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Review

Revisiting the tubulin folding pathway: new roles in centrosomes and cilia

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Abstract

Centrosomes and cilia are critical eukaryotic organelles which have been in the spotlight in recent years given their implication in a myriad of cellular and developmental processes. Despite their recognized importance and intense study, there are still many open questions about their biogenesis and function. In the present article, we review the existing data concerning members of the tubulin folding pathway and related proteins, which have been identified at centrosomes and cilia and were shown to have unexpected roles in these structures.

Keywords: centrosome; cilia; cytosolic chaperonin CCT; tubulin cofactors; tubulin cofactor-related proteins; tubulin folding pathway.

Introduction: the centrosome – an organizing center of multiple activities

The centrosome is the major microtubule-organizing center (MTOC) in animal cells and a key organelle for a variety of cellular functions, being typically composed by a pair of centrioles oriented perpendicularly to each other and connected by fibers in their proximal ends (Figure 1). Centrioles are complex cylindrical-shaped structures, usually formed by nine microtubule triplets arranged radially, which recruit and organize the pericentriolar matrix (1–3).

Since their discovery, centrosomes have been associated with cell division and cell cycle regulation. In fact, by assisting mitotic-spindle formation, centrosomes play a preponderant role in cell division (2). The assembly of the mitotic spindle also relies on acentrosomal pathways (4), but when present centrosomes dominate and their numbers usually define the number of mitotic spindle poles. Furthermore, the centrosome also seems to be required to establish the correct spindle orientation (Figure 1D and G).

Within a centrosome, the two centrioles have different protein compositions and functional features. Thus, each centriole pair in G1 contains an older mature centriole (mother centriole) and an immature centriole (daughter centriole). The mother centriole has elaborate appendages on its distal ends (Figure 1) and is more robust in microtubule anchoring (5). Additionally, the two centrioles show different behaviors in their movement during the cell cycle. In G1 the mother centriole tends to be more fixed at the center of the microtubule aster, whereas the daughter centriole displays a rocking motion (6). At the end of mitosis, however, the movement of the mother centriole to the midbody is required for abscission in animal cells (Figure 1F) (7).

The asymmetry in centriole structure/function is also related to ciliogenesis. In vertebrates, almost every cell type can assemble a primary cilium, usually in G0 or G1. To assemble the cilium the mother centriole docks to the cell membrane becoming a basal body, which nucleates the ciliary axoneme (Figure 1E) (8).

Usually, the immotile primary cilium axoneme is composed of a radial arrangement of nine microtubule doublets (9+0) surrounded by a specialized ciliary membrane (8, 9). By contrast, motile cilia axonemes have a central singlet microtubule pair (9+2), radial spokes and outer and inner dynein arms, features required for their movement (8). Cilia have emerged as important sensory/signaling organelles playing crucial roles in both physiology and development of vertebrates. Indeed, it is now well established that cilia have major roles in signaling pathways such as Hedgehog, Wnt and planar cell polarity pathways (10, 11). Crucially, defects in cilia biogenesis and function are implicated in a plethora of human diseases, collectively known as ciliopathies, which include male infertility, primary cilia dyskinesia, renal cyst formation, blindness, polydactyly, obesity, among others (9).

The asymmetry between the centrioles within a centrosome, and consequently between the duplicated centrosomes within a cell, is a crucial feature for asymmetric cell divisions, which are decisive for cell differentiation and for the maintenance/function of self-renewing stem cells (12). For example, in *Drosophila melanogaster*, male stem cells and neuroblasts present asymmetric divisions that determine which daughter cell retains the stem cell identity or follows the differentiation fate. These asymmetric divisions seem to depend on the regulated orientation of the mitotic spindle (Figure 1G) (13, 14). Interestingly, the centrosome with the oldest, ‘grandmother’, centriole, which localizes cortically close to the stem cell hub, is kept by the germline stem cell,

