Review

CellPress

Targeting of γ-tubulin complexes to microtubule organizing centers: conservation and divergence

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Organisms with closed or open mitosis have differentially evolved various gamma-tubulin complex (γ-TuC) recruiting factors to organize diverse cellular microtubule (MT) arrays, including the mitotic spindle. γ-TuC recruiting factors not only target the γ-TuC to MT nucleation sites, but also regulate MT nucleation activity by generating the template for MT nucleation or promoting the MT nucleation activity of pre-existing γ-tubulin ring complexes (γ-TuRCs). Here we outline the current understanding of MT nucleator assembly and its regulation by γ-tubulin small complex (γ-TuSC) receptors. Moreover, we discuss the emergence of γ-TuC recruiting factors through evolution with augmented complexity and diversity and propose a hypothesis to account for the evolution of these factors in cooperative spindle assembly.

The γ-TuC as an MT nucleation template: self-oligomerization promoted by receptors at MT organizing centers (MTOCs) or assembly with γ-TuRC-specific proteins (GCPs)

MTs are polarized, hollow cylinders that are assembled by the polymerization of heterodimeric α/β-tubulin. In cells, MT nucleation primarily depends on γ-tubulin, a member of the tubulin superfamily. γ-Tubulin associates with defined partner proteins to form γ-TuCs, which then act as scaffolds for α/β-tubulin dimers to initiate polymerization. γ-TuCs are regulated by various factors that recruit γ-TuCs to different cellular locations, spatially controlling MT nucleation activity. Such sites include the canonical MTOCs spindle pole body (SPB) and centrosome (Box 1), the Golgi apparatus, the lateral side of existing MTs, and, in certain polarized cells, the apical membrane. The assembly and recruitment of γ-TuCs and their MT nucleation activity are temporally regulated by cell cycle kinases. This multitude of regulatory mechanisms underpins a range of cellular functions, especially the mitotic spindle assembly.

The budding yeast *Saccharomyces cerevisiae* has the most basic MT nucleation machinery of any model organism. MTs are organized by and attached to the SPB (Figure 1). Although the *S. cerevisiae* genome encodes genes for the γ-TuSC (Box 2), components of the more complex γ-TuC are absent. Structural electron microscopy (EM) studies revealed that, *in vitro*, the γ-TuSC is able to self-oligomerize into ring-like structures in the absence of any additional protein factors under low-salt conditions [1]. The inclusion of the N-terminal fragment of Spc110 (Spc110-N), a γ-TuSC receptor at the nuclear side of the SPB, promoted γ-TuSC oligomerization into filament-like structures under physiological salt conditions. The diameter and pitch of the ring-like complexes are consistent with the conventional 13-protofilament MTs, supporting the template model of MT nucleation. Recent in-depth studies have revealed that the self-oligomerization of γ-TuSCs is both positively and negatively regulated by cell cycle-dependent phosphorylation of Spc110-N [2], suggesting that the formation of MT nucleators is restricted to SPBs and is temporally regulated in budding yeast.

In other eukaryotes, such as *Schizosaccharomyces pombe*, *Aspergillus nidulans*, *Arabidopsis*, *Drosophila melanogaster*, *Xenopus*, and humans, multiple γ-TuSCs assemble with GCP4–6 into the γ-TuRC (Box 2) [3–6]. GCP family members (Spc97/GCP2, Spc98/GCP3, and GCP4–6) each contain the GCP core structure, which has been defined through crystallization of the family member GCP4 [7]. These additional GCP4–6 proteins are not encoded in the *S. cerevisiae* genome. In *S. pombe*, *A. nidulans*, and *D. melanogaster*, RNAi knockdown or gene deletion of GCP4–6 orthologs results in a reduced level of ring-like assemblies, but γ-TuSC is sufficient to be recruited to SPBs or centrosomes and nucleate spindle MTs [3,8–11]. It is likely that, in these cases, γ-TuSC exploits the self-oligomerization pathway utilized in *S. cerevisiae* to form the ring-like MT nucleation template. It is also possible that an incomplete ring-like assembly comprising several γ-TuSCs provides sufficient MT nucleation activity.

Although GCP4–6-dependent γ-TuRC assembly is not essential for spindle formation in these systems, it may be important for the targeting of γ-TuRCs to specific cellular locations. *D. melanogaster* γ-TuRCs localize along interphase cytoplasmic MTs in a GCP4–6-dependent manner. However, these γ-TuRCs do not nucleate MTs but act as points where rescue can occur [12]. Interestingly, GCP4 and GCP5 are essential for *D. melanogaster* germline

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Keywords: microtubule; gamma-tubulin complex; mitotic spindle; centrosome; spindle pole body.

0962-8924/

 http://dx.doi.org/10.1016/j.tcb.2014.12.002

Box 1. Centrosomes and SPBs

The canonical MTOCs involved in spindle formation are centrosomes in higher eukaryotes and SPBs in yeast. The best characterized *Saccharomyces cerevisiae* SPB is a laminar structure that is the functional equivalent of the centrosome. In most fungi, such as *S. cerevisiae* and *Schizosaccharomyces pombe*, mitotic SPBs are embedded in the nuclear envelope with geometrically separated nuclear and cytoplasmic MT nucleation sites. After SPB duplication, receptors located on the nuclear and cytoplasmic sides of the SPBs recruit γ-TuCs. The nuclear side of the SPB nucleates MTs for spindle formation while the cytoplasmic side nucleates MTs for proper nucleus and spindle positioning.

