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Microtubule-Organizing Centers

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Abstract

The organization of microtubule networks is crucial for controlling chromosome segregation during cell division, for positioning and transport of different organelles, and for cell polarity and morphogenesis. The geometry of microtubule arrays strongly depends on the localization and activity of the sites where microtubules are nucleated and where their minus ends are anchored. Such sites are often clustered into structures known as microtubule-organizing centers, which include the centrosomes in animals and spindle pole bodies in fungi. In addition, other microtubules, as well as membrane compartments such as the cell nucleus, the Golgi apparatus, and the cell cortex, can nucleate, stabilize, and tether microtubule minus ends. These activities depend on microtubule-nucleating factors, such as γ-tubulin-containing complexes and their activators and receptors, and microtubule minus end–stabilizing proteins with their binding partners. Here, we provide an overview of the current knowledge on how such factors work together to control microtubule organization in different systems.

Contents

INTRODUCTION

The organization of microtubule networks plays a crucial role in controlling different aspects of cell architecture and function. Microtubules are dynamic polymeric tubes, with the fast-growing plus end, where most of microtubule elongation occurs, and the slow-growing minus end, which is often stabilized and attached to different cellular structures. In dividing animal cells, a major site of microtubule nucleation and anchoring is the centrosome, which thus forms the microtubule-organizing center (MTOC)—the central point of a radial microtubule array (Bornens 2012, Conduit et al. 2015b). However, in contrast to the textbook view, even in cells with a radial microtubule system, many microtubule minus ends are not attached to the centrosome but instead are anchored to membrane organelles, such as the Golgi apparatus, or lie free in the cytoplasm (Chabin-Brion et al. 2001, Efimov et al. 2007, Rodionov et al. 1999). Furthermore, in many types of differentiated animal cells, such as epithelial cells or neurons, microtubule networks are not radial but instead form parallel or antiparallel arrays (Sanchez & Feldman 2017). Linear microtubule arrays are also present in different types of fungi, particularly those with an elongated cell shape (Sanchez & Feldman 2017). Plant cells do not have centrosomes, and a significant part of microtubules in interphase plant cells form sheetlike, semiparallel arrays located underneath the plasma membrane (Hamada 2014). Overall, microtubule organization can vary depending on the cell type and cell cycle or differentiation stage, and the underlying mechanisms strongly rely on the localization and activity of the protein complexes that can nucleate microtubules and subsequently stabilize and anchor their minus ends. Cellular structures that have such a capacity are often termed MTOCs, although some of them, unlike the centrosome, do not cause microtubule minus end focusing into asters.

It is generally believed that the core component responsible for microtubule nucleation is γ-tubulin (for review, see Kollman et al. 2011). In budding yeast, γ-tubulin is part of the so-called

γ-tubulin small complex (γ-TuSC), whereas in other organisms it is part of a larger complex, the γ-tubulin ring complex (γ-TuRC) (**Figure 1*a*,*b***). Importantly, studies in vitro and in cells indicate that the microtubule-nucleating activity of γ-TuRC is strongly regulated by additional factors, many of which typically reside in MTOCs (Petry & Vale 2015). Some of these factors directly bind to γ-TuRC and can also recruit this complex to specific sites (Lin et al. 2014a), whereas others can interact with tubulin dimers or nascent microtubule plus ends, thus promoting microtubule growth from the existing template (Wieczorek et al. 2015). Furthermore, there are indications that γ-TuRC may not be strictly required for all microtubule nucleation events. For example, in fly cells, the depletion of γ-tubulin had no strong effect on the steady-state levels of interphase microtubules (Rogers et al. 2008). In worms, both centrosomal nucleation and formation of noncentrosomal microtubules could be maintained to some extent when γ-tubulin function was strongly diminished (Hannak et al. 2002, Wang et al. 2015). Microtubules easily nucleate spontaneously in solutions of purified tubulin if their concentration is sufficiently high, and many microtubule-associated proteins (MAPs) have been reported to promote this process. Among them, the combination of TPX2 (the targeting protein for Xklp2) and ch-TOG (the human homolog of the microtubule polymerase XMAP215) appears to be very potent, with TPX2 stabilizing early microtubule nucleation intermediates and ch-TOG promoting microtubule growth (Roostalu et al. 2015). However, it is currently unknown whether in cells these proteins can support microtubule nucleation independently of γ-TuRC templates.

By capping microtubule minus ends, γ-TuRC can also potentially stabilize and anchor them. These activities of γ-tubulin can be separated from its role in microtubule nucleation and depend on distinct factors (Anders & Sawin 2011, Muroyama et al. 2016). Furthermore, there are other proteins that can autonomously bind to microtubule minus ends. These include KANSL1/KANSL3 proteins, epigenetic regulators that associate with and stabilize microtubule minus ends in mitotic spindles (Meunier et al. 2015), and the members of the CAMSAP/Patronin family (Goodwin & Vale 2010, Meng et al. 2008; reviewed in Akhmanova & Hoogenraad 2015). CAMSAPs (in vertebrates) and Patronin (in invertebrates) do not nucleate microtubules but associate with their free, uncapped minus ends as they grow and in this way form stretches of stabilized microtubule lattices that can serve as seeds for microtubule regrowth (Hendershott & Vale 2014, Jiang et al. 2014) (**Figure 1*c***). CAMSAP/Patronin-mediated and γ-tubulin-mediated minus end stabilization pathways are thus mutually exclusive. Through their binding partners, CAMSAPs and Patronin can organize microtubule minus ends at different cellular locations. Here, we discuss the mechanisms of microtubule nucleation and anchoring at different types of MTOCs and their functions. We primarily focus on animal cells but also discuss similarities and differences with other eukaryotic systems.

CENTROSOME: THE MAJOR MICROTUBULE-ORGANIZING CENTER IN DIVIDING ANIMAL CELLS

Centrosome Function

The centrosome is composed of two centrioles (the older one and the newer one, termed mother and daughter) and the surrounding pericentriolar material (PCM), which is responsible for microtubule nucleation, as well as for stabilization and attachment of microtubule minus ends. Centrioles have a symmetric cylindrical structure, the core of which is formed by nine microtubule triplets. In addition to playing a role in centrosome formation, centrioles serve as basal bodies of cilia and flagella. The mechanisms of centriole formation and duplication have been the subject of excellent reviews (Avidor-Reiss & Gopalakrishnan 2013, Azimzadeh & Marshall 2010) and are not

