

γ -Tubulin complexes in microtubule nucleation and beyond

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ABSTRACT Tremendous progress has been made in understanding the functions of γ -tubulin and, in particular, its role in microtubule nucleation since the publication of its discovery in 1989. The structure of γ -tubulin has been determined, and the components of γ -tubulin complexes have been identified. Significant progress in understanding the structure of the γ -tubulin ring complex and its components has led to a persuasive model for how these complexes nucleate microtubule assembly. At the same time, data have accumulated that γ -tubulin has important but less well understood functions that are not simply a consequence of its function in microtubule nucleation. These include roles in the regulation of plus-end microtubule dynamics, gene regulation, and mitotic and cell cycle regulation. Finally, evidence is emerging that γ -tubulin mutations or alterations of γ -tubulin expression play an important role in certain types of cancer and in other diseases.

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INTRODUCTION

For many years, the identity of components of microtubule-organizing centers (MTOCs) that nucleate microtubule assembly and establish microtubule polarity was a central unanswered question in the field of mitosis and the cytoskeleton. The discovery of γ -tubulin (Oakley and Oakley, 1989), the key finding that allowed this question to be answered, came from a genetic screen in the fungus *Aspergillus nidulans* designed to identify genes important for microtubule function (Weil *et al.*, 1986). The existence of members of the tubulin superfamily beyond the microtubule proteins α - and β -tubulin was completely unexpected at the time, and the discovery of γ -tubulin is a powerful validation of the value of forward genetics, in which well-designed screens let the cell tell us what is important rather than us imposing our preconceived notions on the cell.

γ -TUBULIN COMPLEXES NUCLEATE MICROTUBULE ASSEMBLY FROM MICROTUBULE-ORGANIZING CENTERS

γ -Tubulin is ubiquitous in eukaryotes, and genome-sequencing projects have revealed that there are one to three γ -tubulin genes in eukaryotic genomes (Findeisen *et al.*, 2014). It localizes to structurally diverse MTOCs and, with few exceptions, is required for microtubule nucleation (reviewed by Job *et al.*, 2003). Ring-shaped structures called γ TuRCs (γ -tubulin ring complexes) that contain γ -tubulin and associated proteins nucleate microtubule assembly in vitro (Zheng *et al.*, 1995) and in vivo (Figure 1, A–D) (reviewed by Teixido-Travesa *et al.*, 2010; Kollman *et al.*, 2011; Lin *et al.*, 2014). Proteins that associate with γ -tubulin have been identified and designated GRIPs (γ -tubulin ring proteins) or GCPs (γ -tubulin complex proteins) (Figure 1D). Five of these proteins, GCP2–6, are structurally related and contain conserved regions called GRIP motifs (Gunawardane *et al.*, 2000; Guillet *et al.*, 2011).

Beyond GCP2–6, γ TuRCs contain three additional, more recently discovered, core subunit proteins, MOZART1 (mitotic spindle-organizing protein associated with a ring of γ -tubulin, or GCP9) and MOZART2A and MOZART2B (GCP8A and GCP8B), which are closely related to each other (Hutchins *et al.*, 2010). MOZART1 is highly conserved in eukaryotes, except in *Saccharomyces cerevisiae* and close relatives (Hutchins *et al.*, 2010; Lin *et al.*, 2014), and it is essential for mitotic spindle assembly and function in humans and *Schizosaccharomyces pombe* (Hutchins *et al.*, 2010; Masuda *et al.*, 2013). *Arabidopsis thaliana* has two MOZART1 homologues, Gip1

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Abbreviations used: γ TuRC, γ -tubulin ring complex; γ TuSC, γ -tubulin small complex; APC/C, anaphase-promoting complex/cyclosome; GCP, γ -tubulin complex protein; GRIP, γ -tubulin ring protein; MTOC, microtubule organizing center; RNAi, RNA interference; SAC, spindle assembly checkpoint; SPB, spindle pole body.

