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Microtubule-organizing centers: from the centrosome to non-centrosomal sites

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Abstract

The process of cellular differentiation requires the distinct spatial organization of the microtubule cytoskeleton, the arrangement of which is specific to cell type. Microtubule patterning does not occur randomly, but is imparted by distinct subcellular sites called microtubule-organizing centers (MTOCs). Since the discovery of MTOCs fifty years ago, their study has largely focused on the centrosome. All animal cells use centrosomes as MTOCs during mitosis. However in many differentiated cells, MTOC function is reassigned to non-centrosomal sites to generate non-radial microtubule organization better suited for new cell functions, such as mechanical support or intracellular transport. Here, we review the current understanding of non-centrosomal MTOCs (ncMTOCs) and the mechanisms by which they form in differentiating animal cells.

Introduction

Microtubules adopt specific spatial arrangements in differentiated cells to perform diverse cellular functions. Early electron microscopy revealed distinct subcellular sites from which microtubules appeared to emanate which were named ‘microtubule-organizing centres’ (MTOCs) [1,2]. Since then, the exact nature of MTOCs has remained somewhat nebulous.

Microtubules have inherent structural polarity, with a dynamic plus end and a comparatively stable and slow-growing minus end [3]. These characteristics of microtubule minus ends are, in part, a function of microtubule structure, but can be influenced *in vivo* by an association with an MTOC. MTOCs can be broadly defined as sites that localize microtubule minus ends, with functions that include microtubule nucleation, stabilization, and/or anchoring.

The best-studied MTOC is the centrosome, a non-membrane bound organelle composed of two centrioles surrounded by pericentriolar material (PCM). The centrosome is often touted as ‘the major microtubule-organizing center of the cell,’ generating a radial organization of microtubules well suited for the division of genomic material between daughter cells. Microtubules are nucleated and anchored within the PCM in dividing animal cells to generate the classic ‘mitotic halo,’ and similar radial arrays form in migrating animal cells

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[4] (reviewed in [5,6]). Microtubules can also be anchored at centriolar appendages; an astral interphase array is generated by subdistal appendages decorating the mother centriole and a lattice-like organization is anchored by and potentially nucleated from the basal foot of basal bodies in multiciliated cells for coordinated ciliary beating [7-12]. As we will discuss below, differentiated cells often generate alternative microtubule organization through the reassignment of MTOC function to non-centrosomal sites following cell division.

Non-centrosomal MTOCs (ncMTOCs)

Differentiated animal cells often establish non-centrosomal MTOCs (ncMTOCs) (Figure 1). In many epithelial cells, MTOC function localizes apically, generating microtubules organized along the apical-basal axis; specific examples include *C. elegans* embryonic intestinal cells, *Drosophila* tracheal, oocyte follicle, and embryonic epithelial cells, *Xenopus* neural epithelial cells, mouse cochlear supporting cells, and various mammalian epithelial cell lines [8,13-20]. Cortical MTOCs have also been observed in mouse and *C. elegans* epidermal cells, and *C. elegans* germ cells [21-24]. Epithelial microtubule arrays appear to be required for organelle positioning and the initiation of apical-basal polarity [13,19,24,25]. In contrast to in epithelia, MTOCs and microtubules in differentiated muscle cells are organized around nuclei and in the cytoplasm parallel to the long axis of the cell [26,27]. Such microtubule arrays in *Drosophila* are essential for nuclear positioning and anchoring [28]. In *Drosophila* oocytes, microtubules grow from the anterior/lateral cortex with plus ends concentrated posteriorly, an arrangement that is critical for directing the localization of mRNAs that establish the embryonic body axis [29] (reviewed in [30]).

In neurons, microtubules are distributed down the lengths of axons and dendrites and are essential for transport, regeneration, and development (reviewed in [31]). Axonal microtubules are uniformly arranged with their plus ends towards the tip, and dendrite microtubules have mixed polarity in vertebrate neurons or a minus end out orientation in *C. elegans* and *Drosophila* [32-34]. A specific ncMTOC in neuronal processes has been elusive. In *Drosophila* class IV dendritic arborization neurons, Golgi outposts appear to act as ncMTOCs in some dendrites [35]. However, removal of Golgi outposts from dendritic arbors had little effect on microtubule organization [36]. Interestingly, in non-neuronal cells, Golgi and mitochondria have also been reported as MTOCs [37,38]. Microtubules in neurons might also arise from the sides of pre-existing microtubules, a scenario that would provide a polarized template to orient newly forming microtubules [39].