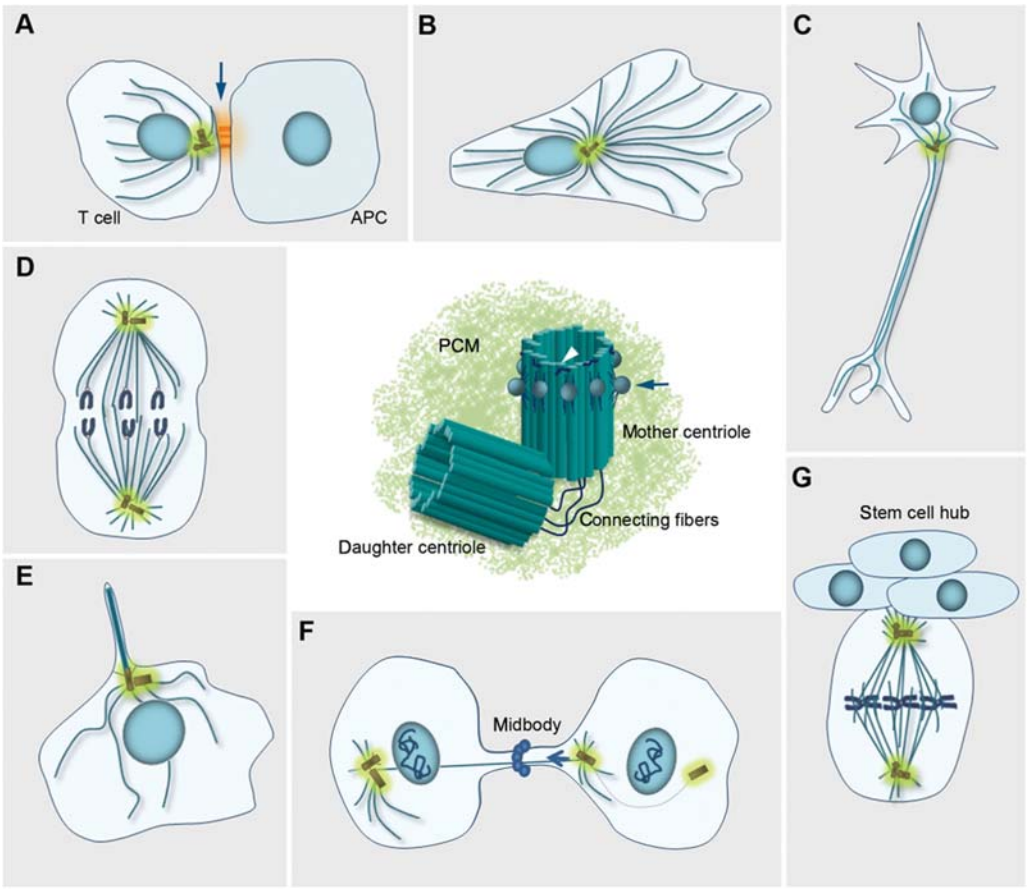

Figure 1 The centrosome as a key player in cell biology.
At the center is a schematic representation of the centrosome where the two centrioles, connected by the connecting fibers and surrounded by the pericentriolar material (PCM), are visible. The mother centriole presents distal (arrow) and subdistal (arrowhead) appendages and is more robust in microtubule anchoring. The centrosome and its positioning have been shown to be involved in several cellular and developmental processes, such as: (A) immune synapse establishment, (B) cell migration, (C) axon growth site specification, (D) mitotic spindle organization, (E) ciliogenesis, (F) cytokinesis, and (G) asymmetric cell division. For detailed explanations see the text.

whereas the daughter centriole is inherited by the cell committing to differentiation (13, 15, 16). This specification relies on the differences between the mother and daughter centrosome MTOC activities (9). The fate of the two daughter cells can be related to the fact that the one inheriting the older centriole is the first to assemble a primary cilium (17), rendering each daughter cell differentially prompted to be challenged by environmental cues.

The position of the centrosome is not only important for cell division; in fact, the position of the centrosome and its association with the nucleus is known to be essential for several cellular functions and for early development (18). In interphase cells, the centrosome is usually at the center of the cell but this positioning is under regulation and changes during cell-state transitions in processes such as immune synapse establishment, cell migration, axon growth site establishment and ciliogenesis (Figure 1) (19–21). Nevertheless, the mechanisms underlying the association of the centrosome to the nucleus and its positioning/reorientation are far from being understood.

Centrosome positioning and cytoplasmic organization are highly dependent on geometrical constraints imposed by both the substratum and cell-cell contacts (22, 23). In addition, forces exerted by microtubules at the cell cortex and forces exercised on them by actomyosin and dynein are also crucial to position the centrosome (24).

Furthermore, several proteins, such as Zyg-12, Emerin and Samp1, establish a physical link between the centrosome and the nuclear envelope (18, 25, 26). This interaction seems to be regulated by a group of distinct kinases like the p160ROCK and the Polo/Greatwall mitotic kinases (27, 28).

The concept that centrosomes function as platforms that allow/promote interactions and recruitment of specific proteins involved in different pathways has been gaining support. For example, several cell cycle regulators accumulate differentially at centrosomes throughout the cell cycle (29, 30). Additionally, DNA repair factors associate with the centrosomes in different cell types and have centrosomal roles (31). For example, the proteins NBS1 and BRCA1 are involved in centrosome number maintenance (32, 33) and

the ATM/ATR kinases phosphorylate the centrosome protein CEP63 and regulate the spindle checkpoint after DNA damage (34).

Recent proteomic analyses of the centrosome revealed new centrosomal proteins reflecting the complexity of its composition, structure and function (35, 36). Nevertheless, this picture is even more complex if we take into account that (i) some of the newly identified centrosomal proteins have well-established cellular roles, but their link to centrosome functions is still obscure; (ii) the extent of the pericentriolar material, and thus the extent of the centrosome, is difficult to establish (37); and (iii) the composition of the pericentriolar material changes during the cell cycle and, probably, among different cell types.

The aim of this article is to give an overview of the available data concerning the group of proteins involved in the tubulin folding pathway and proteins related to them that have been recently shown to localize in the centrosome and/or cilia. The impact of these proteins on the structure/function of these organelles is far from being completely elucidated, but clearly it cannot be ascribed only to their role in tubulin heterodimer maturation. Understanding their puzzling roles in the control of microtubule assembly and dynamics promises to provide new insights on how centrosomes and cilia play their multiplicity of roles.

The tubulin folding pathway: an overview

Microtubules are polar and dynamic polymers of α/β-tubulin heterodimers participating in a wide range of crucial cellular functions such as cell division, cell polarity, cell signaling, cell motility, intracellular spatial organization and transport (38).

The maturation of tubulin heterodimers is a complex multistep process involving the interaction of tubulins with molecular chaperones and tubulin cofactors (TBCA-E) (39) (Figure 2A). The CCT (cytosolic chaperonin-containing TCP1) chaperonin captures tubulin folding intermediates, with significant native-like domain structures, either directly from ribosomes or from the hetero-hexameric chaperone prefoldin (40–42). CCT is a hetero-oligomeric complex formed by two rings connected back-to-back, each composed of eight distinct subunits (CCTα–CCTζ) (43). It is now well established that the CCT complex mediates the folding, driven by ATP binding and hydrolysis, of a wide range of newly synthesized proteins (44) and that tubulins (α, β and γ) (45–47) and actin (48, 49) are its quantitatively major substrates.