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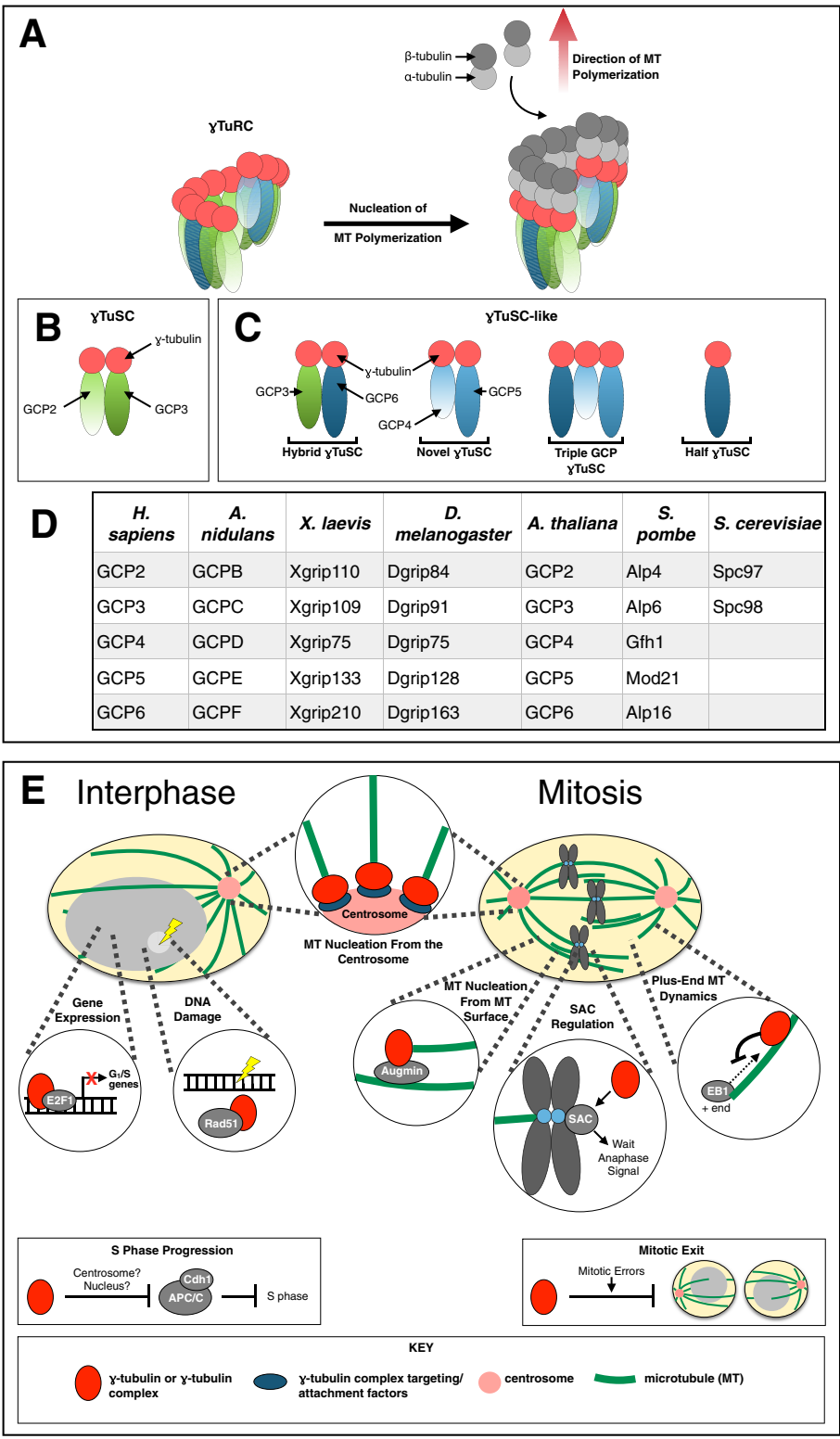


FIGURE 1: γ -Tubulin or γ -tubulin complexes in microtubule nucleation and beyond. (A) Nucleation of microtubule assembly by a γ TuRC. The γ TuRC consists of GCPs that bind to γ -tubulin and to each other, forming a complex in which a ring of γ -tubulin molecules effectively mimics the plus end of a microtubule. It forms a pre-existing nucleus for microtubule assembly. At physiological tubulin concentrations, spontaneous assembly of tubulin into microtubules is rare. Instead, almost all microtubule growth occurs from preformed nucleating structures, principally the γ TuRC. (B) In *S. cerevisiae* only two GCPs exist, Spc97 and Spc98. They assemble with γ -tubulin to form γ TuSCs. (C) The finding that GCP2–6 all bind to γ -tubulin raises the possibility that GCPs and γ -tubulin may assemble into alternative γ TuSC-like structures (Kollman

and Gip2, that interact with GCP3. They are not essential individually, but Gip1/2 double mutants are embryonic lethal (Nakamura *et al.*, 2012). MOZART2A and MOZART2B are apparently restricted to vertebrates, in which they play a role in recruitment of the γ TuRC to the centrosome (Teixido-Travesa *et al.*, 2010; Lin *et al.*, 2014). In addition to the core γ TuRC components, various phyla have proteins, unrelated in sequence to GCP2–9, that are involved in targeting γ -tubulin complexes to MTOCs (reviewed extensively by Lin *et al.*, 2014).

THE STRUCTURE OF THE γ TuRC SUGGESTS A MECHANISM FOR MICROTUBULE NUCLEATION

High-resolution structures of human γ -tubulin (Aldaz *et al.*, 2005; Rice *et al.*, 2008) and GCP4 (Guillet *et al.*, 2011) and lower-resolution structures of small γ -tubulin complexes (γ TuSCs, containing two molecules of γ -tubulin and one molecule each of GCP2 and 3) from *S. cerevisiae* (Kollman *et al.*, 2008, 2010) have been determined, and these and other findings have led to a persuasive model for the structure of the γ TuRC (Kollman *et al.*, 2011). In this model, GCP2–6 each binds directly to γ -tubulin and assemble into a structure that contains 13 γ -tubulin molecules (perhaps containing five γ TuSCs and one molecule each of GCP4–6 bound to γ -tubulin; Figure 1A). GCP3 contains a hinge region, and in the current model, movement about this hinge would alter the positioning of γ -tubulin molecules. In one position, the γ -tubulin molecules would be too far apart to nucleate microtubule assembly, but in the other position, the straight position, they would have exactly the same spacing as microtubule protofilaments and would form a perfect template for microtubule assembly.