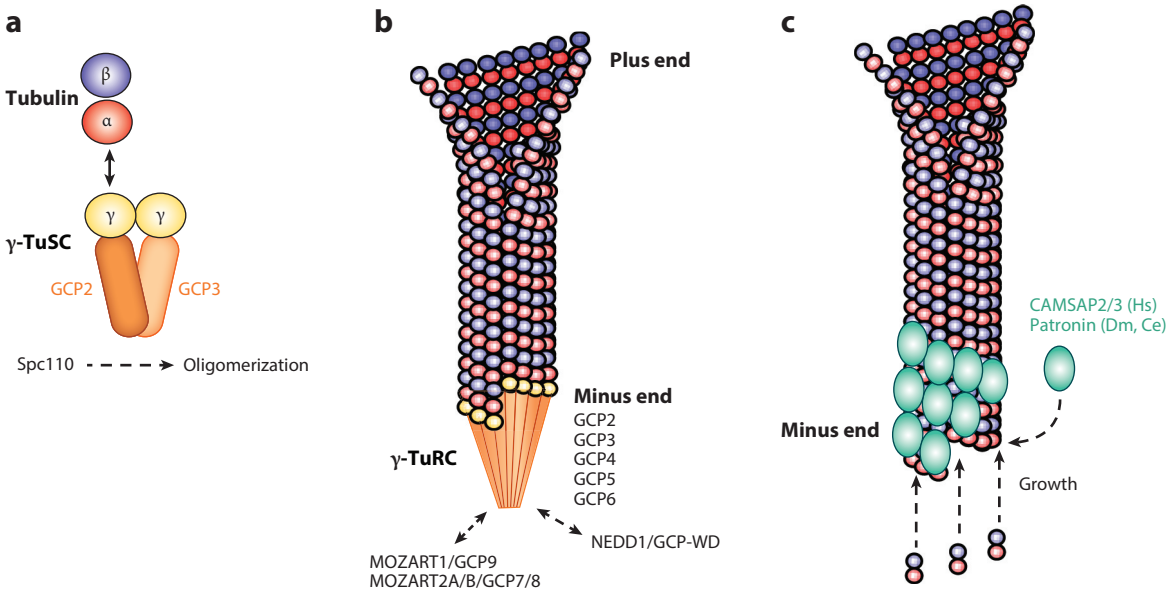

Figure 1

Microtubule minus end binding proteins. (*a*) γ-Tubulin small complex (γ-TuSC). In budding yeast, γ-tubulin is part of γ-TuSC, which consists of two copies of γ-tubulin and two γ-tubulin complex proteins (GCPs), GCP2 and GCP3, which in vitro can oligomerize into ringlike structures; oligomerization is stimulated by the γ-TuSC receptor Spc110, which during mitosis localizes to the nuclear side of the spindle pole body and nucleates spindle microtubules. (*b*) γ-Tubulin ring complex (γ-TuRC). In organisms other than budding yeast, including fission yeast and filamentous fungi, γ-tubulin is part of a larger complex, or γ-TuRC, which includes the additional components GCP4, GCP5, and GCP6. Other proteins tightly associated with γ-TuRC are NEDD1/GCP-WD, MOZART1/GCP9, and MOZART2A/B/GCP7/8. γ-TuRC serves as a cap that blocks minus end dynamics. (*c*) CAMSAP/Patronin family proteins associate with free microtubule minus ends. These proteins are deposited on microtubule minus ends when these ends grow and form stretches of stabilized microtubule lattice, which can serve as seeds for microtubule elongation. Importantly, CAMSAP/Patronin family proteins do not bind to tubulin well and do not nucleate microtubules. Abbreviations: Ce, *Caenorhabditis elegans*; Dm, *Drosophila melanogaster*; Hm, *Homo sapiens*.

discussed here. Importantly, when the activity of the key factors required for centriole duplication, such as Plk4 (Polo-like kinase 4), or of structural centriolar components, such as the microtubule-binding protein Sas-4 [also known as CPAP (centrosomal P4.1-associated protein) or CENPJ in humans], is perturbed either pharmacologically or genetically, centrosomes are typically also lost (Basto et al. 2006, Sir et al. 2013, Wong et al. 2015). Analysis of phenotypes associated with such perturbations showed that centrosomes are not strictly required for cell survival. During cell division, centrosomes have a dominant role in organizing bipolar spindles in somatic animal cells. However, even physical ablation of centrosomes with all the associated components shortly before mitosis does not prevent subsequent spindle formation and cell division (Khodjakov et al. 2000). Centrosomes are required, however, for rapid, robust, error-free chromosome separation (for review, see Meraldi 2016). Centrosomes are also needed for formation of astral microtubules (Basto et al. 2006, Bazzi & Anderson 2014), which, in turn, are important for spindle positioning (see Wu et al. 2017 for a recent review). Still, development of flies and mice proceeds surprisingly far in the absence of centrioles (i.e., morphologically normal adult flies are formed, and mice can make it to embryonic day 9.5), and the main defects are caused by the lack of cilia (Basto et al. 2006, Bazzi & Anderson 2014). Furthermore, centrioles play no role in meiotic spindle assembly,

which instead depends on self-organization of multiple acentriolar MTOCs, and the same is true for the first cleavages in mouse embryos (see Courtois et al. 2012 and references therein).

During interphase, the radial, centrosome-based microtubule organization plays a crucial role in positioning of different membrane organelles. Central, pericentrosomal positioning of organelles such as the cell nucleus, the Golgi apparatus, or recycling endosomes requires the activity of the minus end–directed motor dynein. The relative positioning of the centrosome and the nucleus has a role in determining cell polarity (Bornens 2012). For example, in mesenchymal cells, the centrosome and the Golgi apparatus are positioned in front of the nucleus (Luxton & Gundersen 2011), and centrosome repositioning in epithelial cells correlates with epithelial-mesenchymal transition and cell scattering (Burute et al. 2017). In cytotoxic T lymphocytes, centrosome movement to the plasma membrane mediates delivery of secretory granules to the immunological synapse (Stinchcombe et al. 2006).

In addition to such microtubule-dependent cell architecture–related functions, centrosomes act as signaling hubs, display cross talk with DNA damage response pathways, and even organize actin filaments (Conduit et al. 2015b, Farina et al. 2016, Mullee & Morrison 2016). Centriole loss causes a p53-dependent arrest in G1 phase of the cell cycle (Bazzi & Anderson 2014, Wong et al. 2015). Some components of this pathway are known (Fong et al 2016, Lambrus et al. 2016, Meitinger et al. 2016), but it is still not entirely clear whether cell cycle arrest is just a consequence of prolonged mitosis or reflects a more direct connection between the centrosomes and p53-dependent cell cycle control.

Evolution of Centrosomes and Centrosome-Like Structures

The core centrosome components, centrioles, serve as basal bodies for the assembly of cilia and flagella, and as such were likely present in the last eukaryotic common ancestor because they are found in all eukaryotic supergroups (Hodges et al. 2010). However, centrally localized, centriole-containing MTOCs important for cell division appear to be of later origin (Azimzadeh 2014, Gräf et al. 2015). Among animals, the planarian *Schmidtea mediterranea*, which is capable of forming cilia, does not form centrosomes and lacks conserved animal centrosome proteins (Azimzadeh et al. 2012). This could be due to the fact that embryonic development in this worm does not rely on oriented cell divisions and thus does not require astral microtubules, the formation of which depends on centrosomes.

Centrosome-like structures are also present in lineages that lack centrioles, such as fungi and Amoebozoa. In yeast, the role of centrosomes is fulfilled by spindle pole bodies (SPBs), which are embedded into the nuclear envelope, with distinct γ-tubulin receptors present at the two sides to control assembly of cytoplasmic and spindle microtubules (the latter are located in the cell nucleus because yeast have closed mitosis) (for review, see Kilmartin 2014, Lin et al. 2014a). In *Dictyostelium*, a representative of Amoebozoa, the acentriolar MTOC, known as the nucleus-associated body, is a box-shaped three-layered structure surrounded by a PCM-like corona that can concentrate γ-tubulin (Euteneuer et al. 1998). The molecular components of yeast SPBs have been extensively studied (Kilmartin 2014), and some proteins composing the *Dictyostelium* centrosome have been identified (see Meyer et al. 2017 and references therein). Many other organisms, for example, diatoms or Apicomplexa such as *Toxoplasma*, also possess MTOCs (Azimzadeh 2014, Morlon-Guyot et al. 2017), but their molecular makeup is much less understood.