After interacting with CCT, tubulins follow two different folding pathways: α-tubulin is captured by cofactor B (TBCB) and β-tubulin by cofactor A (TBCA) (50, 51). Then, cofactors E (TBCE) and D (TBCD) capture α- and β-tubulin, respectively. The two pathways converge and α-tubulin, β-tubulin, TBCE and TBCD form a supercomplex. Cofactor C (TBCC) interacts with this complex and promotes GTP hydrolysis by tubulin and the consequent release of α/β-tubulin-GDP heterodimers. Upon exchange of GDP by GTP these heterodimers become competent to polymerize into microtubules (Figure 2A) (52).

Tubulin cofactors were first described as participating in the tubulin folding pathway, mainly by *in vitro* folding assays and supported by genetic studies in yeast (39). However, when these proteins started to be studied in mammalian models, it became clear that they play crucial functions, not always directly related to their expected role in the tubulin folding pathway but still related to the cytoskeleton. For example, TBCA, which in yeast has been proposed to function as a reservoir of excess of β-tubulin, is dispensable for tubulin folding *in vitro* (53, 54). Nevertheless, TBCA is an essential gene in human cell lines with a preponderant role in the recycling of mature tubulin heterodimers (54) (Nolasco et al., unpublished). TBCB, TBCE and TBCD have the ability of dissociating the tubulin dimer, causing microtubule depolymerization when overexpressed in mammalian cells (55–58). TBCB overexpression also leads to a decrease in the number of microtubules in plant cells (59). The dissociation of native tubulin heterodimers by TBCD is prevented through its interaction with Arl2 (57). Arl2 is a highly conserved protein in eukaryotes that belongs to the ADP-ribosylation factor family of small GTPases. This protein seems to play important roles in the regulation of microtubule-dependent processes (60, 61). Therefore, by interacting with TBCD, Arl2 regulates the tubulin folding pathway and consequently microtubule dynamics (57, 62) (Figure 2). Zhou et al. (61) have also shown that Arl2 localizes at the centrosome throughout the cell cycle. The expression of a dominant activating Arl2 mutant, unable to hydrolyze GTP, caused microtubule loss, centrosome fragmentation and cell cycle arrest in M phase. The authors have thus proposed that the Arl2 mutants can prevent tubulin polymerization at centrosomes by binding and inhibiting or sequestering an essential component.

This picture became more complex when proteins related in sequence, and sharing key functional domains, with tubulin cofactors were described. For example, the protein designated by E-like, based on its sequence similarity to TBCE also promotes microtubule depolymerization when overexpressed in mammalian cells and commits tubulin to proteosomal degradation (63). Additionally, two TBCC-related proteins were also identified, RP2 and TBCCD1, although only RP2 presents a functional overlap with TBCC. Therefore, the tubulin cofactors and their related proteins are not only involved in tubulin heterodimers maturation but also assist tubulin recycling and tubulin degradation (64, 65), which places them in the center of the regulation of tubulin pools availability and quality.

Centrosomal microtubule nucleation and assembly depends on tubulin folding pathway members

The involvement of CCT chaperonin and tubulin cofactors in tubulin synthesis, and recycling/degradation of heterodimers, makes them excellent candidates to regulate microtubule nucleation/assembly and dynamics. This concept is