et al., 2011) that assemble along with γ TuSCs to form the γ TuRC. (D) Designations for GCPs in various organisms in which they have been studied extensively. (E) Functions of γ -tubulin. γ -Tubulin complexes nucleate microtubules from the centrosome and other MTOCs in both interphase and mitosis or from microtubule surfaces in mitosis. In interphase and mitosis, γ -tubulin has been shown to regulate microtubule plus-end dynamics. In mitosis, it plays a role in the spindle assembly checkpoint (SAC) and the control of mitotic exit. In interphase, γ -tubulin plays an important role in inhibiting APC/C^{Cdh1}, thereby promoting the transition from the G₁ to the S phase. It also appears to regulate E2F1-mediated gene expression and complexes with Rad51, suggesting a role in the Rad51-mediated DNA damage response.

Control of the conformation of GCP3 could therefore regulate the ability of the γ TuRC to nucleate microtubule assembly. Important unanswered questions in this area are the exact arrangement of GCP2–6 in the γ TuRC and the positioning of MOZART1, 2A, and 2B in the complex. The hinge model, although persuasive, needs to be tested rigorously, and if it is correct, it raises the question of what controls the rotation of GCP3 around its hinge.

γ -TUBULIN COMPLEXES NUCLEATE MICROTUBULES FROM THE SIDES OF EXISTING MICROTUBULES

Although microtubule nucleation from MTOCs is extremely important, in many phyla MTOCs are not apparent. Even in vertebrate mitosis, centrosomes are not always required for the formation of a functional spindle (Khodjakov *et al.*, 2000), and when centrosomes are present, many microtubules may be nucleated independent of the centrosome. Similarly MTOC-independent nucleation of cytoplasmic microtubules occurs in many phyla.

Murata *et al.* (2005) showed convincingly that cortical microtubules in higher plant cells are nucleated from the sides of existing microtubules at a characteristic angle of 42° with respect to existing microtubules, that γ -tubulin is at the branch points, and that lateral microtubule nucleation is γ -tubulin dependent. Similarly, Janson *et al.* (2005) demonstrated that microtubules are nucleated from γ -tubulin complexes at the sides of cytoplasmic microtubules in *S. pombe*. These data established the principle that γ -tubulin complexes function in nucleation of microtubules from the sides of existing microtubules. Data obtained over the intervening decade have identified the augmin complex as a key player in lateral microtubule nucleation.

Augmin is an eight-subunit complex that is important for localizing γ -tubulin to mitotic spindles and for centrosome-independent microtubule generation in mitotic and meiotic spindles (Goshima *et al.*, 2008; Ho *et al.*, 2011; Petry *et al.*, 2011; Hsia *et al.*, 2014). In higher plants, augmin colocalizes with GCPs, and they are corecruited to cortical microtubules. Knockdown of augmin with a microRNA reduces branching lateral nucleation of microtubules (Liu *et al.*, 2014). In *Xenopus* extracts, augmin and γ TuRCs along with the microtubule assembly factor TPX2 and the GTP-bound RanGTPase nucleate microtubule assembly, forming fan-like structures consistent with the possibility that the microtubules are in branched arrays (Petry *et al.*, 2013). Microtubule nucleation persists if augmin or TPX2 are depleted, but fan-like arrays do not form. Monitoring the plus-end microtubule-tracking protein EB-1 revealed that new microtubules are nucleated from the sides of existing microtubules and that the newly nucleated microtubules have the same polarity as the microtubules from which they are nucleated. Such lateral nucleation of microtubule assembly in a mitotic apparatus would result in the proliferation of spindle microtubules but, importantly, would not disrupt the polarity of microtubules in the spindle. These and related findings support a persuasive although not completely proven model that the augmin complex binds to microtubule walls and recruits the γ TuRC through NEDD1 or other proteins to initiate new microtubule nucleation (Uehara *et al.*, 2009; Zhu *et al.*, 2009; Johmura *et al.*, 2011). The fact that augmin apparently plays a role in lateral microtubule nucleation in both plants and animal suggests that augmin-mediated lateral microtubule nucleation is widespread in eukaryotes. However, many organisms do not have homologues of all augmin subunits (Lin *et al.*, 2014), and the fact that the polarity of microtubules nucleated from the sides of microtubules in *S. pombe* is opposite to that of augmin-mediated nucleation (Janson *et al.*, 2005) suggests there are at least two lateral nucleation mechanisms.

The theme that has emerged from two decades of research is that γ -tubulin complexes are almost universally involved in micro-

tubule nucleation. Mechanisms have evolved, however, to control and target these complexes, such that microtubule nucleation occurs when and where it is needed.