Pericentriolar Material and γ-TuRC Recruitment to Centrosomes

Centrosomes are self-assembling structures, the proteome of which has been well characterized (Andersen et al. 2003, Muller et al. 2010). Super-resolution microscopy studies revealed how

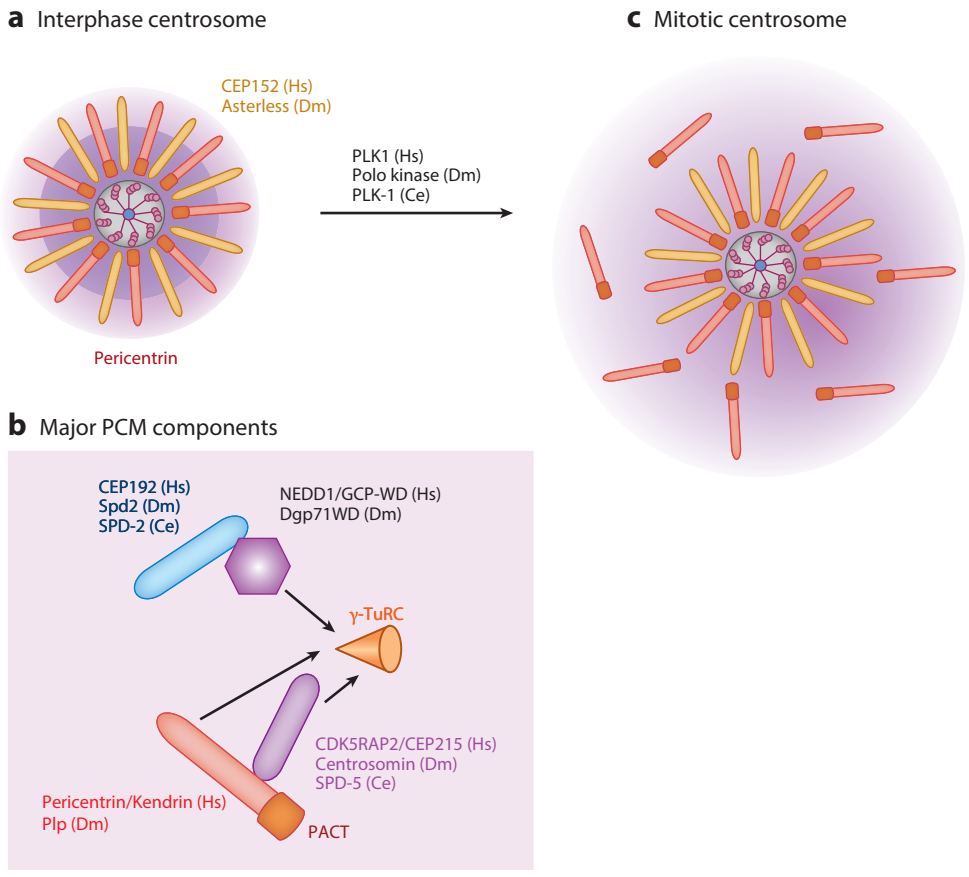

Figure 2

Centrosome organization in interphase and mitosis. (*a*) Organization of pericentriolar material (PCM) around the mother centriole in interphase. Pericentrin and CEP152 form interdigitating elongated fibers extending from the centriole wall; the C-terminal PACT domain of pericentrin is responsible for targeting the protein to the centriole wall. Other PCM proteins form orderly toroid structures around the centriole. (*b*) Major PCM components and their nomenclature in *Homo sapiens* (Hs), *Drosophila melanogaster* (Dm), and *Caenorhabditis elegans* (Ce). NEDD1, CDK5RAP2, and pericentrin can interact with γ-TuRC. CDK5RAP2 is also a potent γ-TuRC activator. The worm SPD-5 is a functional counterpart of CDK5RAP2 but is not a homolog. (*c*) During cell division, mitotic kinases, particularly PLK1, phosphorylate PCM components and promote their polymerization into an extended amorphous matrix.

centrosomes in fly and human cells assemble by attachment of PCM around the mother centriole (Lawo et al. 2012, Mennella et al. 2012) (**Figure 2*a***). One of the key players in PCM organization in mammalian cells is an elongated coiled-coil protein, pericentrin [also known as kendrin in mammals and pericentrin-like protein (Plp) in flies] (Delaval & Doxsey 2010). Centrosome recruitment of pericentrin depends on its C-terminal PACT (pericentrin-AKAP450 centrosomal targeting) domain, which interacts with calmodulin (Gillingham & Munro 2000), but the mechanism of centrosome targeting by PACT is not yet known. In interphase centrosomes, clusters of pericentrin form elongated fibrils that attach with their PACT-containing C terminus to the centriole in a pattern that may follow its ninefold symmetry (Mennella et al. 2012) (**Figure 2*a*,*b***). Pericentrin is

required for efficient recruitment and organization of several other centrosomal proteins, including CDK5 regulatory subunit–associated protein 2 [CDK5RAP2, also known as CEP215 in mammals and centrosomin (Cnn) in flies], CEP192 (Spd2 in flies and SPD-2 in worms), and its binding partner NEDD1 (Neural precursor cell expressed, developmentally downregulated 1; also known as GCP-WD in mammals and as Dgp71WD in flies). These proteins form toroids of different diameters around the centriole (reviewed in Conduit et al. 2015b, Mennella et al. 2014, Woodruff et al. 2014). Another conserved PCM component is CEP152 [Asterless (Asl) in flies], which does not depend on pericentrin and which in flies also forms elongated fibers that interdigitate with those of pericentrin (Mennella et al. 2012) (**Figure 1*a***). Asl/CEP152 does not seem to be essential for interphase PCM organization but controls formation of a scaffold that initiates formation of a new centriole and participates in mitotic PCM assembly (Dzhindzhev et al. 2010; reviewed in Conduit et al. 2015b).

Several PCM components can recruit γ-tubulin. These include pericentrin, the N-terminal part of which binds to γ-TuRC (Takahashi et al. 2002), and CDK5RAP2, which interacts with pericentrin through the conserved C-terminal centrosomin motif 2 (CM2) (Wang et al. 2010), whereas its N-terminal centrosomin motif 1 (CM1) represents a γ-TuRC-binding site (Choi et al. 2010, Sawin et al. 2004). NEDD1 is another factor that tightly associates with γ-TuRC. NEDD1 is essential for γ-TuRC recruitment to centrosomes in human cells (Haren et al. 2006, Luders et al. 2006), although not in flies (see Reschen et al. 2012 and references therein). Interestingly, there is a clear functional difference between the γ-TuRC-binding regions of CDK5RAP2 and NEDD1. The CM1 motif of CDK5RAP2 can not only recruit γ-TuRC but also activate γ-tubulin-mediated microtubule nucleation, even when it is positioned ectopically at the cell cortex; therefore, the γ-TuRC-binding site of CDK5RAP2 is also termed γ-TuRC-mediated nucleation activator (γ-TuNA) (Choi et al. 2010, Muroyama et al. 2016). In contrast, the γ-TuRC-binding region of NEDD1 can recruit γ-TuRC to ectopic sites but does not cause microtubule nucleation; however, NEDD1 is needed for anchoring of microtubules, including those nucleated by CDK5RAP2 (Muroyama et al. 2016).

Other important players in controlling γ-TuRC localization and activity are the small proteins MOZART1 (mitotic spindle–organizing protein associated with a ring of γ-tubulin, or GCP9) and MOZART2A/B (GCP7/8), which participate in γ-TuRC recruitment to the centrosome in mitosis and interphase, respectively (Hutchins et al. 2010, Teixido-Travesa et al. 2010). Recent work showed that MOZART1 binds to the CM1 region of CDK5RAP2, and the loss of MOZART1 affected the integrity of γ-TuRC as well as CDK5RAP2-induced microtubule nucleation (P. Liu et al. 2014). NME7, another factor that copurifies with γ-TuRC, has kinase activity, but its exact function is not yet understood (P. Liu et al. 2014) (**Figure 1*b***).