Some organisms, including yeast and higher plants, lack centrosomes altogether, thus microtubule organization by definition is non-centrosomal (Figure 1). Yeast have an analogous structure to the centrosome called the spindle pole body (SPB). Although the SPB is the only MTOC in budding yeast, in fission yeast, ‘interphase MTOCs’ generate non-SPB microtubules in the cytoplasm, on the nucleus, and on other microtubules, and ‘equatorial MTOCs’ organize microtubules around the cell division site at the end of mitosis (reviewed in [40,41]). Finally, cells of higher plants completely lack centrosomes or analogous structures, yet have elaborate cortical microtubule arrays that appear to be largely generated by microtubule-based microtubule nucleation and are required for growth and

morphogenesis [42,43]. Despite the wide range of ncMTOCs in many diverse cell types, their composition and mechanisms of assembly are just beginning to be understood.

ncMTOC structure and composition

The complexity of the centrosome obscures our understanding of the specific structure or proteins that alone impart MTOC function. Similarly, the structure or composition of an ncMTOC is unclear. ncMTOCs could be layered structures composed of shells of proteins, similar to PCM (reviewed in [44]) or could be composed of discrete populations of microtubules held together by site-specific adapters (Figure 2). In either case, ncMTOCs should in principle contain 1) proteins that interact with microtubule minus ends, and 2) adapter proteins that link these proteins to specific subcellular sites.

(1) Microtubule minus end proteins

Unlike the large number of proteins that have been shown to interact with microtubule plus ends, only a handful of minus end-associated proteins has been identified. These proteins act as microtubule nucleators, stabilizers, and anchors, examples of which are discussed below.

Nucleators: γ–tubulin ring complex (γ–TuRC)—Although it is clear that microtubules form spontaneously in the absence of accessory proteins, nucleators help to enhance microtubule assembly and so are key components of MTOCs. γ–tubulin was the first microtubule minus end protein discovered and was shown to play a role in centrosomal microtubule nucleation; a γ–tubulin mutation in *Aspergillus nidulans* blocks mitotic spindle assembly and γ–tubulin depletion inhibits microtubule growth from the centrosome *in vitro* [45-48]. γ–tubulin is part of a larger complex, termed the γ–tubulin ring complex (γ–TuRC), which nucleates microtubules and inhibits their minus end growth and depolymerization [49,50]. However, γ–TuRC may not be the only complex involved in microtubule nucleation as microtubules are still nucleated in *Drosophila* and *C. elegans* cycling cells after γ–tubulin depletion, albeit at a reduced rate [51-53].

The role of γ–TuRC in nucleating non-centrosomal microtubules has often been inferred from localization observations. For example, γ–tubulin localizes to the cell cortex of *C. elegans* germ cells, at hemidesmosomes of *C. elegans* epidermal cells, surrounding nuclei of cultured muscle cells, at the Golgi membrane in RPE1 cells, and along the apical membrane of *Drosophila* tracheal cells, *C. elegans* intestinal cells, and Caco-2 and WIF-B epithelial cell lines [13,14,20,23,24,27,37,54]. Microtubules appear to regrow from these sites following induced depolymerization, suggesting that γ–TuRCs might control microtubule nucleation there. Indeed, alterations in γ–tubulin expression suggest that γ–TuRC nucleates non-centrosomal microtubules in the axons and dendrites of neurons, at the nuclear envelope in myotubes, and from Golgi membranes in RPE1 cells [27,35-37,39,55]. Altogether, these data suggest a microtubule nucleation function of γ–TuRC at ncMTOCs, but do not rule out its role in stabilization and/or capping. Regardless of the exact function of γ–TuRC in ncMTOCs, other microtubule minus end proteins must exist because not all minus ends associate with γ–TuRCs [53,55].

Stabilizers: The CAMSAP/Patronin family—In addition to nucleating microtubules, MTOCs stabilize microtubules through an association with minus end stabilizing proteins. The CAMSAP/Nezha/Patronin family, characterized by evolutionarily conserved CKK domains, has been shown to specifically serve this function for non-centrosomal microtubules (reviewed in [56]). Like γ–tubulin, CAMSAP/Patronin has been shown to stabilize and protect microtubule minus ends from depolymerization [57,58]. However, unlike γ–tubulin, CAMSAP/Patronin does not nucleate microtubules or associate with centrosomes, and its depletion specifically reduces non-centrosomal microtubule number and/or organization in cultured cells and in living organisms [19,25,55,58,59]. CAMSAP/Patronin is predicted to form stabilized ‘seeds’ of microtubules for microtubule outgrowth; more specifically, CAMSAP/Patronin stabilizes polymerizing minus ends, slowing the rate of minus end depolymerization and increasing the rate of microtubule plus end outgrowth [15,57,58]. The ability of CAMSAP/Patronin to support microtubule plus end outgrowth may obviate the need for microtubule nucleators at ncMTOCs, and could instead depend on microtubule-severing enzymes to amplify minus ends. This mechanism has been proposed for microtubule outgrowth from Patronin foci at ncMTOCs in *Drosophila* oocytes [15]. However, the possibility remains that CAMSAP/Patronin stabilizes microtubules nucleated and released by γ–TuRC, as CAMSAP2 localizes to the minus ends of microtubules released from the centrosome in cultured epithelial cells [58].