Differing from the planer SPBs of yeast, the centrosomes of the Metazoa comprise two orthogonally arranged centrioles surrounded by pericentriolar material (PCM). In contrast to the conventional view of the PCM as an amorphous protein network, recent advances in super-resolution microscopy have revealed that it is highly structured with consecutive layers of proteins responsible for MT nucleation and γ-TuC anchorage [104–107] including γ-tubulin, pericentrin (PCNT/kendrin), CDK5RAP2 (CEP215), CG-NAP (AKAP9/AKAP450), and NEDD1 (GCP-WD). On entry into mitosis, the nuclear envelope breaks down and a centrosome maturation process is initiated that leads to the recruitment of three to five times more γ-tubulin than is present on interphase centrosomes and enhances MT nucleation activity [108,109].

development [13], suggesting a more important role for the γ-TuRC in meiosis.

In contrast to these model systems, in human cells γ-TuRC function is absolutely required for centrosomal MT nucleation and mitotic spindle formation. Depletion of either GCP5 or GCP6 prevents γ-TuRC assembly and dramatically reduces γ-tubulin recruitment to spindle poles, leading to frequent mitotic failure with monopolar spindles [14,15]. GCP6 is also important for the apical distribution of γ-tubulin in interphase epithelial cells [16].

The γ-TuSC self-oligomerization promoted by receptors at the SPBs of *S. cerevisiae* and the requirement of γ-TuRC assembly for centrosomal localization of γ-tubulin in human cells highlight the coupling of γ-TuC recruitment and the generation of ring-like template assembly. However, there are differences in the hierarchy of control (Figure 2). While γ-TuSC self-oligomerization is dependent on the recruitment of γ-TuSC to SPBs, the GCP4–6-dependent assembly of the γ-TuRC is a prerequisite for its subsequent recruitment to centrosomes. The MT nucleation activity of the γ-TuRC can be further enhanced through the interactions with γ-TuC recruiting factors such as CDK5RAP2 and NEDD1 [17,18]. Such clear differences may not only reflect speciation, but are likely to be driven by a need to evolve more complex γ-TuC recruiting factors to meet the increasing demand of more complex and diverse MT cytoskeletons in higher eukaryotes. However, the basic machinery and principles behind MT nucleation are conserved from yeasts to humans. This review summarizes recent progress in the study of γ-TuC recruiting factors in various model organisms and provides a hypothetical model of how spindle assembly systems have evolved.

Budding yeast represents the simplest MT nucleation system: self-oligomerization of γ-TuSCs promoted by γ-TuSC receptors

In *S. cerevisiae*, Spc110 and Spc72 are the only γ-TuSC recruiting factors targeting γ-TuSCs to the nuclear and cytoplasmic side of the SPB, respectively (Figure 1) [19–23]. The N-terminal region of Spc110 directly interacts with Spc98/GCP3 to recruit γ-TuSCs to the nuclear side of SPBs [20,24]. Overexpression of yeast γ-TuSC components does not induce ectopic MT nucleation [25], supporting the view that γ-TuSC activating factors reside at SPBs. Spc110-N *in vitro* data raise the possibility that Spc110 promotes γ-TuSC assembly into rings at SPBs [1].

A recent publication has shed light on how the activity of Spc110 is controlled during the cell cycle by showing that phosphorylation of Spc110-N both positively and negatively regulates its own activity to promote γ-TuRC oligomerization [2]. Phosphorylation of Spc110-N by S-phase Cdk1–Clb5 kinase and Mps1 kinase at SPBs promotes γ-TuSC oligomerization to initiate MT nucleation. By contrast, subsequent phosphorylation of T18 by mitotic Cdk1–Clb2 dominantly counteracts the promoting activity of the S-phase phosphorylation sites, indicating that the cell cycle-dependent activity of Spc110 in promoting γ-TuRC oligomerization is coordinated with the MT nucleation activity of SPBs required for mitotic events. In S phase, the newly assembled SPB nucleates MTs to drive spindle formation and SPB separation; however, once chromosome biorientation has been established in metaphase, the nucleation activity for new MTs at SPBs is reduced to ensure that the interdigitating halves of the spindle slide apart without any further nucleation through anaphase [26,27].

Spc110 has two conserved elements that are essential for γ-TuSC oligomerization *in vitro* and MT nucleation *in vivo*. Centrosomin (Cnn) motif 1 (CM1) is a highly conserved element of γ-TuSC receptors [28]. The newly discovered Spc110–Pcp1 motif (SPM) is present on only a subset of γ-TuSC receptors that share both structural and functional homology with pericentrin [2]. The activating phosphosites of Spc110 reside in the putative disordered linker that connects SPM and CM1 elements. By contrast, the inactivating phosphosite T18 resides within the SPM.

Although the exact regions of Spc98/GCP3 that are directly contacted by the SPM and CM1 of Spc110 remain unclear, the activating phosphorylations on the flexible linker make it tempting to propose a model in which the SPM and CM1 constitute a bipartite docking site for the γ-TuSC in which CM1 binding to the N-Spc98/GCP3 is complemented by SPM binding to the stem region of the GCP core body of Spc98/GCP3. The activating phosphorylations would regulate the spatial configuration of the SPM and CM1 to promote γ-TuSC oligomerization. By contrast, phosphorylation within the SPM would disrupt its binding to Spc98, thereby further inactivating γ-TuSC oligomerization. Spc98 is also a phosphoprotein and several phosphorylation sites were identified in the putative linker connecting Spc98-N and the GCP core body [29,30]. It is therefore possible that the phosphorylation of Spc110-N and the linker of Spc98 cooperatively modulate the interaction between Spc110-N and Spc98-N to boost MT nucleation activity.

Interestingly, Spc110–Spc98/GCP3 interaction and γ-TuSC oligomerization are not completely reliant on phosphorylation of Spc110 and Spc98-N. *In vitro* N-terminal truncated Spc98/GCP3 supports γ-TuSC self-oligomerization into ring-like structures under low-salt conditions,