γ -TUBULIN BEYOND MICROTUBULE NUCLEATION

The traditional view has been that each protein has a single function, but increasingly, proteins have been shown to be multifunctional. In this regard, increasing evidence indicates that γ -tubulin has functions beyond microtubule nucleation (Cuschieri *et al.*, 2007). In many ways, this makes sense, because γ -tubulin is at the hub of the microtubule cytoskeleton and, along with other MTOC components, is in a unique position to receive and send signal molecules transported by microtubule motors. The problem with interpreting some of these data, however, is that, because γ -tubulin has a critical role in mitosis and disruption of mitosis has many consequences, it is difficult to distinguish putative non-microtubule-nucleation functions of γ -tubulin from consequences of mitotic failure or alteration of microtubule nucleation. Nevertheless, we believe that there is ample evidence that γ -tubulin has important functions beyond microtubule nucleation (summarized in Figure 1E).

γ -TUBULIN PLAYS A ROLE IN THE REGULATION OF MICROTUBULE PLUS-END DYNAMICS

There have been repeated reports from multiple phyla that mutations or deficiencies in γ -tubulin or GCPs alter plus-end microtubule dynamics (Paluh *et al.*, 2000; Vogel *et al.*, 2001; Zimmerman and Chang, 2005; Bouissou *et al.*, 2009) and that γ -tubulin alters the distribution of the plus end-tracking protein Bim1 in *S. cerevisiae* (Cuschieri *et al.*, 2006) and its homologue, EB1, in *Drosophila* (Bouissou *et al.*, 2014). This is surprising, since γ -tubulin is most obviously located at the minus end. Two non-mutually exclusive explanations have been proposed. Considering that there is constant bidirectional transport along microtubules, one idea is that γ -tubulin complexes at MTOCs could bind catastrophe or rescue factors or the motor molecules that transport them. This would facilitate loading of molecules that affect microtubule dynamics onto motor molecules, thereby affecting their transport to microtubule plus ends and, consequently, affecting microtubule dynamics at plus ends. Consistent with this idea, γ -tubulin mutations alter binding of motor proteins and/or microtubule stability factors (Vogel *et al.*, 2001; Zimmerman and Chang, 2005; Cuschieri *et al.*, 2006). For example, some γ -tubulin mutations alter the binding of kinesin-14 and kinesin-5 to γ -tubulin complexes, while others alter the interaction of cytoplasmic dynein with spindle pole bodies (SPBs; Li *et al.*, 2005; Rodriguez *et al.*, 2008; Olmsted *et al.*, 2014), although the binding of type 5 and 14 kinesins may be involved in regulating microtubule nucleation rather than plus-end microtubule dynamics (Olmsted *et al.*, 2014).

The second proposed explanation comes from work in *Drosophila*, in which γ TuRC proteins localize along astral microtubules and are proposed to act as rescue factors that arrest microtubule disassembly (Bouissou *et al.*, 2009, 2014). It is worth noting that the proposed rescue activity of γ TuRCs distal to MTOCs might not be intrinsic to the γ TuRC but a function of its binding to rescue factors, as discussed above. Another possibility that is unfortunately difficult to test is that γ -tubulin mutations alter the microtubule lattice and thereby alter microtubule dynamics.

γ -TUBULIN FUNCTIONS IN THE REGULATION OF MITOSIS AND THE CELL CYCLE

There have also been persuasive reports of perturbation of the regulation of mitotic exit by alterations of γ -tubulin or GCPs. Alanine-scanning mutants of human γ -tubulin expressed as the sole γ -tubulin

in *S. pombe* allowed cells to go through anaphase and cytokinesis when spindle formation was disrupted (Hendrickson *et al.*, 2001). Mutations in the GCP4 and GCP5 homologues of *S. pombe* caused a failure of mitotic arrest in the presence of the antimicrotubule agent thiabendazole (Vardy and Toda, 2000). Similarly, a γ -tubulin mutation in *A. nidulans* caused mitotic exit before successful completion of mitosis in a strain in which the establishment of spindle bipolarity was delayed by a type 14 kinesin deletion (Prigozhina *et al.*, 2001). Another allele caused a premature mitotic exit even when the microtubule cytoskeleton was completely disassembled with benomyl (Prigozhina *et al.*, 2004). Likewise, RNA interference (RNAi) of Dgrip84 (GCP2) in *Drosophila* caused an untimely mitotic exit in the presence of colchicine (Colombie *et al.*, 2006). Thus genetic or RNAi perturbations of γ -tubulin or GCPs in three organisms and four laboratories all point to a microtubule nucleation-independent role for γ -tubulin complexes in the control of mitotic exit.

A possibly related area in which results from several labs have indicated a non-microtubule nucleation function for γ -tubulin is coordination of mitotic events and/or the spindle assembly checkpoint (SAC). In a systematic alanine mutagenesis screen, a number of γ -tubulin mutants were shown to cause premature anaphase entry and defective cytokinesis, suggesting that γ -tubulin regulates the SAC and orderly mitotic exit (Hendrickson *et al.*, 2001). An *A. nidulans* γ -tubulin allele caused late mitotic events (chromosomal disjunction, spindle elongation, and mitotic exit) to become disordered (Prigozhina *et al.*, 2004). The failure of chromosomal disjunction in this instance was not due to lack of microtubule assembly or force generation, because chromosomes were often stretched dramatically in anaphase, even though they did not disjoin. With respect to mitotic regulation and the SAC, it is worth noting that there are reports that the SAC/mitotic regulatory proteins CDC20 and BubR1 are in a complex with γ -tubulin in mammalian cells (Muller *et al.*, 2006) and that Mad2 binds to Alp4p (GCP2) in *S. pombe* (Mayer *et al.*, 2006).