Anchors: Ninein—Newly nucleated and stabilized microtubules need a mechanism for anchorage at MTOCs. In theory, nucleators and stabilizers could themselves anchor minus ends, but proteins specific for anchoring likely exist. The coiled-coil protein ninein has not been shown to interact directly with microtubule minus ends, but appears to anchor minus ends in many contexts. Ninein was first identified as a centrosomal protein localizing to subdistal appendages of the mother centriole, and thereafter was described to have a microtubule anchoring function: ninein overexpression in mouse fibroblasts inhibited microtubule release from the centrosome and ninein inhibition in U2OS cells resulted in a perturbation in microtubule organization [8,60-62]. Ninein interacts directly with γ–TuRC and potentially recruits it to the centrosome; however, ninein's microtubule anchoring capacity appears to be separable from its ability to localize γ–TuRC to the centrosome [63].

Ninein is also hypothesized to anchor microtubules at non-centrosomal sites. Ninein localizes near microtubule minus ends at apical sites of mouse cochlear cells, at the cell cortex in differentiated mouse epidermal cells, and surrounding nuclei in myotubes [8,21,27]. A putative ninein homologue in *C. elegans* (NOCA-1) localizes exclusively to ncMTOCs and is important for microtubule organization at these sites [25,64]. Strikingly, *noca-1* mutants exhibit severe sterility and gonad morphology defects, and microtubules in adult germ cells are highly disorganized [25]. In epidermal cells, NOCA-1 depletion also perturbs microtubule organization, but to a lesser extent due to an apparent parallel function of PTRN-1 in maintaining these arrays [25].

(2) Site-specific adapters

The attachment of microtubules to a specific subcellular site requires site-specific adapters that interact with minus end proteins (Figure 2A). Several putative adapter proteins have

been identified in different cell types, although in most cases a direct link to minus end proteins has not been demonstrated. In *Drosophila* tracheal cells, the transmembrane protein Piopio is required to localize γ–TuRC to the apical membrane [14]. CAMSAP3/Nezha was originally discovered because of its association with the Zonula Adherens protein PLEKHA7, depletion of which mislocalizes CAMSAP3 from adherens junctions [59]. In *Drosophila* oocytes, the actin binding protein Short stop recruits Patronin foci to the cortex, which in turn localize microtubules [15]. Finally, in differentiated keratinocytes, ninein localization to desmosomes requires the desmosome component desmoplakin [21].

The interplay between minus end proteins and adapters at ncMTOCs is an open and exciting question in the field. Pairwise localization studies of γ–TuRC, CAMSAP/Patronin, and ninein *in vitro* and *in vivo* suggest that their localization is independent of one another and/or non-overlapping, and genetic studies in *C. elegans* epidermal cells suggest that NOCA-1 and PTRN-1 operate in parallel pathways [15,21,25,59]; however, biochemical studies and higher resolution microscopy will be required to better clarify this issue. It is likely that additional minus end proteins and adapters exist, the discovery of which will provide significant insight into MTOC biology.

ncMTOC formation

ncMTOC formation requires the attenuation of MTOC function at the centrosome, the designation of a non-centrosomal site, and the proper localization of MTOC components to that site. The mechanisms of ncMTOC site designation are largely unknown and the localization of MTOC components depends on whether the ncMTOC simply anchors or both anchors and nucleates microtubules. Early studies predicted a division of labor between microtubule assembly and localization, suggesting that microtubules in differentiated cells might be nucleated at the centrosome, released, and then captured at non-centrosomal sites (reviewed in [65]) (Figure 3A). This mechanism necessitates the reassignment of anchoring function to a non-centrosomal site coupled with a mechanism for transporting microtubules there. Alternatively, nascent ncMTOCs might form at sites away from the centrosome, such as on residual centrosomal microtubules, which could facilitate transport to non-centrosomal sites (Figure 3B). Finally, ncMTOCs might form independently of centrosomes, with microtubules growing directly from and remaining at non-centrosomal sites (Figure 3C).

