasmic MT arrays, respectively (Knop and Schiebel, 1997; Knop and Schiebel, 1998). The interaction between Spc110p and the Tub4p complex is mediated by Spc98p/GCP3 and is regulated by phosphorylation of Spc98p (Knop and Schiebel, 1997).

Two potential orthologs of Spc110p, Pcp1p and Mto1p, exist in S. pombe and both have been implicated in γ-TuC tethering (Sawin et al., 2004; Venkatram et al., 2004). Mto1p appears to recruit γ-TuCs to all cytoplasmic MT nucleation sites including the outer plaque of the fission yeast SPB (Sawin et al., 2004; Venkatram et al., 2004). The finding that pcp1 is an essential gene (Flory et al., 2002) leads us to speculate that Pcp1p is involved in recruiting γ-tubulin to the nuclear side of the SPB, but no experimental evidence exists to support this hypothesis. Indeed, it is currently not known how the γ-TuC is tethered on the nuclear side of the SPB in S. pombe.

The recruitment and regulation of cytoplasmic γ-tubulin complexes are more complex in fission yeast compared with budding yeast, reflecting more complex MT arrays. Whereas the SPB of budding yeast is responsible for all MT nucleation and organization throughout the cell cycle, several iMTOCs and the eMTOC also contribute to the nucleation and organization of MT arrays in fission yeast (Hagan and Petersen, 2000). The iMTOC is a specialized MTOC that may be analogous to the midbody in animal cells. It is assembled on the cytokinetic ring during late anaphase, where it nucleates the post-anaphase MT array. Following septation, the eMTOC disassembles through the activity of the Hsp70-based chaperone system when γ-TuCs and other components of the eMTOC are redistributed to form several iMTOCs that are located on the surface of the nucleus and along MTs (Zimmerman et al., 2004). iMTOCs nucleate cytoplasmic MTs that are highly bundled and run along the long axis of the cell (Hagan and Petersen, 2000). Recently, the Mto1p-interacting protein Mto2p has been shown to cooperate with Mto1p to regulate the interphase MT array

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**Fig. 6.** Schematic diagrams of γTuRC recruitment to interphase centrosomes, mitotic centrosomes during centrosome maturation, and to mitotic spindle MTs. (A) During interphase, centrosomal γ-tubulin is exchanged with the cytoplasmic pool. (B) Additional γTuRC is recruited to centrosomes as cells enter mitosis in a process called centrosome maturation. (C) During mitosis, γTuRC is recruited to centrosomes as well as to spindle MTs (indicated by arrows; notice that γTuRC is not drawn to scale). How γ-tubulin is recruited to different sites and in a cell-cycle-dependent manner is only beginning to emerge. The mechanisms of γTuRC recruitment to centrosomes during interphase and mitosis are probably mediated by distinct sets of proteins (e.g. Casenghi et al., 2003).
by mediating the interaction between γ-TuRC and MTs (Janson et al., 2005; Samejima et al., 2005; Venkatram et al., 2005).

The targeting of γ-tubulin complexes in Drosophila
Despite the conservation of γTuRC components, γ-tubulin may be targeted to the centrosome by different mechanisms in different organisms. This is indicated by the finding that the Drosophila γTuRC cap subunits are dispensable for γTuRC recruitment: when the levels of all four cap subunits of the Drosophila γTuRC (Dgrip163, Dgrip128, Dgrip75 and Dgp71WD) are simultaneously reduced in S2 cells by RNAi, the remaining γTuSC components are still recruited to the centrosome, albeit at reduced levels (Vérollet et al., 2006). In fact, the mitotic defects in the RNAi-treated cells are surprisingly mild. Clearly, retention of centrosome function could be a result of efficient recruitment of residual γTuRC that remains after RNAi, but an obvious interpretation of this result is that γTuSC can be recruited to centrosomes on its own. The observation that mutant flies that lack Dgrip163, Dgrip75 or Dgp71WD reach adulthood despite appreciable cell-division defects supports the latter notion (Vérollet et al., 2006).

Although Dgrip163, Dgrip128, Dgrip75 and Dgp71WD may not be required for the localization of γ-tubulin to centrosomes, they are required for their binding along the MTs of the mitotic spindle (Fig. 6) and for its recruitment to the midbody (Vérollet et al., 2006). Thus, the recruitment of γ-tubulin-containing complexes in Drosophila is similar to that in fission yeast. In both organisms, the small γ-tubulin complex appears to be sufficient for centrosome binding. One interesting difference is that Gh1p, Alp16p and Mto1p localize to the SPB independently of Alp4 and Alp6p (Venkatram et al., 2004), whereas centrosomal targeting of the Drosophila cap subunits requires the integrity of the γTuRC (Vérollet et al., 2006).