The regulators and receptors of γ-tubulin are well conserved in evolution. As indicated in the Introduction, budding yeast *Saccharomyces cerevisiae* has a relatively simple microtubule nucleation system. This system includes γ-TuSCs and their receptors Spc110 and Spc72, which are responsible for the SPB-based organization of spindle and cytoplasmic microtubules, respectively. Spc110 is the yeast counterpart of pericentrin and contains a C-terminal calmodulin-binding domain; in addition, it also contains a CM1 domain that directly participates in γ-TuSC oligomerization in a manner that depends on cell cycle–dependent phosphorylation (Lin et al. 2014b; reviewed in Lin et al. 2014a). Spc72 is related to CDK5RAP5, with which it shares a CM1 domain; similar to its fission yeast homolog Mto1, it also has a conserved C-terminal MTOC-targeting sequence (Samejima et al. 2010). Budding yeast lacks γ-TuRC components GCP4, -5, and -6, as well as NEDD1 and MOZART homologs. These proteins, however, are present in other fungi, where they participate in recruitment of γ-tubulin to microtubule nucleation sites, but are often not essential (Masuda & Toda 2016 and references therein). Interestingly, recent detailed analysis of the

MOZART1 homolog in the fungus *Candida albicans* showed that this protein acts together with CM1-containing γ-TuRC receptors to induce efficient oligomerization of γ-TuSCs and thus promote formation of active templates for microtubule nucleation (Lin et al. 2016). Taken together, these findings indicate that γ-tubulin-dependent microtubule nucleation at MTOCs requires a set of conserved components that can be used in different combinations in various species.

Notably, oligomers or rings of γ-tubulin are not sufficient for efficient microtubule nucleation. Formation of steadily elongating microtubule plus ends is positively affected by microtubule polymerases and stabilizers and is inhibited by catastrophe-promoting factors (Wieczorek et al. 2015). Therefore, many plus end regulators—including the members of the XMAP215/ch-TOG family and their partners in the TACC (transforming acidic coiled-coil) family, as well as End-Binding (EB) proteins and their numerous interactors such as CLASPs (Akhmanova & Steinmetz 2015)—can bind to PCM components and are enriched at the centrosomes, where they regulate microtubule formation and stability.

Expansion of Pericentriolar Material in Mitosis

During cell division, the centrosome undergoes maturation, a strongly increased recruitment of PCM components, and as a result, instead of a single orderly PCM layer surrounding the centriole in interphase cells, an extended amorphous PCM is formed during mitosis (**Figure 2*c***). A key regulator of this process is the mitotic kinase PLK1, which localizes to the centrosomes and phosphorylates PCM components to promote their assembly; another centrosomal kinase, Aurora A, also contributes to centrosome maturation (reviewed in Conduit et al. 2015b, Mennella et al. 2014, Woodruff et al. 2014). The molecular details of mitotic PCM assembly differ between species, but the basic principle appears to be the same: Assembly involves formation of an extended scaffold, the core of which consists of a few key proteins that can multimerize. In worms, such key components are the coiled-coil proteins SPD-2 and SPD-5 (the latter is the functional counterpart to, although not a homolog of, CDK5RAP2/Cnn). These proteins are mostly monomeric in the cytoplasm, but phosphorylation of SPD-5 converts it to an assembly-competent state, in which it can form networks, even in a purified form in vitro (Woodruff et al. 2015, Wueseke et al. 2016). Networks of multimerized SPD-5 recruit Plk1 and SPD-2, which in turn accelerate further PCM assembly (Woodruff et al. 2015). SPD-2 also acts as a limiting component that determines centrosome size during early worm development (Decker et al. 2011). Certain features of PCM assembly can be modeled as formation of centriole-organized autocatalytic liquid droplets (Zwicker et al. 2014).

In flies, the CDK5RAP5 homolog Cnn and Spd2 are also the key players in mitotic centrosome assembly (Conduit et al. 2015b). Cnn contains a leucine zipper region, which can multimerize in a manner that is likely dependent on Plk1 phosphorylation; moreover, overexpression of a phosphomimicking Cnn mutant can induce formation of acentrosomal MTOCs (Conduit et al. 2014). The dynamics of mitotic PCM components in fly cells is complex: Cnn is initially recruited to the centriole wall and then undergoes outward flux in a manner that is dependent on microtubules. Cnn recruitment in mitosis depends on Asl and Spd2 (Conduit et al. 2010); Spd2 also initially binds close to the centrioles and shows outward movement, albeit a microtubule-independent one (Conduit & Raff 2015 and references therein). The centriole-dependent recruitment and outward flux could represent a mechanism to regulate centrosome size; however, this mechanism does not appear to be general because SPD-5 in worms incorporates into PCM in an isotropic fashion (Laos et al. 2015).

In mammals, the crucial players in mitotic PCM expansion are pericentrin and CDK5RAP2. Pericentrin is phosphorylated by PLK1, leading to enhanced recruitment of several PCM components (Lee & Rhee 2011). Pericentrin may have some self-assembling properties, as its

overexpression leads to increased PCM accumulation (Loncarek et al. 2008). In interphase, CDK5RAP2 is dependent on pericentrin for centrosome recruitment; however, during mitosis, both proteins depend on each other during PCM expansion (Kim & Rhee 2014). In flies, pericentrin (Plp)-Cnn interaction also plays a role in outer PCM formation, but the function of this interaction is less important than in mammalian cells (Richens et al. 2015). Mammalian CEP192 synergizes with pericentrin and CDK5RAP2 for PCM accumulation (Gomez-Ferreria et al. 2007, Zhu et al. 2008), and its binding partner NEDD1 is needed to recruit γ-TuRC (Haren et al. 2006, Luders et al. 2006).

The exact mechanism of PCM recruitment to the centriole wall remains an open question. During mitosis, the daughter centriole is converted into a mother centriole competent for PCM recruitment, and in both fly and human cells, this process requires sequential loading of several factors, including Cep135, Ana1 (CEP295), and Asl (CEP152) (Fu et al. 2016). Another protein that has been implicated in PCM recruitment is Sas-4/CPAP. This protein is essential for the early steps of centriole formation (reviewed in Avidor-Reiss & Gopalakrishnan 2013). In worms, reduced levels of SAS-4 lead to the reduction of PCM accumulation (Kirkham et al. 2003), and it has been proposed that in flies Sas4 forms complexes with PCM components that are recruited to centrioles (Gopalakrishnan et al. 2012). However, subsequent work has not confirmed corecruitment of Sas4 and PCM proteins; still, the Sas4 pool that is associated with the centriole can participate in recruiting Asl, a factor that is important for PCM binding around the mother centriole (Conduit et al. 2015a). Along the same lines, the mouse Asl homolog Cep152, when expressed together with Plk4, can trigger acentriolar MTOCs in fly oocytes (Coelho et al. 2013). Furthermore, a new worm centriole component, the coiled-coil protein SAS-7, was very recently shown to act upstream of SPD-2 for centriole recruitment and to participate in PCM formation (Sugioka et al. 2017). The full complexity of centriole-dependent PCM recruitment needs to be further unraveled.

Microtubule Anchoring at Centrosomes

Besides participating in microtubule nucleation, another important role of PCM is microtubule anchoring at the centrosome. This function is essential for the maintenance of a radial interphase microtubule array. In contrast, during cell division, although centrosomes are embedded in the spindle poles and drive their formation, the majority of spindle microtubules are not directly anchored at the centrosomes. Instead, spindle microtubule minus ends are assembled into spindle poles through the combined activities of MAPs and the microtubule minus end–directed motors dynein and kinesin-14 (reviewed in Maiato & Logarinho 2014). The minus ends of spindle microtubules undergo slow disassembly, resulting in their continuous poleward translocation, the process termed spindle flux (Rogers et al. 2005). Different centrosomal and spindle pole proteins participate in linking the two structures (reviewed in Chavali et al. 2015); for example, CDK5RAP2 binds to the kinesin-14 HSET (human spleen, embryo, and testes expressed protein), and this complex attaches centrosomes to the pole and drives clustering of supernumerary centrosomes into pseudobipolar spindles (Chavali et al. 2016).

Because microtubule nucleation and anchoring are mechanistically distinct processes, microtubules can be promptly released from their nucleation sites. Experiments with washout of microtubule-depolymerizing drugs such as nocodazole, which are frequently used to assess MTOC activity, can thus be misleading because they can provide information on the cellular sites that can initiate formation of new microtubules but do not necessarily have the capacity to retain them.