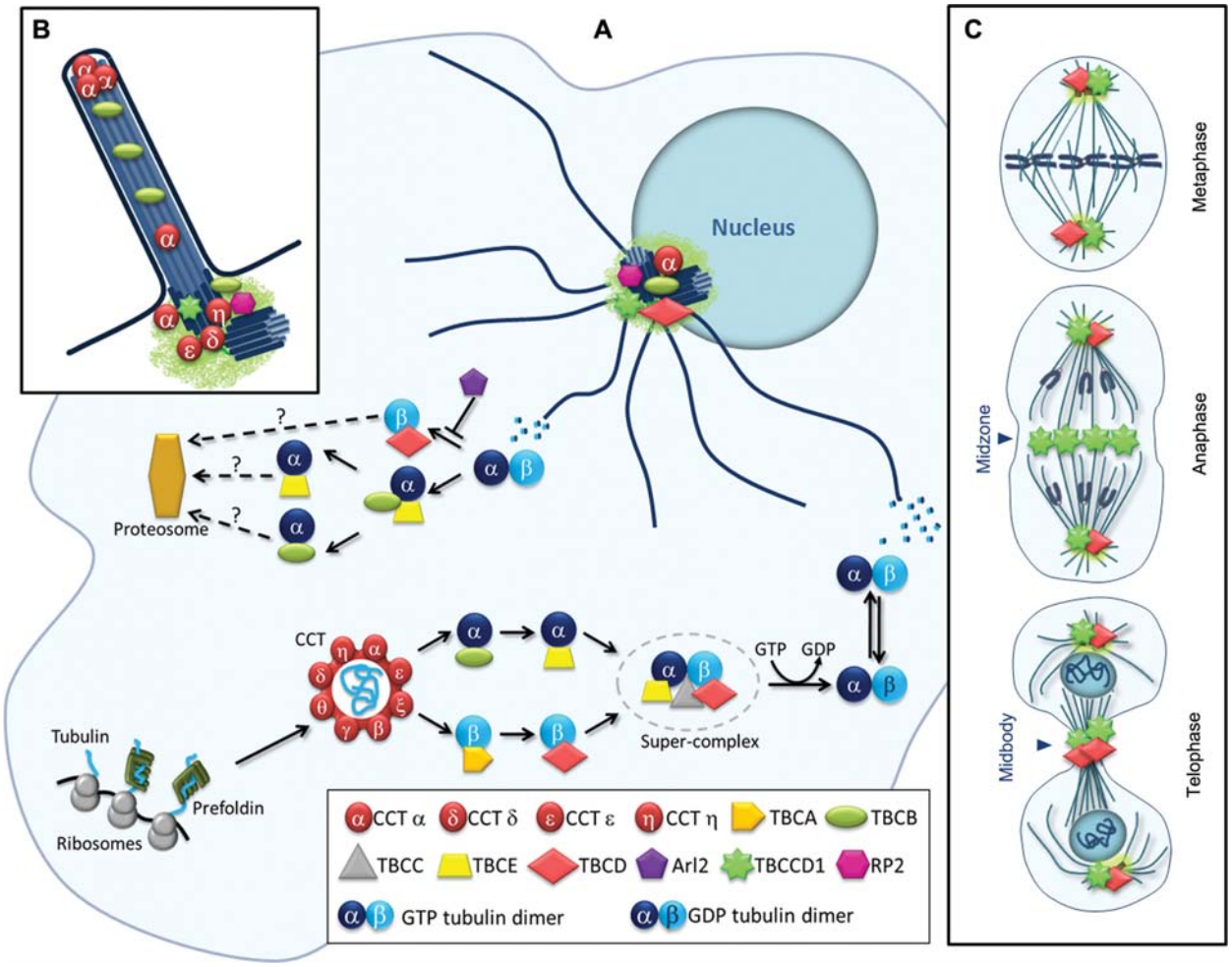

Figure 2 The tubulin folding pathway and related proteins in several cellular contexts.
(A) Schematic representation of the tubulin folding and native dimer disassembly pathways (adapted from 39). The maturation of tubulin is a complex multistep process that culminates in the release of α/β-tubulin dimers in a GTP form and thus competent to polymerize into microtubules. Native dimers can be dissociated by TBCB, TBCD and TBCE. TBCB and TBCE form a complex and cooperate in the dissociation process. In (A) the localization of some members of these pathways and related proteins at the centrosome is also represented. (B) Representation of the cilium and basal body localizations of CCT subunits, TBCB, RP2 and TBCCD1. (C) Representation of the TBCD and TBCCD1 localizations during mitosis. These proteins are visible in the centrosome throughout the cell cycle, being TBCCD1 also identified in the midzone of the mitotic spindle. In late mitosis, TBCD and TBCCD1 localize in the midbody.

strengthened by the localization of these proteins at MTOCs such as the centrosome.

CCTα and CCTζ subunits have been localized at the centrosome throughout the cell cycle in mammalian cells (66, 67). In agreement, in the ciliate *Tetrahymena pyriformis*, the CCTα, CCTε, CCTδ and CCTη subunits localize in basal bodies, the oral apparatus, and contractile vacuole pores (68), all structures known to nucleate/organize microtubules (69). In plants, the nuclear surface actively supports microtubule outgrowth and the CCTε subunit was found at the nuclear envelope of maize coleoptiles (70). Moreover, Brown et al. (67) have shown that the microinjection of an anti-CCTα antibody inhibits the recovery of the microtubule aster from the centrosome in mammalian cells pretreated with nocodazole, after drug washout. These observations suggest that the presence of these subunits in the centrosome/MTOCs facilitates tubulin polymerization by assisting microtubule nucleation. In addition, CCT subunits can be required at the centrosome to interact with other centrosomal proteins. For example, many CCT interacting proteins contain WD40 domains [for review see (40)] which are also present in a variety of centrosomal proteins such as the p80 subunit of the microtubule severing protein katanin (71); the centrosomal protein POC1, involved in ciliogenesis and in centriole duplication, stability and length control (72, 73); and NEDD1/GCP-WD, a γ-tubulin ring complex component (74). CCT subunits, either as free entities or as components of microcomplexes (75), have also been implicated in the regulation of cytoskeleton assembly and dynamics (66, 68, 76, 77), supporting the concept that their function extends beyond the folding assistance or the maintenance of correct tubulin heterodimers concentration.

The participation of tubulin cofactors in the control of microtubule nucleation/assembly has also been described. Indeed, both TBCB and TBCD were shown to localize at the centrosome. Human TBCD contains two domains involved in this specific localization (residues 311–610; residues 803–1054) (78) and also a microtubule-binding domain (residues 888–1200) (79). Specifically, during the cell cycle, this tubulin cofactor is found in the daughter centriole at G1, on procentrioles at S phase, and disappears from older centrioles at telophase, when the protein is recruited to the midbody (79). These observations suggest the involvement of TBCD in centriologenesis.

Interestingly, both TBCB and TBCD overexpression cause supernumerary acentriolar MTOCs containing γ-tubulin (79, 80), which in the case of TBCD is accompanied by aberrant mitotic figures (78). In fact, TBCD high levels promote a G1 delay and the loss of the γ-TuRC, GCP-WD and pericentrin, but not centrin-2, from centrosomes, which correlates with microtubule release from this structure, either during mitosis or in interphase (78, 79).