The recruitment of γTuRC in vertebrates
Recruitment of the γTuRC to centrosomes in vertebrates appears to differ in important ways from that in yeast and flies. Analysis of the human Dgp71WD ortholog GCP-WD (Lüders et al., 2005), also known as Nedd1 (Haren et al., 2006), revealed that recruitment of the γTuRC to centrosomes and spindle poles is abolished in cultured human cells depleted of GCP-WD by RNAi. This suggests that GCP-WD is required for correct targeting of the γTuRC. However, these experiments need to be interpreted with caution, because depletion of GCP-WD also affects centriole integrity (Haren et al., 2006). Because PCM assembly (and hence γTuRC recruitment) requires centrioles (Leidel and Gönczy, 2005), the failure to observe an association of the γTuRC with centrosomes in GCP-WD-depleted cells could reflect a defect in centriole function that leads to failure to organize the PCM properly, rather than a direct effect on γTuRC recruitment. Interestingly, both the association of the γTuRC with spindle MTs and spindle assembly depend on phosphorylation of GCP-WD, whereas its recruitment to the centrosome is independent of GCP-WD phosphorylation (Lüders et al., 2005).

A careful analysis of the requirement for the other cap subunits of the γTuRC in human cells has not been reported, but studies using Xenopus egg extracts suggest that the largest cap subunit, Xgrip210 (the Xenopus Dgrip163 ortholog), is required for both γTuRC assembly and the recruitment of γ-tubulin to centrosomes assembled from sperm centrioles (Zhang et al., 2000). Therefore, although vertebrate and invertebrate γTuRCs share many similarities, the mechanism of recruitment to centrosomes, and the role of the cap subunits in γTuRC function, may be divergent.

The structural basis of MT nucleation mediated by γ-tubulin complexes
Models for γTuRC-mediated MT nucleation
Two models attempt to explain how the γTuRC might be involved in MT nucleation (Fig. 7). As its name suggests, the ‘template model’ postulates that the γTuRC serves as a template for the assembly of tubulin subunits (Zheng et al., 1995). In this model, adjacent γ-tubulin subunits interact laterally with each other in the ring. By contrast, the ‘protofilament model’ postulates a perpendicular arrangement of γ-tubulin subunits. Consequently, γ-tubulin subunits contact their neighbors through head-to-tail interactions (Fig. 7) (Erickson and Stoffler, 1996).

Within the recently obtained crystals of human γ-tubulin, adjacent subunits make contacts that resemble the lateral interactions between neighboring αβ-tubulins in the MT lattice (Aldaz et al., 2005). This finding supports the notion that γ-tubulins assume shoulder-to-shoulder contacts in the ring complex, as predicted by the template model. Further support for this model comes from the finding that the GTP-binding pocket of γ-tubulin in the γTuRC is accessible for nucleotide exchange. The GTP-binding pockets of all but the terminal γ-tubulin subunit would be covered and thus presumably unable to exchange nucleotide if γ-tubulin were arranged as proposed in the protofilament model. Keep in mind, however, that although the template model enjoys wide support in the field,
definitive experimental evidence to support or refute either model is still lacking.

Guanine nucleotides, γ-tubulin, and MT nucleation

The tubulin superfamily comprises a unique class of guanine-nucleotide-binding proteins. α- and β-tubulin each bind one molecule of GTP, but α-tubulin lacks the ability to hydrolyze its GTP. Furthermore, the opening of the GTP-binding pocket on α-tubulin is covered by β-tubulin in the dimer (Nogales et al., 1999), which renders the GTP on α-tubulin non-exchangeable. Consequently, only the GTP on β-tubulin can undergo exchange and hydrolysis during MT assembly.

Structural studies suggest that β-tubulins exist in distinct conformations, depending on the bound guanine nucleotide (Wang and Nogales, 2005). Whereas GTP-bound β-tubulin makes relatively straight inter- and intra-αβ-tubulin contacts within the tubulin protofilament, GDP binding induces kinks in both the intra- and inter-dimer contacts. These kinks are thought to facilitate MT disassembly through the protofilament peeling that is observed during MT depolymerization (Fig. 2) (Mandelkow et al., 1991; Nogales and Wang, 2006). Comparisons of the structural differences between GDP-bound and GTP-bound αβ-tubulins provide evidence that binding of GTP to β-tubulin straightens both intra- and inter-tubulin dimer contacts (Wang and Nogales, 2005). This explains why only GTP-bound αβ-tubulin can be incorporated into growing MTs.