The mechanism of microtubule anchoring at the centrosome is traditionally believed to involve capping of microtubule minus ends by γ-TuRCs. Recent work in mammalian cells showed that free, uncapped microtubule minus ends rapidly recruit members of the CAMSAP family,

even when microtubules are nucleated at and subsequently released from the centrosome (Jiang et al. 2014). Because no CAMSAPs can be detected at the centrosome in steady-state conditions, these data suggest that all centrosomal microtubule minus end are indeed capped by γ-TuRC or other factors. An important centrosomal component required for microtubule anchoring is ninein. Ninein, a coiled-coil protein, requires pericentrin for its centrosomal localization (Chen et al. 2014); it participates in γ-TuRC and microtubule retention at the centrosome (Delgehyr et al. 2005). Immobilized aggregates of NOCA-1, the worm counterpart of ninein, can capture microtubule ends in vitro (Wang et al. 2015), but it is not known whether the protein can specifically recognize microtubule minus ends in either a free or a γ-TuRC-bound form. Ninein is enriched at the tips of subdistal appendages of the mother centriole, which are involved in microtubule anchoring (Mogensen et al. 2000), and increased expression of ninein can inhibit microtubule release from the centrosome (Abal et al. 2002).

Other proteins important for microtubule anchoring at centrosomes and SPBs in organisms ranging from yeast to mammals are members of the MSD1/SSX2IP (mitotic spindle disanchored 1/synovial sarcoma, X breakpoint 2 interacting protein) family (for review, see Hori & Toda 2017). In fission yeast, the complex of Msd1 with another conserved protein, Wdr8, is transported to the SBPs by the minus end–directed kinesin-14 and is required for minus end tethering (Yukawa et al. 2015). In vertebrates, MSD1/SSX2IP and Wdr8 also form a complex that interacts with γ-TuRC and that is required for centrosomal microtubule organization; the minus end–directed transport of this complex depends on dynein (Hori et al. 2014, Inoue et al. 2017). Furthermore, several other proteins are required for centrosomal anchoring; these include trichoplein and ODF2, which may cooperate with ninein (Ibi et al. 2011), and EB1 (Askham et al. 2002), which acts together with the centrosomally localized proteins CAP350 and FOP (Yan et al. 2006).

Another major player in both centrosome-dependent microtubule retention and protein delivery to the centrosomes and cilia is cytoplasmic dynein (Balczon et al. 1999, Burakov et al. 2008). Many centrosome and ciliary proteins, including ninein and MSD1/SSX2IP, are delivered to centrosomes as a part of centriolar satellites, mobile protein particles organized by the self-assembling coiled-coil protein PCM-1 (reviewed in Hori & Toda 2017). Other centrosome components such as pericentrin do not associate with centriolar satellites and interact with dynein directly (Purohit et al. 1999). Another pathway of dynein-mediated transport of PCM components, such as γ-tubulin, depends on Rab11-positive recycling endosomes and is active in mitosis (Hehnly & Doxsey 2014). PCM-based organization of microtubule minus ends and dynein-based minus end–directed transport of PCM components thus create a positive feedback loop that supports centrosome formation and maintenance.

Centrosome Perturbations in Human Disease

In agreement with the fact that centrosome function is important but not essential for different cellular processes, mutations in many centriolar and centrosome components do not cause lethality but rather lead to severe development defects. A major type of such developmental disorders is primary autosomal recessive microcephaly (microcephaly primary hereditary), in which patients are born with small brains and simplified gyri and display different levels of intellectual disability (reviewed in Barbelanne & Tsang 2014, Chavali et al. 2014). Some mutations in centrosome proteins also lead to primordial dwarfism, a condition whereby patients are born with small brains and small stature. For example, mutations in the gene encoding pericentrin can lead to dwarfism, microcephaly, and mental retardation (reviewed in Delaval & Doxsey 2010), whereas mutations in the gene coding for CDK5RAP2 cause Seckel syndrome, which is characterized by prenatal proportionate short stature, severe microcephaly, and intellectual disability (Yigit et al. 2015).

Moreover, mutations in genes encoding centrosomal protein CEP152 as well as centriole assembly factors CPAP, STIL, and CEP135 cause microcephaly (Barbelanne & Tsang 2014). The spindle misorientation and mitotic delay caused by centrosome defects likely affect the balance between proliferation and differentiation of progenitor cells or cause their apoptosis, resulting in a smaller number of cells in the brain or the whole body. The observation that most tissues develop relatively normally in these patients suggests that the centrosome is more important for controlling cell numbers than for cell polarity and differentiation.

Increased centrosome numbers are also deleterious because they can cause formation of multipolar spindles, and although such structures are typically transient and evolve into bipolar spindles through clustering of extra poles, they can affect the fidelity of mitosis and result in genome instability, which is often associated with cancer. Moreover, enhancing MTOC function by increasing centrosome numbers promotes invasive behavior of tumor cells (Godinho et al. 2014). These topics and the potential of using centrosome proteins as anticancer targets have been extensively reviewed recently (Maiato & Logarinho 2014, Milunovic-Jevtic et al. 2016) and are not discussed here.

THE GOLGI APPARATUS AS A MICROTUBULE-ORGANIZING CENTER IN MAMMALIAN CELLS

Even in cells with a seemingly radial microtubule system, such as fibroblasts, a significant proportion of microtubules do not converge in a single ~1-μm-large site, indicating that they are not attached to the centrosome. The Golgi apparatus represents the second major mammalian MTOC, which functions in both microtubule nucleation and anchoring (Chabin-Brion et al. 2001; reviewed in Rios 2014, Zhu & Kaverina 2013). In certain types of mammalian cells, such as retinal pigment epithelium cells (RPE1 cells), nearly half of all cellular microtubules initiate at the Golgi apparatus (Efimov et al. 2007). The ability of the Golgi apparatus to organize microtubules is functionally important for several reasons. First, in mammalian cells, Golgi membranes are positioned close to the centrosome by dynein-mediated transport (Corthesy-Theulaz et al. 1992), and Golgi-anchored microtubules help to assemble dispersed Golgi stacks into the Golgi ribbon after mitosis (Miller et al. 2009; reviewed in Rios 2014, Zhu & Kaverina 2013). Second, in contrast to the centrosome, which forms a symmetric array, Golgi-derived microtubule networks are polarized and can thus drive asymmetric vesicle transport and promote overall cell polarity (Hurtado et al. 2011; Vinogradova et al. 2009, 2012). Recent work showed that in mesenchymal cells, the presence of Golgi-attached microtubules accelerates reorientation of the whole microtubule network, including the centrosome, in the direction of migration (Wu et al. 2016). This suggests that the centrosome may be the passenger and not the driver during cell polarization. Golgi-derived microtubules become particularly important when mesenchymal-type migration of cancer cells is examined in a soft three-dimensional matrix (Wu et al. 2016), which represents a more natural substrate for these cells than the conventional hard two-dimensional substrates, such as coverslips. This finding supports the idea that microtubule organization and dynamics are much more important for cell migration in soft 3D substrates than on hard 2D surfaces (Bouchet et al. 2016; reviewed in Bouchet & Akhmanova 2017). Furthermore, Golgi membranes control formation of noncentrosomal microtubule arrays in differentiated cells, including pancreatic β-cells and muscle cells (Oddoux et al. 2013, Zhu et al. 2015). In dendrites of fly neurons, the so-called Golgi outposts regulate neurite outgrowth and branching and are associated with γ-tubulin-binding factors (Ori-McKenney et al. 2012, Yalgin et al. 2015). However, a significant part of γ-tubulin-dependent microtubule nucleation in fly dendrites occurs in a Golgi-independent manner (Nguyen et al. 2014).