TBCB is a substrate of p21-activated kinase (Pak1) and its phophorylated form colocalizes with this kinase at the centrosome. The overexpression of Pak1 causes a phenotype similar to that of TBCB. Phosphorylation of TBCB is essential for the polymerization of new microtubules. Indeed, mutated forms of TBCB at its phosphorylation sites inhibit microtubule regrowth from the centrosome after nocodazole treatment and washout (80). More recently, it has been shown that TBCB, just like *tau* and α-tubulin, undergoes nitration, which inhibits the polymerization of new microtubules (81). The inhibition of microtubule regrowth in this case seems to be related to the fact that tyrosine nitration of TBCB inhibits its ability to undergo phosphorylation. These studies show the existence of an important crosstalk between those two TBCB post-translational modifications in the control of microtubule polymerization.

Regarding TBCD, this protein is also required for the correct organization of microtubular structures and cell cycle progression. This is supported by observations in *Schizosaccharomyces pombe* where mutations in the *alp1* gene, the TBCD homolog, cause G1/S arrest (82–84). Furthermore, TBCD depletion by RNAi in mammalian cells resulted in the formation of mono- and multipolar spindles, disorganized/short spindles, long midbodies and failure in cytokinesis (78, 79). Interestingly, these dramatic alterations in spindle organization are not accompanied by the disappearing of TBCD from the centrosome, despite a sharp decrease in the cytoplasmic levels of the protein. It is noteworthy that both the knockdown and the expression of dominant acting TBCD mutants do not produce changes in the steady-state levels of γ-, α- or β-tubulins, indicating that the phenotypes associated with depletion are not merely explained by altered tubulin pools (78, 79). This is consistent with the observation that, in budding yeast, TBCD (Cin1p) is not essential for cell viability, but its deletion increases sensitivity to antimitotic drugs and cold, as well as chromosome instability (85, 86). These results indicate that, in *Saccharomyces cerevisiae*, TBCD/Cin1p is not essential for tubulin heterodimer maturation but its role still affects mitotic segregation in this organism, probably through microtubule-associated defects. However, at this point, it is still unclear if the centrosomal phenotypes observed when TBCD is overexpressed or depleted are related to its ability to interact directly with tubulin heterodimers and/or microtubules or to an additional unknown TBCD function.

In *S. pombe*, there is a genetic interaction between TBCD mutant forms and specific kinetochore (like the CENP-B-like protein) and spindle components (82), suggesting that TBCD might be a key factor in the interface between structure of MTOCs and microtubule nucleation/polymerization by assisting the turnover of tubulin heterodimers in these structures. Although the binding of TBCD to microtubules has been reported for the *S. pombe* ortholog Alp1p (84), this has never been observed for mammalian TBCD.

As already referred, the activity of TBCD in the tubulin folding pathway is regulated by its interaction with Arl2. Additionally, TBCD and Arl2 in brain extracts are components of a 300 kDa complex that contains the heterotrimeric protein serine/threonine phosphatase 2A (PP2A), a major eukaryotic phosphatase that is highly conserved from yeast to human (87). The presence of PP2A in this complex suggests additional roles for protein phosphorylation/dephosphorylation in tubulin folding regulation and/or microtubule nucleation/assembly and dynamics. This also suggests a link between TBCD and Arl2 and other molecules/pathways that are involved in the regulation of microtubule dynamics. For example, it is known that PP2A interacts with the microtubule-associated protein *tau* (88) and is also present at kinectochores (89). Moreover, it was shown that PP2A is involved in the regulation of the apical junctional complex assembly/disassembly in polarized MDCK epithelial cells (90), a process in which TBCD/Arl2 have been recently implicated in. TBCD overexpression causes disassembly of the tight and adherent junctions followed by cell dissociation from the epithelial monolayer (91). The authors have also shown that TBCD localization in cell-cell adhesions is dependent on microtubules and that the dissociation of the junctional complex is inhibited by the overexpression of the Arl2. At first glance, these data suggest that the disassembly of the junctional complex might occur through the ability of TBCD to dissociate tubulin heterodimers (91). However, this explanation is simplistic if we take into consideration that overexpression of TBCD leads to α-tubulin degradation and the depolymerization of microtubules on which its localization at the junctions depends on (56, 57).

Several lines of evidence implicate members of the tubulin maturation machinery in centrosome activities such as microtubule nucleation, assembly and, consequently, dynamics. If these centrosomal activities depend on the ability of these proteins to directly regulate tubulin heterodimers assembly/dissociation remains unclear. The studies focused on the role and regulation of TBCB and TBCD also highlighted the fact that changes in concentration of both tubulin cofactors affect centrosomal γ-tubulin, which could explain the described perturbations in nucleation and polymerization of microtubules. However, this picture is only fragmentary.

The ciliary roles of tubulin folding pathway members and related proteins

In addition to their localization at the centrosome, tubulin folding pathway components and related proteins are not restricted to centrosomes and some were also found in basal bodies and in cilia axonemes. Furthermore, their ciliary localization seems to be crucial for the assembly and maintenance of cilia structure.