Finally, there is incontrovertible evidence that γ -tubulin plays a role in the regulation of cell cycle progression in interphase. In *A. nidulans*, a γ -tubulin allele, *mipAD159*, causes failure of inactivation of the anaphase-promoting complex/cyclosome (APC/C) at the G₁/S boundary (Nayak *et al.*, 2010). This failure correlates with failure of the APC/C activator Cdh1 to dissociate from the SPB, and it can be overridden by deletion of Cdh1 (Edgerton-Morgan and Oakley, 2012). Importantly, this failure occurs if microtubules are present or if they are completely depolymerized, so it is not a microtubule nucleation phenomenon (Nayak *et al.*, 2010). This allele also causes mislocalization of the SAC proteins Bub1/R1 (*A. nidulans* has a single gene with functional domains of Bub1 and BubR1) and Mps1, thereby abrogating the SAC (Edgerton *et al.*, 2015). Note also that analyses of an *alp4* (GCP2) mutation indicate Alp4 has an essential function in G₁ in *S. pombe* (Vardy and Toda, 2000). This mutation, moreover, can cause septation, even when mitosis is arrested, by allowing inappropriate recruitment of the Sid1 kinase to the SPB (Vardy *et al.*, 2002). There are also reports that γ -tubulin modulates the activity of E2F transcription factors, which control the expression of a number of genes that are necessary for DNA replication and centrosome duplication (Hoog *et al.*, 2011; Ehlen *et al.*, 2012). The key challenge in understanding the various nonnucleation functions of γ -tubulin will be to move from phenomenology to molecular mechanism.

γ -TUBULIN IN DISEASE

Although the functional significance is not yet clear, there is strong and intriguing evidence of the association of γ -tubulin with tumor

suppressor proteins, particularly those involved in DNA repair. It binds to Rad51 in nuclei in response to DNA damage (Lesca *et al.*, 2005) and coimmunoprecipitates with ATR, BRCA1, and C53 from nuclei (Zhang *et al.*, 2007; Hubert *et al.*, 2011; Horejsi *et al.*, 2012). BRCA1/BARD1 binds to and monoubiquitinates γ -tubulin, and there is evidence that this ubiquitination is important in the regulation of centrosome number (Hsu *et al.*, 2001; Starita *et al.*, 2004), a particularly interesting finding, since centrosome amplification is important to cancer progression.

γ -Tubulin expression or localization pattern is altered in a variety of cancers, including some multiple myelomas, non-small cell lung cancers (Maounis *et al.*, 2012; Dementyeva *et al.*, 2013), ductal hyperplasia and breast cancer (Niu *et al.*, 2009; Cho *et al.*, 2010), gliomas and glioblastoma cell lines (Katsetos *et al.*, 2006), and medulloblastomas and medulloblastoma cell lines (Caracciolo *et al.*, 2010). Spontaneous mutations in one of the two human γ -tubulin genes, *TUBG1*, are associated with lissencephaly and microcephaly (Poirier *et al.*, 2013; Bahi-Buisson *et al.*, 2014). Further effort devoted to studies of both the microtubule nucleation-dependent and nucleation-independent functions of γ -tubulin in different cell and tissue contexts should shed light on the relevance of this cytoskeleton protein in health and disease.

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REFERENCES

- Aldaz H, Rice LM, Stearns T, Agard DA (2005). Insights into microtubule nucleation from the crystal structure of human γ -tubulin. *Nature* 435, 523–527.
- Bahi-Buisson N, Poirier K, Fourniol F, Saillour Y, Valence S, Lebrun N, Hully M, Bianco CF, Boddaert N, Elie C, *et al.* (2014). The wide spectrum of tubulinopathies: what are the key features for the diagnosis? *Brain* 137, 1676–1700.
- Bouissou A, Verollet C, de Forges H, Haren L, Bellafche Y, Perez F, Merdes A, Raynaud-Messina B (2014). γ -Tubulin ring complexes and EB1 play antagonistic roles in microtubule dynamics and spindle positioning. *EMBO J* 33, 114–128.
- Bouissou A, Verollet C, Sousa A, Sampaio P, Wright M, Sunkel CE, Merdes A, Raynaud-Messina B (2009). γ -Tubulin ring complexes regulate microtubule plus end dynamics. *J Cell Biol* 187, 327–334.
- Caracciolo V, D'Agostino L, Dráberová E, Sládková V, Crozier-Fitzgerald C, Agamanolis DP, de Chadarévian JP, Legido A, Giordano A, Dráber P, Katsetos CD (2010). Differential expression and cellular distribution of γ -tubulin and β III-tubulin in medulloblastomas and human medulloblastoma cell lines. *J Cell Physiol* 223, 519–529.
- Cho EH, Whipple RA, Matrone MA, Balzer EM, Martin SS (2010). Delocalization of γ -tubulin due to increased solubility in human breast cancer cell lines. *Cancer Biol Ther* 9, 66–76.
- Colombie N, Verollet C, Sampaio P, Moisan A, Sunkel C, Bourbon HM, Wright M, Raynaud-Messina B (2006). The *Drosophila* γ -tubulin small complex subunit Dgrip84 is required for structural and functional integrity of the spindle apparatus. *Mol Biol Cell* 17, 272–282.
- Cuschieri L, Miller R, Vogel J (2006). γ -tubulin is required for proper recruitment and assembly of Kar9-Bim1 complexes in budding yeast. *Mol Biol Cell* 17, 4420–4434.
- Cuschieri L, Nguyen T, Vogel J (2007). Control at the cell center: the role of spindle poles in cytoskeletal organization and cell cycle regulation. *Cell Cycle* 6, 2788–2794.
- Dementyeva E, Kryukov F, Kubiczko L, Nemeč P, Sevcikova S, Ichnatova I, Jarkovsky J, Minarik J, Stefanikova Z, Kuglik P, Hajek R (2013). Clinical implication of centrosome amplification and expression of centrosomal functional genes in multiple myeloma. *J Transl Med* 11, 77.