The pathway of microtubule organization at the Golgi apparatus is well understood and shows interesting similarities and differences with centrosomes (**Figure 3*a***). The core component

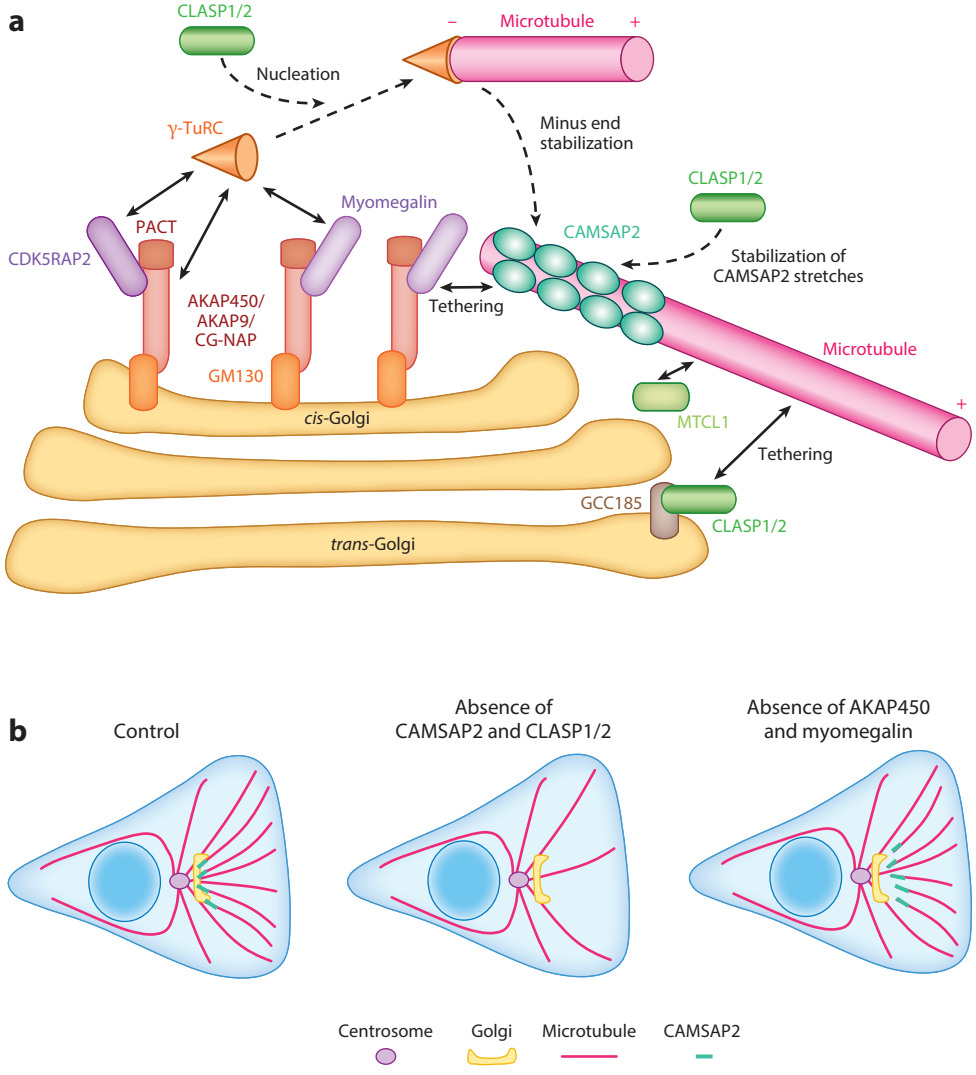

Figure 3

Microtubule organization at the Golgi apparatus. (*a*) Microtubule nucleation and tethering at the Golgi apparatus. GM130, localized to the *cis*-Golgi, binds to AKAP450, a PACT domain protein similar to pericentrin (alternative mammalian protein names are indicated). AKAP450 recruits CDK5RAP2 as well as its homolog, myomegalin. AKAP450, CDK5RAP2, and myomegalin can recruit γ-TuRC; among these, CDK5RAP2 is the most potent, but AKAP450 is sufficient for γ-TuRC recruitment and Golgi-dependent microtubule nucleation. CLASP1/2 promotes microtubule nucleation. Microtubules released from γ-TuRC are stabilized from their minus ends by CAMSAP2; CLASP1/2 promotes formation or stability of CAMSAP2-decorated microtubule stretches. A complex of AKAP450 and myomegalin tethers CAMSAP2-decorated microtubules to the Golgi apparatus; CLASP1/2, which is bound to the *trans*-Golgi through GCC185, and the microtubule binding protein MTCL1 can participate in microtubule tethering. (*b*) Phenotypes associated with the loss of different factors. In control cells, a centrosomal aster and a polarized Golgi-associated microtubule array are present. The latter is lost in the absence of CAMSAP2 or CLASP1/2. In the absence of AKAP450 or myomegalin, CAMSAP2-stabilized microtubule minus ends detach from the Golgi apparatus.

responsible for microtubule organization at the Golgi apparatus is AKAP450 (also known as AKAP9 or CG-NAP) (reviewed in Rios 2014). This large coiled-coil protein is similar to pericentrin, as it has a C-terminal PACT domain, and it can also associate with centrosomes and CDK5RAP2 (Gillingham & Munro 2000, Wang et al. 2010). However, although the role of AKAP450 at the centrosome is relatively minor, it strongly accumulates at the *cis*-Golgi by binding with its N terminus to the Golgi protein GM130 (Hurtado et al. 2011, Rivero et al. 2009). AKAP450 can recruit γ-tubulin either directly, through a binding site in its C-terminal part, or indirectly, by targeting to the Golgi the γ-TuRC-binding proteins CDK5RAP2 and its paralog, myomegalin (Roubin et al. 2013; Wang et al. 2010, 2014; Wu et al. 2016) (**Figure 3*a***). In the absence of AKAP450, no microtubule nucleation at the Golgi takes place (Rivero et al. 2009, Wu et al. 2016).

Interestingly, the complex of AKAP450, CDK5RAP2, and myomegalin is insufficient to anchor microtubules to the Golgi (Wu et al. 2016). An important player in this latter process is CAMSAP2, which forms stable stretches at the free, growing microtubule minus ends (Jiang et al. 2014). These stretches are tethered to the Golgi membranes by the complex of AKAP450 and myomegalin, whereas CDK5RAP2 does not participate in this function (Wu et al. 2016) (**Figure 3*a***). Microtubules that originate from either the Golgi or the centrosome and that have lost their γ-TuRC cap can be stabilized by CAMSAP2 and attached to Golgi membranes through this mechanism.

Another factor important for Golgi microtubule organization is the microtubule plus end–binding and –stabilizing protein CLASP (which is represented in mammals by two paralogs, CLASP1 and CLASP2) (Efimov et al. 2007). CLASPs promote microtubule nucleation at the Golgi, possibly by reducing the kinetic barrier for microtubule outgrowth from γ-TuRC templates (Sanders & Kaverina 2015), and they are also needed for the stabilization of CAMSAP2-decorated microtubule stretches (Wu et al. 2016). CLASPs can also tether microtubules to the Golgi apparatus by binding to the *trans*-Golgi protein GCC185 (Efimov et al. 2007) (**Figure 3*a***). In the absence of CAMSAP2 or CLASPs, the majority of noncentrosomal microtubules are lost, and the only remaining microtubules are those emanating from the centrosome (Efimov et al. 2007, Jiang et al. 2014) (**Figure 3*b***). In contrast, in the absence of AKAP450, numerous noncentrosomal, CAMSAP2-stabilized microtubules are present, but they are not connected to Golgi membranes (Wu et al. 2016) (**Figure 3*b***). Another MAP, MTCL1, which appears to interact with both AKAP450 and CLASPs, also contributes to microtubule tethering to the Golgi apparatus (Sato et al. 2014). Furthermore, the centrosomal protein CAP350 was implicated in the stabilization of Golgi microtubules (Hoppeler-Lebel et al. 2007). Together, these data demonstrate that, whereas microtubule nucleation at the Golgi apparatus is similar to that at the centrosome and uses an overlapping set of factors, microtubule tethering to Golgi membranes is γ-TuRC independent and requires multiple MAPs.