How does guanine nucleotide binding regulate γ-tubulin-mediated MT nucleation? Monomeric γ-tubulin binds to both GDP and GTP, as does γ-tubulin within the context of the γTuRC or the γTuSC. Interestingly, γ-tubulin appears to prefer GDP over GTP within the Drosophila γTuSC (Oegema et al., 1999), whereas purified monomeric human γ-tubulin exhibits similar affinities for both GDP and GTP (Aldaz et al., 2005). The reason for this observed difference is unknown, but the recently solved crystal structure of monomeric γ-tubulin bound to GTP provides a glimpse into how GTP/GDP binding might affect the MT-nucleating activity of γ-tubulin (Aldaz et al., 2005). Within the crystal, GTP-γ-tubulin assumes a curved conformation (Aldaz et al., 2005) similar to that seen in unpolymerized GDP-bound tubulin (Ravelli et al., 2004) and in GTP-bound tubulin in curved protofilaments (Wang and Nogales, 2005). If we assume that the interface between γ-tubulin and αβ-tubulin resembles that between αβ-tubulin dimers, it is possible that bent (GTP-bound) γ-tubulin interacts with αβ-tubulin dimers at an angle that disfavors subsequent lateral interactions. Incorporation of γ-tubulin into γ-tubulin complexes, or binding of γ-tubulin to GDP (or both), might induce the necessary conformational straightening to favor MT nucleation. It will be important to determine how GDP-bound monomeric γ-tubulin as well as γ-tubulin that is associated with its partner proteins in the γ-tubulin complexes, which should further our understanding of how guanine nucleotides on γ-tubulin regulate MT nucleation.

Other MT nucleators

Perturbing γ-tubulin function results in misorganized mitotic spindles and formation of fewer mitotic MTs in all cell types examined, but in many cases MT assembly is not completely eliminated under these conditions. This suggests that other proteins can act as MT nucleators. Recent work has implicated members of three types of MT-associated proteins in MT nucleation: TACCs (transforming acidic coiled coil proteins), TOGs (tumor overexpressed genes) and TPX2 (targeting protein for the Xenopus kinesin-like protein 2). The exact molecular mechanisms of how they promote MTs assembly is not known, but probably differ from γ-tubulin-mediated MT nucleation (Srayko et al., 2005).

Microtubule nucleation from centrosomes requires a protein complex containing TACC and TOG proteins. The founding member of the TOG family, the Xenopus microtubule-associated protein 215 (XMAP215), was first isolated almost 20 years ago from Xenopus egg extracts as a protein that can modulate MT dynamics in vitro (Gard and Kirschner, 1987). Orthologs of XMAP215 have subsequently been found in evolutionarily distant species from fungi and invertebrates to humans (Gard et al., 2004). All XMAP215/TOG family members localize to MTOCs and have been implicated in regulation of MT nucleation and elongation from MTOCs. Two types of activity have been described for TOG proteins that could explain their MT-stabilizing properties. First, TOGs promote the formation of long MTs by antagonizing the activity of the MT depolymerase, Xenopus catastrophe modulator 1 (XXCM1; also known as MCAK, which stands for mitotic centromere-associated kinesin) (Tournebize et al., 2000). For example, disrupting TOG proteins in Drosophila, C. elegans or Xenopus leads to disorganized spindles and clusters of short MTs, and this phenotype can be rescued by simultaneous disruption of MCAK and TOG (Cullen et al., 1999; Kemphues et al., 1986; Matthews et al., 1998; Tournebize et al., 2000). Second, human and yeast TOG proteins bind not only to MTs but also to tubulin dimers (Al-Bassam et al., 2006; Spittle et al., 2000), and Stu2p has been proposed to facilitate MT growth by delivering tubulin subunits to the MT plus ends (Al-Bassam et al., 2006).