The importance of CCT subunits in cilia biogenesis emerged from reciliation studies in the ciliate *T. pyriformis*. These studies have shown that the CCT genes are upregulated concomitantly with tubulin genes during cilia regeneration [(68, 92) and unpublished results]. Later on, it was observed that, as reciliation proceeds, CCT subunits are recruited to basal bodies and growing axonemes. Accordingly, CCTα is found in rabbit tracheal cilia and is recruited to cilia during sea urchin embryonic cilia regeneration (93). By contrast, reciliation affects the oligomeric state of CCT subunits with tubulin being preferentially associated with smaller CCT oligomeric species in early reciliation stages (68). A recent study showed that the knockout of CCTα or CCTδ in the ciliate *Tetrahymena thermophila* caused a loss of cell body microtubules, failure to assemble new cilia and cell death (94). Additionally, CCT subunit depletion leads to axoneme shortening and splaying of cilia tips. Interestingly, an epitope-tagged CCTα, which was localized primarily to the tips of cilia, rescued the gene knockout phenotype. Moreover, the mutation G346E in CCTα impaired its cilia localization and caused defects in cilia structure. Nevertheless, this mutated form of CCTα supports cell survival, suggesting that tubulin is not limiting in the mutant (94). The amino acid residue G346 is conserved in the CCTα-related centrosomal protein BBS6, which is implicated in the Bardet-Biedel syndrome, a disorder associated with defects in primary cilia (95). These studies demonstrate that CCT subunits are essential and required for cilia assembly and maintenance of the axoneme structure, especially at cilia tips.

In *T. thermophila*, cilia tips display a complex structure designated by cap, which connects axonemal microtubule ends to the cilia membrane (96). These caps were suggested to be involved in the regulation of assembly/disassembly of axonemal microtubules (97–99). The preferential CCTα localization in cilia tips suggests that CCTs are associated with either the distal ends of microtubules and/or with caps, probably controlling tubulin turnover and assisting the incorporation of new tubulin in the axoneme structure, by acting as end-binding microtubule-associated proteins (MAPs). Additionally, CCTs might stabilize microtubule anchoring to MTOCs/caps thus helping these structures to cope, for example, with mechanical stress. In fact, some CCT subunits (α, γ, ζ and θ) associate with microtubules polymerized *in vitro*, behaving as typical MAPs (66). Alternatively, CCTs could be involved in the interactions between microtubules and the ciliary membrane given that cilia caps connect these two systems. Interestingly, there is some evidence that CCT subunits interact with membranes. For example, the adrenal medullary form of CCT (chromobindin A) binds efficiently to chromaffin granule membranes (100). Furthermore, in human erythrocytes, CCTα is translocated to the plasma membrane following a heat shock (101). Moreover, CCT subunits CCTα, β, γ, δ, ε and CCTθ were shown to form a complex with the chaperonin-like BBS6, BBS10 and BBS12 proteins (vertebrate-specific BBS genes) (102). This complex was recently described to be required for the BBSome assembly. The BBSome is an oligomeric complex of BBS (BBS1–2, BBS4–5, BBS7–9) proteins that have been directly implicated in ciliogenesis by promoting vesicle trafficking to the cilia membrane (103).

Although several studies suggest that CCT subunits have additional roles outside the chaperonin, acting either alone or in microcomplexes, studying them has been difficult due to the major folding activity of the whole complex. Nevertheless, it would be interesting to clarify if all the subunits are present in centrosomes and cilia, what their oligomerization state is and if it is altered, for example, during the changes these organelles undergo during the cell cycle.

Similarly to CCT subunits, tubulin cofactors were also shown to be present at basal bodies and axonemes of cilia; however, their exact role in these structures is unknown. In fact, TBCB was detected in the axonemes of primary cilia present in primary cultures of mouse brain, in the basal bodies of cilia in the respiratory tract (104) and in *T. thermophila* motile cilia (105). In mouse brain ependymal cell primary cultures, Fanarraga and coworkers (79) showed that TBCD is recruited into ‘centriolar rosettes’ during basal body assembly, which suggests that TBCD also plays a role in cilia biogenesis. Remarkably, TBCD knockdown leads to long primary cilia (79). Finally, TBCC, which is the least studied of the tubulin cofactors and acts together with TBCD as a β-tubulin GTPase-activating protein (GAP) (52, 106), is localized predominantly in the connecting cilium of rod and cone photoreceptors in the human retina (107). Bearing in mind the roles of tubulin cofactors in the tubulin folding pathway, it is tempting to suggest that their ciliary function is related to control the quality and/or tubulin turnover of tubulin heterodimers. In support of this view, a TBCC-related protein called retinitis pigmentosa 2 protein (RP2) localizes at the basal body of *Trypanossoma brucei* flagellum, where it was proposed to participate in a tubulin quality control mechanism prior to incorporation of tubulin heterodimers in the axoneme (108). In vertebrates, RP2 has been extensively studied due to its involvement in the X-linked condition retinitis pigmentosa, a pathology characterized by a progressive degeneration of photoreceptor cells (109, 110). Similar to TBCC, RP2 also contains a TBCC and a CARP domain in its N-terminal region, suggesting a possible functional overlap between these two proteins. Indeed, both TBCC and RP2 act as GAP proteins towards tubulin in a TBCD-dependent manner. In a yeast complementation assay, RP2 was shown to partially complement the *CIN2* (*tbcc* homolog) deletion. However, unlike TBCC, RP2 does not promote tubulin heterodimerization *in vitro* (111). The GAP activity of RP2 and TBCC relies on the TBCC domain and many of the RP2 mutations involved in the retinitis pigmentosa pathology occur in this domain (109). Among the amino acid residues