The understanding of the Golgi-microtubule association pathway allowed for critical reassessment of the idea that centrosomal and Golgi-derived microtubules are needed for the assembly of a single Golgi apparatus in mammalian cells. Interestingly, in cells that could neither nucleate nor tether microtubules at the Golgi membranes due to the absence of AKAP450, and that were also depleted of centrosomes by using a Plk4 inhibitor, a single Golgi apparatus could still form and reform from a dispersed state after microtubules were disassembled and repolymerized (Wu et al. 2016). Surprisingly, in these conditions, the Golgi apparatus appeared overly compact and exhibited an increased number of associated vesicles (Wu et al. 2016). These data showed that microtubule-based transport, but not microtubule anchoring at the Golgi, is essential for formation of a single Golgi apparatus; however, association with a dense microtubule network is likely required for normal vesicle transport to and from the Golgi area.

MICROTUBULE ORGANIZATION AT THE NUCLEAR ENVELOPE

In plant cells, as well as in certain differentiated animal cells such as muscle cells, microtubule minus ends are organized at the nuclear envelope (reviewed in Petry & Vale 2015). Plant cells lack centrosomes and instead nucleate microtubules from other microtubules, the cell cortex, or the nuclear envelope (Masoud et al. 2013). Homologs of major PCM proteins such as pericentrin or CDK5RAP2 are absent in plants (Yamada & Goshima 2017). However, the counterparts of MOZART proteins, the GCP3-interacting proteins GIP1 and GIP2, are present and participate in anchoring γ-TuRCs to the nuclear envelope, possibly through a plant-specific transmembrane anchor (Batzenschlager et al. 2013). GIP–γ-tubulin complexes are important for different aspects of plant microtubule organization and contribute to controlling nuclear architecture (Batzenschlager et al. 2014). A plant homolog of NEDD1 was also reported to localize to the nuclear envelope in prophase *Arabidopsis* cells (Zeng et al. 2009).

In muscle cells, the nuclear envelope, which is associated with γ-tubulin, pericentrin, and ninein, functions as an MTOC responsible for both microtubule nucleation and anchoring (Bugnard et al. 2005, Tassin et al. 1985). Pericentrin is redistributed from the centrosome to the nuclear envelope during myoblast differentiation (Fant et al. 2009). LINC (linker of nucleoskeleton and cytoskeleton) complexes, which consist of SUN domain proteins in the inner nuclear membrane and KASH domain proteins in the outer membrane (reviewed in Kim et al. 2015), are good candidates for the recruitment of centrosomal proteins to the nuclear envelope. A recent study reported that Emery-Dreifuss muscular dystrophy patients with mutations in SUN1 and SUN2 have defects in the recruitment of pericentrin to the nuclear envelope in muscle cells; consequently, microtubule nucleation from the nuclear envelope is impaired (Meinke et al. 2014). Myotube nuclei organize microtubules in a semiparallel array, with most microtubules aligned along the long cell axis. Interestingly, muscle microtubules nucleate also from the Golgi membranes, and γ-tubulin and pericentrin have been detected on these membranes (Oddoux et al. 2013), suggesting that the nuclear envelope and Golgi membranes cooperate in microtubule organization in muscle cells.

In yeast, the SPBs are embedded in the nuclear envelope, and yeast SUN and KASH proteins participate in SPB insertion into the nuclear envelope, as well as in SPB duplication and function (see Kim et al. 2015 and Walde & King 2014 and references therein). LINC complexes also play an important role in the indirect connection between the nucleus and centrosomes through the microtubule cytoskeleton (Kim et al. 2015).

MICROTUBULE ORGANIZATION BY CHROMATIN AND KINETOCHORES

During mitosis, microtubule nucleation is strongly increased around chromatin, which stimulates the formation of a gradient of Ran-GTP that in turn activates several spindle assembly factors, such as TPX2 (reviewed in Clarke & Zhang 2008). Besides the Ran-GTP pathway, the chromosomal passenger complex, which consists of Aurora B kinase and several scaffolding proteins, can promote the generation of microtubules from chromatin (Clarke & Zhang 2008). It has been proposed that nuclear pore components bind to chromatin or kinetochores, interact with γ-TuRC, and act as seeds for microtubule assembly (Mishra et al. 2010, Yokoyama et al. 2014). However, it is also possible that a diffuse pool of γ-tubulin concentrated around chromosomes promotes microtubule nucleation or capping. Chromatin-mediated microtubule nucleation depends on NEDD1 (Luders et al. 2006). This function is positively regulated through NEDD1 phosphorylation by Aurora A, which in turn is activated by TPX2 (Pinyol et al. 2013, Scrofani et al. 2015). In addition, TPX2

acts as a scaffold for a subset of γ-TuRCs, which are defined by the presence of a specific cofactor, RHAMM, that is involved in chromatin-dependent microtubule nucleation (Scrofani et al. 2015).

MICROTUBULE GROWTH FROM PREEXISTING MICROTUBULES

In addition to the centrosome and membrane organelles, preexisting microtubules can also nucleate new microtubules. In animal and plant cells, this process critically depends on the evolutionarily conserved complex augmin (reviewed in Sanchez-Huertas & Luders 2015). Augmin plays an essential role in microtubule nucleation from spindle microtubules and thus controls spindle microtubule density (Goshima et al. 2008). In human cells, augmin is composed of eight subunits that are named HAUS (homologous to augmin subunits) (Uehara et al. 2009). In vitro reconstitutions showed that augmin is a Y-shaped structure that has affinity for microtubules (Hsia et al. 2014). In plants, the augmin complex is also composed of eight subunits, two of which are plant specific (Hotta et al. 2012).

Augmin localizes to interphase centrosomes and to mitotic spindle microtubules, and its depletion causes multiple mitotic defects (Lawo et al. 2009, Uehara et al. 2009). Augmin is also critical for organizing spindles in *Xenopus* eggs and in *Arabidopsis* (Ho et al. 2011, Petry et al. 2011). Recently, augmin-based microtubule nucleation was also shown to occur in nonmitotic cells, such as interphase plant cells and neurons (T. Liu et al. 2014, Sanchez-Huertas et al. 2016). The mechanism of how augmin promotes microtubule-based microtubule nucleation was initially believed to depend mostly on γ-TuRC (Petry et al. 2011, Uehara et al. 2009). Use of *Xenopus* egg extracts allowed investigators to observe a strikingly clear branching microtubule nucleation and demonstrated that this process also depends on TPX2, a Ran effector (Petry et al. 2013).

Augmin-nucleated microtubule branches grow toward the plus ends of mother microtubules. In contrast, in fission yeast, where microtubule-based microtubule nucleation is also an important mechanism, the newly formed microtubules grow in the direction opposite to that of the mother microtubule. The key players in microtubule nucleation in fission yeast are Mto1 (a homolog of CDK5RAP2) and Mto2, which together form a multimeric complex that recruits and activates γ-tubulin (Lynch et al. 2014). The antiparallel organization of fission yeast microtubules is determined not by γ-tubulin receptors, but by motors and MAPs (Janson et al. 2007).

MICROTUBULE ORGANIZATION AT THE CELL CORTEX

In plants, a very significant proportion of microtubules is organized into sheetlike arrays associated with the cell cortex, but the mechanisms underlying such organization are incompletely understood (Hamada 2014). In animals, epithelial cells often acquire acentrosomal, longitudinally organized parallel microtubule arrays with microtubule minus ends attached to the cell cortex; in cells showing apico-basal polarity, microtubule minus ends are located at the apical side and the plus ends at the basal side (reviewed in Sanchez & Feldman 2017). Such microtubule organization is very important for cell polarization because plus end– and minus end–directed transport ensures asymmetric distribution of structural and signaling components. Retaining centrosomal microtubule organization in epithelial cells can inhibit cell polarization, for example, by blocking dynein-based apical vesicle delivery (Noordstra et al. 2016).