- Edgerton H, Paolillo V, Oakley BR (2015). Spatial regulation of the spindle assembly checkpoint and anaphase-promoting complex in *Aspergillus nidulans*. *Mol Microbiol* 95, 442–457.
- Edgerton-Morgan H, Oakley BR (2012). γ -Tubulin plays a key role in inactivating APC/C^{dh1} at the G₁-S boundary. *J Cell Biol* 198, 785–791.
- Ehlen A, Rossello CA, von Stedingk K, Hoog G, Nilsson E, Pettersson HM, Jirstrom K, Alvarado-Kristensson M (2012). Tumors with nonfunctional retinoblastoma protein are killed by reduced γ -tubulin levels. *J Biol Chem* 287, 17241–17247.
- Findeisen P, Muhlhausen S, Dempewolf S, Hertzog J, Zietlow A, Carlomagno T, Kollman M (2014). Six subgroups and extensive recent duplications characterize the evolution of the eukaryotic tubulin protein family. *Genome Biol Evol* 6, 2274–2288.
- Goshima G, Mayer M, Zhang N, Stuurman N, Vale RD (2008). Augmin: a protein complex required for centrosome-independent microtubule generation within the spindle. *J Cell Biol* 181, 421–429.
- Guillet V, Knibiehler M, Gregory-Paaron L, Remy MH, Chemin C, Raynaud-Messina B, Bon C, Kollman JM, Agard DA, Merdes A, Mourey L (2011). Crystal structure of γ -tubulin complex protein GCP4 provides insight into microtubule nucleation. *Nat Struct Mol Biol* 18, 915–919.
- Gunawardane RN, Martin OC, Cao K, Zhang L, Dej K, Iwamatsu A, Zheng Y (2000). Characterization and reconstitution of *Drosophila* γ -tubulin ring complex subunits. *J Cell Biol* 151, 1513–1524.
- Hendrickson TW, Yao J, Bhadury S, Corbett AH, Joshi HC (2001). Conditional mutations in γ -tubulin reveal its involvement in chromosome segregation and cytokinesis. *Mol Biol Cell* 12, 2469–2481.
- Ho CM, Hotta T, Kong Z, Zeng CJ, Sun J, Lee YR (2011). Augmin plays a critical role in organizing the spindle and phragmoplast microtubule arrays in *Arabidopsis*. *Plant Cell* 23, 2606–2618.
- Hoog G, Zarrizi R, von Stedingk K, Jonsson K, Alvarado-Kristensson M (2011). Nuclear localization of γ -tubulin affects E2F transcriptional activity and S-phase progression. *FASEB J* 25, 3815–3827.
- Horejsi B, Vinopal S, Sladkova V, Draberova E, Sulimenko V, Sulimenko T, Vosecka V, Philimonenko A, Hozak P, Katsetos CD, Draber P (2012). Nuclear γ -tubulin associates with nucleoli and interacts with tumor suppressor protein C53. *J Cell Physiol* 127, 367–382.
- Hsia KC, Wilson-Kubalek EM, Dottore A, Hao Q, Tsai KL, Forth S, Shimamoto Y, Milligan RA, Kapoor TM (2014). Reconstitution of the augmin complex provides insights into its architecture and function. *Nat Cell Biol* 16, 852–863.
- Hsu LC, Doan TP, White RL (2001). Identification of a γ -tubulin-binding domain in BRCA1. *Cancer Res* 61, 7713–7718.
- Hubert T, Vandekerckhove J, Gettemans J (2011). Cdk1 and BRCA1 target γ -tubulin to microtubule domains. *Biochem Biophys Res Commun* 414, 240–245.
- Hutchins JR, Toyoda Y, Hegemann B, Poser I, Heriche JK, Sykora MM, Augsburg M, Hudecz O, Buschhorn BA, Bulkescher J, et al. (2010). Systematic analysis of human protein complexes identifies chromosome segregation proteins. *Science* 328, 593–599.
- Janson ME, Setty TG, Paoletti A, Tran PT (2005). Efficient formation of bipolar microtubule bundles requires microtubule-bound γ -tubulin complexes. *J Cell Biol* 169, 297–308.
- Job D, Valiron O, Oakley B (2003). Microtubule nucleation. *Curr Opin Cell Biol* 15, 111–117.
- Johmura Y, Soung NK, Park JE, Yu LR, Zhou M, Bang JK, Kim BY, Veenstra TD, Erikson RL, Lee KS (2011). Regulation of microtubule-based microtubule nucleation by mammalian polo-like kinase 1. *Proc Natl Acad Sci USA* 108, 11446–11451.
- Katsetos CD, Reddy G, Dráberová E, Smejkalová B, Del Valle L, Ashraf Q, Tadevosyan A, Yelin K, Maraziotis T, Mishra OP, et al. (2006). Altered cellular distribution and subcellular sorting of γ -tubulin in diffuse astrocytic gliomas and human glioblastoma cell lines. *J Neuropathol Exp Neurol* 65, 465–477.
- Khodjakov A, Cole RW, Oakley BR, Rieder CL (2000). Centrosome-independent mitotic spindle formation in vertebrates. *Curr Biol* 10, 59–67.
- Kollman JM, Merdes A, Mourey L, Agard DA (2011). Microtubule nucleation by γ -tubulin complexes. *Nat Rev Mol Cell Biol* 12, 709–721.
- Kollman JM, Polka JK, Zelter A, Davis TN, Agard DA (2010). Microtubule nucleating γ -TuSC assembles structures with 13-fold microtubule-like symmetry. *Nature* 466, 879–882.
- Kollman JM, Zelter A, Muller EG, Fox B, Rice LM, Davis TN, Agard DA (2008). The structure of the γ -tubulin small complex: implications of its architecture and flexibility for microtubule nucleation. *Mol Biol Cell* 19, 207–215.