conserved between RP2 and TBCC that are important for their function, it is an arginine residue crucial for their GAP activity, which when mutated provokes retinitis pigmentosa (112). RP2 localizes at the cytoplasm and at the cellular and cilia membranes depending on these localizations on post-translational modifications such as myristoylation and palmitoylation of its amino terminus (110). In mammalian cells, RP2 depletion does not affect cilia biogenesis. However, *rp2* silenced cells present swelled cilia with accumulation of the calcium release channel polycystin-2, suggesting that RP2 plays a role in polycystin-2 secretion from the cilia (113). Indeed, in cilia, RP2 interacts with polycystin-2 whose mutations/silencing cause left-right defects and kidney cysts. Also, RP2 interacts with, and is a GAP to, Arl3, a small GTP-binding protein whose function is still unclear but has a microtubule cytoskeleton related role. In mammalian cells, Arl3 localizes at the centrosome throughout the cell cycle, mitotic spindle, midzone, midbody and cilia (61). Furthermore, in mouse photoreceptor cells, it localizes mainly in the connecting cilium (107). Indeed, in the parasite *Leishmania donovani* and in mice Arl3 was shown to be required for the assembly of cilia/flagella (114). The deregulation of Arl3 caused by *rp2* silencing in mammalian cells leads to Golgi apparatus fragmentation. Interestingly, RP2 is needed for vesicle trafficking from the Golgi into the cilium, which when affected hinders the transport and location of ciliary proteins, such as IFT20 (115). Additionally, morpholino-mediated RP2 inhibition in zebrafish has severe consequences in development, causing defects such as *situs-inversus*, hydrocephalus and kidney cysts, phenotypes already well established as related to ciliopathies (113). Finally, it was reported that RP2 also interacts with the N-ethylmaleimide sensitive factor (NSF), a protein involved in vesicle-membrane fusion (116).

Collectively, these data implicate RP2 in cilia biogenesis/function through a possible role in tubulin quality control, suggested by localization of RP2 at basal bodies and its GAP function towards tubulin, and/or an involvement in protein trafficking possibly through the regulation of Arl3 and an interaction with NSF. A crucial role for RP2 in cilia is further supported by the fact that RP2 orthologs are only present in ciliated organisms (108). However, the RP2 functions are beyond its ciliary role because it relocalizes to the nucleus in response to ionizing radiation-induced DNA damage where it binds to damaged DNA (single-stranded or nicked DNA) and exerts an exonuclease activity involved in DNA repair (117). This and the accumulation of RP2 in the centrosome reinforce the concept that the centrosome is a meeting point for proteins involved in DNA repair.

Centrosome-nucleus connection requires TBCCD1, a TBCC-related protein

Recently, a new TBCC-related protein, called TBCCD1 (TBCC domain-containing 1), was described both in the biflagellated green alga *Chlamydomonas reinhardtii* and human cells (118, 119).

Similar to TBCC and RP2, TBCCD1 contains the functional domains, CARP and TBCC. This led to the hypothesis that TBCCD1 could share the GAP function of TBCC and RP2 and possibly have a microtubule-related role. However, this is contradicted by the inability of TBCCD1 to rescue the phenotypes of TBCC/CIN2 deletion in yeast (119). Indeed, TBCCD1 has an atypical TBCC domain in which the amino acid residues crucial for the GAP activity of TBCC and RP2 are not conserved (118, 119). In human cells, TBCCD1 localizes at the centrosome throughout the cell cycle, at the spindle midzone during anaphase, at the midbody during cytokinesis and at the basal bodies of primary and motile cilia (119). In *C. reinhardtii*, TBCCD1 localizes in the centrioles/basal bodies and in rhizoplasts, structures that connect them to each other and to the nucleus (118). TBCCD1 depletion by RNAi in human RPE-1 cells caused a marked increase in the nucleus-centrosome distance, with the centrosome often found at the cell periphery (119).

These observations placed TBCCD1 as an important player in centrosome positioning and therefore in processes such as cell division, cell migration, organelle positioning, ciliogenesis, immune synapse establishment and axon growth site specification, which all depend on centrosome positioning (21) (Figure 1). Supporting this, *tbccd1* silencing in RPE-1 cells also caused a cell cycle delay in G1, the disorganization of the Golgi apparatus, a decreased efficiency in the assembly of primary cilia and slowed-down cell migration. In *C. reinhardtii*, TBCCD1 loss of function caused similar phenotypes to the ones observed in human cells. *Asq*2 mutant cells containing an insertion in the *tbccd1* gene have centriole positioning defects, which lead to the formation of mitotic spindles with incorrect orientations (118). Furthermore, the absence of TBCCD1 can lead to aberrant numbers of centrioles and flagella. *asq2* cells can have up to seven flagella in the absence of TBCCD1, showing that this protein is not necessary for the formation of these structures but is probably involved in regulation of the centriole assembly pathway (118). This phenotype has not been reported for *tbccd1* silencing in human cells. This could be due to the presence of residual protein levels after silencing, which would mask certain phenotypes that would be revealed in its complete absence. Alternatively, other mechanisms regulating the *de novo* assembly pathway in human cells could compensate for the decrease in TBCCD1 levels. Moreover, specificities of each model might account for the observed differences. Nevertheless, the data coming from the two studies clearly establish TBCCD1 as a centrosomal/basal body protein required for the correct positioning of these organelles in the cell. This finding is of great relevance because the mechanisms governing centrosome positioning and its association to the nucleus are still poorly understood.

Expert opinion

A large amount of information concerning the structure, composition and function of the centrosome is already available. However, the involvement of this structure in crucial events of eukaryotic cells, such as cilia assembly/function,

asymmetric cell division and cell fate and differentiation, intrinsic cell polarity and cell motility, still maintain it as one of the most attractive organelles to investigate. The fine regulation of these processes is required for the successful development of the organism and their misregulation is intrinsic to several human diseases.