Both γ-tubulin- and CAMSAP/Patronin-dependent pathways play a role in organizing cortical microtubule minus ends (**Figure 4**). During cell differentiation, cell cycle exit is often accompanied by the loss of centrosome activity. In mouse keratinocytes, such loss occurs because NEDD1 expression is downregulated and the centrosome cannot anchor microtubules (Muroyama et al. 2016). In differentiated epithelial cells, γ-TuRC is relocalized to the apical surface; this event

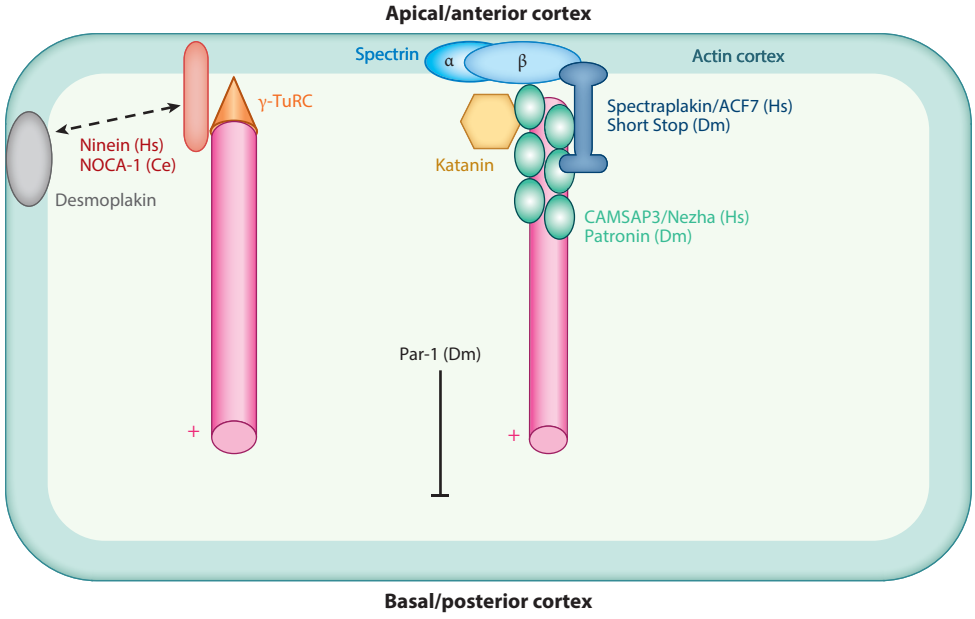

Figure 4

Mechanisms of cortical microtubule minus end anchoring. Microtubule minus ends can be stabilized and anchored at the cortex through two pathways. One of them involves γ-TuRC and ninein. How γ-TuRC is recruited to the apical cortex is not clear, but this process does not require ninein. Ninein can be attached to cell-cell junctions by desmoplakin; how it attaches to the apical cell cortex in epithelial cells is not clear, but γ-TuRC appears to be involved. The second pathway, which operates in human and fly epithelia and the anterior cortex of fly oocyte, involves CAMSAP3 or Patronin, as well as the actin-microtubule cross-linking factor spectraplakin. In fly epithelial cells, spectrin is involved. In fly oocytes, the microtubule-severing protein katanin is also present in the complex; fly spectraplakin is excluded from the posterior cortex through the activity of Par-1 kinase. Abbreviations: Ce, *Caenorhabditis elegans*; Dm, *Drosophila melanogaster*; Hm, *Homo sapiens*.

depends on cortical proteins, such as the polarity regulator Par3 in worms, the transmembrane protein Piopio in flies, and the actin-associated factor Shroom in *Xenopus* (Brodu et al. 2010, Feldman & Priess 2012, Lee et al. 2007). The nature of the γ-tubulin receptors at the cortex requires further elucidation. Furthermore, the microtubule-anchoring protein ninein is an important player in microtubule organization in epithelia; in keratinocytes, it localizes to cell-cell junctions by interacting with desmoplakin (Lechler & Fuchs 2007), whereas in intestinal cells, it binds to the apical cortex (Goldspink et al. 2017). In both mammalian and worm cells, cortical ninein acts downstream of γ-tubulin, possibly by anchoring γ-tubulin-nucleated microtubules (Goldspink et al. 2017, Wang et al. 2015).

An alternative pathway of cortical microtubule anchoring depends on the mammalian protein CAMSAP3 or its invertebrate homolog Patronin. In mammalian intestinal cells, loss of CAMSAP3 causes severe disorganization of apico-basal arrays, although such loss is not lethal in mice (Toya et al. 2016). In hair cells of the organ of Corti, a specific CAMSAP3 isoform colocalizes with cortical noncentrosomal MTOCs (Zheng et al. 2013). Targeting of CAMSAP3 to the cell cortex depends on the spectraplakin ACF7 (Ning et al. 2016, Noordstra et al. 2016) (**Figure 4**). A similar protein complex exists in fly epithelial cells, where spectrins located at the apical cortex

participate in recruitment of the complex of Patronin and the spectraplakin homolog Short Stop (Shot) (Khanal et al. 2016); Shot is, however, not essential for apical localization of Patronin (Nashchekin et al. 2016). Cortical noncentrosomal MTOCs, which contain Patronin, Shot, and the microtubule-severing protein katanin, are also formed in fly oocytes (Nashchekin et al. 2016) (**Figure 4**). Similarly, CAMSAPs and katanin interact in mammalian cells (Jiang et al. 2014), but it is currently unknown whether katanin participates in cortical minus end organization in mammals.

Importantly, genetic work in worm demonstrated that the ninein homolog NOCA-1, which acts together with γ-tubulin, is essential for microtubule organization in the germline but is redundant with Patronin in larval epidermis (Wang et al. 2015). It would be important to find out whether ninein and CAMSAP3 control two redundant pathways of microtubule minus end anchoring in mammals, explaining why CAMSAP3 is not essential in mice.

FUTURE DIRECTIONS: UNDERSTANDING TRANSITIONS BETWEEN DIFFERENT TYPES OF MICROTUBULE ORGANIZATION

The examples described above demonstrate that the activities of different types of MTOCs are strongly regulated during cell cycle progression and differentiation. For example, during mitosis, the activity of the centrosome is upregulated, whereas the ability of the Golgi apparatus to nucleate microtubules is inhibited (Maia et al. 2013). In innate immune cells, centrosomes undergo Plk1-independent maturation in interphase in response to inflammation and promote cytokine production (Vertii et al. 2016). In contrast, in many other differentiating cell types, such as epithelial cells and neurons, centrosomes are switched off; as described above, this process is accompanied by the relocation of γ-TuRC and by downregulation or inactivation of certain PCM components (Muroyama et al. 2016, Yang & Feldman 2015, Yau et al. 2014). During differentiation of neuronal progenitors into neurons, cells switch between centrosome-associated and noncentrosomal ninein splice isoforms (Zhang et al. 2016). Furthermore, CAMSAP2, which is inactive during mitosis due to phosphorylation, becomes active in interphase and is important for the development of dense, noncentrosomal microtubule arrays in differentiating neurons (Yau et al. 2014).

Competition between different structures with MTOC properties may play a role in controlling the balance between their activities. For example, whereas γ-tubulin is difficult to detect at the Golgi apparatus in normal cells, it becomes clearly enriched at Golgi membranes of cells in which centrioles and centrosomes are removed due to Plk4 inhibition (Wu et al. 2016). Interestingly, cells lacking centrosomes form unfocused, noncentrosomal microtubule arrays that depend on CAMSAP2; however, cells that lack both centrioles and CAMSAP2 assemble a single acentriolar PCM cluster, which drives formation of a radial microtubule array (Wu et al. 2016). These data emphasize the self-assembling properties of PCM and show that microtubule minus end stabilization pathways can compete with each other. Future studies will reveal how cells employ posttranslational modifications, expression of specific regulators, and competitive relationships between microtubule minus end–organizing sites to control specific types of microtubule arrays.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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