- Lesca C, Germanier M, Raynaud-Messina B, Pichereaux C, Etievant C, Emond S, Bulet-Schiltz O, Monsarrat B, Wright M, Defais M (2005). DNA damage induce γ -tubulin-RAD51 nuclear complexes in mammalian cells. *Oncogene* 24, 5165–5172.
- Li S, Oakley CE, Chen G, Han X, Oakley BR, Xiang X (2005). Cytoplasmic dynein's mitotic spindle pole localization requires a functional anaphase-promoting complex, γ -tubulin, and NUDF/LIS1 in *Aspergillus nidulans*. *Mol Biol Cell* 16, 3591–3605.
- Lin TC, Neuner A, Schiebel E (2014). Targeting of γ -tubulin complexes to microtubule organizing centers: conservation and divergence. *Trends Cell Biol* 25, 296–307.
- Liu T, Tian J, Wang G, Yu Y, Wang C, Ma Y, Zhang X, Xia G, Liu B, Kong Z (2014). Augmin triggers microtubule-dependent microtubule nucleation in interphase plant cells. *Curr Biol* 24, 2708–2713.
- Maounis NF, Draberova E, Mahera E, Chorti M, Caracciolo V, Sulimenko T, Riga D, Trakas N, Emmanouilidou A, Giordano A, et al. (2012). Overexpression of γ -tubulin in non-small cell lung cancer. *Histol Histopathol* 27, 1183–1194.
- Masuda H, Mori R, Yukawa M, Toda T (2013). Fission yeast MOZART1/Mzt1 is an essential γ -tubulin complex component required for complex recruitment to the microtubule organizing center, but not its assembly. *Mol Biol Cell* 24, 2894–2906.
- Mayer C, Filopei J, Batac J, Alford L, Paluh JL (2006). An extended anaphase signaling pathway for Mad2p includes microtubule organizing center proteins and multiple motor-dependent transitions. *Cell Cycle* 5, 1456–1463.
- Muller H, Fogeron ML, Lehmann V, Lehrach H, Lange BM (2006). A centrosome-independent role for γ -TuRC proteins in the spindle assembly checkpoint. *Science* 314, 654–657.
- Murata T, Sonobe S, Baskin TI, Hyodo S, Hasezawa S, Nagata T, Horio T, Hasebe M (2005). Microtubule-dependent microtubule nucleation based on recruitment of γ -tubulin in higher plants. *Nat Cell Biol* 7, 961–968.
- Nakamura M, Yagi N, Kato T, Fujita S, Kawashima N, Ehrhardt DW, Hashimoto T (2012). *Arabidopsis* GCP3-interacting protein 1/MOZART 1 is an integral component of the γ -tubulin-containing microtubule nucleating complex. *Plant J* 71, 216–225.
- Nayak T, Edgerton-Morgan H, Horio T, Xiong Y, De Souza CP, Osmani SA, Oakley BR (2010). γ -Tubulin regulates the anaphase-promoting complex/cyclosome during interphase. *J Cell Biol* 190, 317–330.
- Niu Y, Liu T, Tse GM, Sun B, Niu R, Li HM, Wang H, Yang Y, Ye X, Wang Y, Yu Q, Zhang F (2009). Increased expression of centrosomal α , γ -tubulin in atypical ductal hyperplasia and carcinoma of the breast. *Cancer Sci* 100, 580–587.
- Oakley CE, Oakley BR (1989). Identification of γ -tubulin, a new member of the tubulin superfamily encoded by *mipA* gene of *Aspergillus nidulans*. *Nature* 338, 662–664.
- Olmsted ZT, Colliver AG, Riehlman TD, Paluh JL (2014). Kinesin-14 and kinesin-5 antagonistically regulate microtubule nucleation by γ -TuRC in yeast and human cells. *Nat Commun* 5, 5339.
- Paluh JL, Nogales E, Oakley BR, McDonald K, Pidoux AL, Cande WZ (2000). A mutation in γ -tubulin alters microtubule dynamics and organization and is synthetically lethal with the kinesin-like protein pkl1p. *Mol Biol Cell* 11, 1225–1239.
- Petry S, Groen AC, Ishihara K, Mitchison TJ, Vale RD (2013). Branching microtubule nucleation in *Xenopus* egg extracts mediated by augmin and TPX2. *Cell* 152, 768–777.
- Petry S, Pugieux C, Nedelec FJ, Vale RD (2011). Augmin promotes meiotic spindle formation and bipolarity in *Xenopus* egg extracts. *Proc Natl Acad Sci USA* 108, 14473–14478.
- Poirier K, Lebrun N, Broix L, Tian G, Saillour Y, Boscheron C, Parrini E, Valence S, Pierre BS, Oger M, et al. (2013). Mutations in TUBG1, DYNC1H1, KIF5C and KIF2A cause malformations of cortical development and microcephaly. *Nat Genet* 45, 639–647.
- Prigozhina NL, Oakley CE, Lewis A, Nayak T, Osmani SA, Oakley BR (2004). γ -Tubulin plays an essential role in the coordination of mitotic events. *Mol Biol Cell* 15, 1374–1386.
- Prigozhina NL, Walker RA, Oakley CE, Oakley BR (2001). γ -Tubulin and the C-terminal motor domain kinesin-like protein, KLPA, function in the establishment of spindle bipolarity in *Aspergillus nidulans*. *Mol Biol Cell* 12, 3161–3174.
- Rice LM, Montabana EA, Agard DA (2008). The lattice as allosteric effector: structural studies of $\alpha\beta$ - and γ -tubulin clarify the role of GTP in microtubule assembly. *Proc Natl Acad Sci USA* 105, 5378–5383.
- Rodriguez AS, Batac J, Killilea AN, Filopei J, Simeonov DR, Lin I, Paluh JL (2008). Protein complexes at the microtubule organizing center regulate bipolar spindle assembly. *Cell Cycle* 7, 1246–1253.