Recently, several components of the tubulin folding pathway have been shown to be centrosomal proteins. Their ability to control the maturation of tubulin makes them candidate factors to regulate microtubule nucleation/assemble and dynamics *in vivo*, making their centrosomal localization not surprising. This picture is probably more complex because, for example, the depletion and overexpression of tubulin cofactors give phenotypes not always interpreted by their role in tubulin folding. Although tubulin cofactors seem to have tubulin heterodimer-independent roles, participating, for example, in the proper recruitment/organization of γ-tubulin at the centrosome, their action on tubulin dimers is certainly important in several aspects.

Their ability to dissociate tubulin heterodimers, contributing to tubulin recycling/degradation, promises to play an important role in microtubule cytoskeleton remodeling and assembly/disassembly of specific microtubule structures. Therefore, it is easy to conceive that these properties are important for centrosome and cilia biogenesis/maintenance and functions.

Tubulin cofactors might even be key players in the modulation of tubulin pools in terms of their tubulin isotype composition. *In vivo*, tubulin pools diversity can be generated by the expression of distinct genes, originating different tubulin isotypes that can be differentially post-translationally modified. These isotypes share a considerable degree of sequence homology, being their C-terminal the most divergent region, which confers them their identity. Some of these isotypes are constitutively expressed, whereas others are either specifically or preferentially expressed in different tissues, for example, neurons and testis. In vertebrates, the different classes of isotypes are considerably conserved, suggesting that they must have functional significance. Although the precise functions of all the isotypes have yet to be determined, some of them have been associated, for example, with anticancer drug resistance (βIII) (120), neuronal differentiation (βII) and cilia/flagella assembly (βIV; βI) (121, 122). It is conceivable that in response to certain cues/demands cells will polymerize microtubules enriched in certain isotypes that will confer them specific features. Considering the role of tubulin cofactors in tubulin heterodimers assembly/disassembly and recycling, we can hypothesize that these proteins are able to discriminate between the different tubulin isotypes and favor the assembly of tubulin heterodimers with specific isotype compositions. In addition, the dissociation of tubulin heterodimers is thermodynamically very unfavorable in the absence of tubulin cofactors and GTP hydrolysis (123). Therefore, one of the most important roles for tubulin cofactors would be the dissociation of tubulin heterodimers that would allow not only to control tubulin heterodimers quality but also to exchange tubulin subunits between native tubulin heterodimers. The diversity in tubulin pools is also generated by a variety of tubulin post-translational modifications and it is also unknown if these affect the ability of tubulin cofactors to deal with tubulin dimers. These proposed roles for tubulin cofactors would also fit perfectly with centrosome and cilia functions and are definitely some of the most attractive questions in the tubulin cofactors field. In this context, it is also very interesting that tubulin cofactor-related proteins do not participate directly in tubulin folding but instead, in the case of E-like, regulate heterodimer stability and degradation and, in the case of RP2, regulate GTP hydrolysis by tubulin. By contrast, TBCCD1 does not seem to fit this scheme of tubulin-related functions. Indeed, its function as a GAP is still unclear and its putative regulatory role crucial for centrosome positioning is yet to be uncovered.

Outlook

From the data reviewed here, it is clear that CCT subunits, tubulin cofactors and related proteins have important, but still unclear, roles at the centrosome and cilia. For example, not all of the components of the tubulin folding machinery were detected in these organelles, raising the question if those detected there are involved in tubulin maturation/recycling or, alternatively, are playing other roles. Taking into account that perturbations in the amounts of these proteins are not always accompanied by alterations in tubulin levels, one can speculate that, in cilia and centrosomes, they could be assisting tubulin heterodimer transport to growing microtubule ends and/or behaving as specialized microtubule-associated proteins.

The ability of CCTs and RP2 to interact with membranes also raises the hypothesis that these proteins have the ability to establish an interface between tubulin/microtubules and Golgi, cytoplasmic and ciliary membranes. As referred above, tubulin cofactors could be involved either in the differential incorporation of certain isotypes into newly assembled tubulin dimers and/or the exchange of tubulin subunits between native dimers. By contrast, it is not yet possible to discard the hypothesis that these proteins could have tubulin-independent roles. Finally, it is important to investigate how far the functional overlap between tubulin cofactors and their related proteins goes. These hypotheses deserve further investigation and could constitute future areas of research involving these proteins.

The clarification of these issues will certainly contribute to elucidate their role in several cellular contexts. With the recent transfer of tubulin folding pathway components and tubulin cofactor-related proteins research from the test tube to the cellular and organism contexts, we have already, and will in the future, gain new insights about the functions of these proteins, which are in a crossroad of different microtubule-dependent processes and centrosome/cilia activities.

Highlights

- CCT subunits play a role outside of the chaperonin complex.

- CCTs might assist tubulin turnover at cilia and cilia structure protection against damage.
- CCTs, tubulin cofactors and related proteins might establish an interface between microtubules and cellular membranes.
- Tubulin cofactors are implicated in tubulin heterodimer maturation and recycling/degradation and are key regulators of microtubule dynamics.
- Tubulin cofactors might be involved in tubulin isotypes interchange between mature heterodimers that can occur at centrosome.
- Tubulin cofactor-related proteins are regulators of microtubule-dependent processes by direct/indirect interaction with tubulin heterodimers.
- Tubulin cofactors and related proteins can play specific centrosomal roles, e.g., centrosome positioning and ciliogenesis/cilia function.

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