Although TOGs bind directly to MTs in vitro, their efficient association with centrosomes in vivo appears to require the activity of the TACC family of proteins (Lee et al., 2001; Cullen and Ohkura, 2001). This highly conserved family is defined by a ~200 amino acid C-terminal coiled-coil domain (Gergely et al., 2000a; Gergely et al., 2000b; Raff, 2002) and an overall acidic pl (Still et al., 1999). A number of studies suggest that the TACC and TOG proteins coordinate with each other and with the γTuRC to regulate MT assembly. For example, depletion of the Xenopus TACC results in poorly functional centrosomes despite the efficient recruitment of γ-tubulin to them (O’Brien et al., 2005). Xenopus TACC regulates the localization and enhances the activity of XMAP215 (Kinoshita et al., 2005; Peset et al., 2005). In Xenopus egg extracts, XMAP215 promotes centrosome-mediated MT nucleation, presumably by stabilizing γTuRC-nucleated MTs (Popov et al., 2002). In C. elegans embryos, γ-tubulin is required for localization of TAC-1 (the nematode TACC ortholog) to centrosomes to regulate MT growth in conjunction with ZYG-9, the C. elegans TOG (Bellanger and Gönczy, 2003; Le Bot et al., 2003; Srayko et al., 2003). The TACC-like protein in budding yeast (Still et al., 2004), Spc72p, brings both the γ-tubulin complex (Knop and Schiebel, 1998) and Stu2p (yeast TOG) (Chen et al., 1998) to the SPB to regulate astral MT anchorage and dynamics.
mitotic chromosomes. In addition to centrosome-initiated MTs that are captured and stabilized by kinetochores, MTs are nucleated in random orientations near the chromosomes and are sorted into bipolar arrays by the action of MT motors (reviewed by Gadde and Heald, 2004). TPX2 was first described as a MT-loading factor for the mitotic Xenopus protein XKip2 (Wittmann et al., 2000). Although it is not required for MT nucleation by the centrosome, TPX2 was found to be essential for mitotic spindle assembly, functioning as a crucial regulator of MT assembly in the vicinity of mitotic chromosomes (Gruss et al., 2001; Gruss et al., 2002; Schatz et al., 2003). Depletion of TPX2 from Xenopus egg extracts results in poorly formed spindles when centrosomes are present, but in the absence of TPX2 formation of MTs is completely abolished when centrosomes are not available. The potential role for TPX2 in MT nucleation is underscored by the observation that a complex containing both TPX2 and γ-tubulin is underscored by the observation that a complex containing both TPX2 and γ-tubulin has been isolated from Xenopus egg extracts (Groen et al., 2004).

Although it has proven difficult to define the precise mechanism underlying the role of TPX2 in spindle assembly, TPX2 might impact MT assembly in more ways than one: TPX2 functions as an activator for the mitotic kinase Aurora-A and is required for its targeting to the spindle pole (Kufer et al., 2002; Tsai et al., 2003; Trieselman et al., 2003; Eyers et al., 2003; Özlü et al., 2005). Aurora-A kinase itself plays multiple roles in mitosis. For example, Aurora-A kinase regulates MT nucleation and bipolar spindle assembly (Giet and Prigent, 2000; Liu and Ruderman, 2006; Tsai et al., 2003; Tsai and Zheng, 2005) and is required for centrosome maturation (Hannak et al., 2002). Substrates of Aurora-A kinase include TPX2 and TACC proteins (Kufer et al., 2002; Wittmann et al., 2000; Peset et al., 2005; Kinoshita et al., 2005; Barros et al., 2005).

Conclusions and perspectives

The discovery of γ-tubulin and its associated proteins has significantly advanced our understanding of MTOC function in different organisms. Yet, the picture that is beginning to emerge suggests that, despite their considerable conservation, γ-tubulin complexes might be regulated differently in different organisms. This may reflect differences in the complexity of MT arrays and underscores the importance of studying γ-tubulin complexes in a variety of organisms.

Despite significant advances over the past few years, many important questions remain. For example, the contacts made between γ-tubulin and α/β-tubulin remain to be defined. Our knowledge of the arrangement of subunits within γ-tubulin complexes, their assembly and their interactions with MTOCs remains largely limited. The contributions of the Grips to the mechanism of microtubule nucleation remain equally enigmatic. Similarly, the contributions of other microtubule-binding proteins, such as TPX2 and TACC complexes, to microtubule nucleation remain to be determined in molecular detail. Another major advance has been the appreciation that Aurora-A kinase is an important regulator of MT nucleation and organization. The major challenge ahead is to decipher how different MT nucleators work together to generate different MT arrays in interphase and mitosis.

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