- Starita LM, Machida Y, Sankaran S, Elias JE, Griffin K, Schlegel BP, Gygi SP, Parvin JD (2004). BRCA1-dependent ubiquitination of γ -tubulin regulates centrosome number. *Mol Cell Biol* 24, 8457–8466.
- Teixido-Travesa N, Villen J, Lacasa C, Bertran MT, Archinti M, Gygi SP, Caelles C, Roig J, Luders J (2010). The γ yeixi revisited: a comparative analysis of interphase and mitotic human γ TuRC redefines the set of core components and identifies the novel subunit GCP8. *Mol Biol Cell* 21, 3963–3972.
- Uehara R, Nozawa RS, Tomioka A, Petry S, Vale RD, Obuse C, Goshima G (2009). The augmin complex plays a critical role in spindle microtubule generation for mitotic progression and cytokinesis in human cells. *Proc Natl Acad Sci USA* 106, 6998–7003.
- Vardy L, Fujita A, Toda T (2002). The γ -tubulin complex protein Alp4 provides a link between the metaphase checkpoint and cytokinesis in fission yeast. *Genes Cells* 7, 365–373.
- Vardy L, Toda T (2000). The fission yeast γ -tubulin complex is required in G₁ phase and is a component of the spindle assembly checkpoint. *EMBO J* 19, 6098–6111.
- Vogel J, Drapkin B, Oomen J, Beach D, Bloom K, Snyder M (2001). Phosphorylation of γ -tubulin regulates microtubule organization in budding yeast. *Dev Cell* 1, 621–631.
- Weil CF, Oakley CE, Oakley BR (1986). Isolation of *mip* (microtubule-interacting protein) mutations of *Aspergillus nidulans*. *Mol Cell Biol* 6, 2963–2968.
- Zhang S, Hemmerich P, Grosse F (2007). Centrosomal localization of DNA damage checkpoint proteins. *J Cell Biochem* 101, 451–465.
- Zheng Y, Wong ML, Alberts B, Mitchison T (1995). Nucleation of microtubule assembly by a γ -tubulin-containing ring complex. *Nature* 378, 578–583.
- Zhu H, Fang K, Fang G (2009). FAM29A, a target of Plk1 regulation, controls the partitioning of NEDD1 between the mitotic spindle and the centrosomes. *J Cell Sci* 122, 2750–2759.
- Zimmerman S, Chang F (2005). Effects of γ -tubulin complex proteins on microtubule nucleation and catastrophe in fission yeast. *Mol Biol Cell* 16, 2719–2733.