(1) Attenuation of MTOC function at the centrosome

The attenuation of MTOC function at the centrosome can generally be considered as the process of removing microtubule nucleation and/or anchoring potential. The degree to which either of these occurs depends on the cell type. For example, cells exclusively containing ncMTOCs completely inactivate microtubule nucleation and anchoring at the centrosome as is seen in myotubes, rat and *Drosophila* neurons, and in some *C. elegans* and *Drosophila* epithelial cells [27,66,67]. Centrosomes in *C. elegans* embryonic intestinal cells or *Drosophila* tracheal cells lose PCM association and the ability to nucleate microtubules [13,14,54,68]. In both cell types, γ–TuRCs move away from the centrosome, and in *Drosophila* tracheal cells, this release requires the microtubule severing protein spastin. Interestingly, the inactivation of MTOC function at the centrosome in *C. elegans* embryonic

intestinal cells does not appear to be permanent, since centrosomal MTOC function in a differentiated cell can be rapidly reactivated upon fusion with a mitotic cell [54].

Alternatively, attenuation of MTOC function at the centrosome might involve the retention of nucleating potential and the inactivation of anchoring function. Loss of anchoring function at the centrosome has been inferred from localization studies, for example in cochlear and epidermal cells where γ–tubulin is exclusively localized to the centrosome and anchoring proteins such as ninein are localized to non-centrosomal sites [8,21]. Mouse keratinocytes were shown to have distinct microtubule nucleating and anchoring complexes CDK5RAP2-γ–TuRC and Nedd1-γ–TuRC, respectively [69]. During keratinocyte differentiation, centrosomes inactivate anchoring function concomitant with a loss of Nedd1, suggesting that centrosomal MTOC attenuation is partly due to the loss of this factor [69]. Attenuation appears to be coupled to cell cycle exit as serum starvation or CDK inhibitor treatment induced loss of Nedd1–γ–TuRC from the centrosome [69].

(2) Activation of MTOC function at non-centrosomal sites

The attenuation of MTOC function at the centrosome must be paired with the designation and activation of MTOC function at a non-centrosomal site. This process undoubtedly begins with a change in cell state that is permissive for the association of microtubules with new sites. For example, mitotic cytoplasm can rapidly remove the apical ncMTOC in an intestinal epithelial cell, indicating that MTOC location is responsive to cell cycle state [54]. The rapidity of this switch suggests that post-translational modifications might control MTOC location. Activation of MTOC function at non-centrosomal sites can also be coupled to differentiation. For example, in *Drosophila* tracheal cells, a transcription factor required for tracheal fate specification, Trachealess, and its target, Piopio, are required for apical ncMTOC formation [14]. Whether this requirement for differentiation relies solely on the transcription of specific adaptors or might also involve changes in post-translation modification of MTOC components remains to be tested.

Once the cell has achieved a state permissive for ncMTOC formation, MTOC components need to become properly localized. Cells that use a ‘release and capture’ mechanism must relocate anchoring proteins (Figure 3A). For example, in differentiating mouse cochlear cells, ninein localization is initially restricted to the centrosome early in development, but later localizes to cytoplasmic, and then apical sites [70]. It is tempting to speculate that ninein is released from the centrosome and moved apically, however, live imaging and/or photomarking experiments in differentiating cells would be needed to test this hypothesis. Ninein has been shown to traffic along microtubules in cultured cells lines, suggesting that ncMTOC formation involves ninein transport along centrosomal microtubules to the apical surface [70]. However, this model is complicated by the fact that cells would have to both release anchoring factors from the centrosome and retain microtubules on which to transport them.

Cells with ncMTOCs capable of both microtubule nucleation and anchoring inactivate these functions at the centrosome and transfer them to the non-centrosomal site. Imaging studies in *C. elegans* suggest that this task is accomplished through the hand-off of a physical ‘plume’ of γ–TuRCs and microtubules from the centrosome to the membrane, potentially

coupling centrosome inactivation and ncMTOC establishment [13]. Plume formation requires the centrosome, microtubules, and the conserved polarity protein PAR-3 [13]. Interestingly, γ–TuRCs and microtubules still form following PAR-3 depletion but at apparently random locations, indicating that in the absence of a specific 'landing pad', these factors coalesce at inappropriate sites [13,71]. γ–TuRC proteins also move from the centrosome to the apical surface of *Drosophila* tracheal cells [14]. As in *C. elegans*, the initiation of this process requires microtubules, however, in both systems microtubules become dispensable for apical γ–TuRC localization later in development [13,14]. The exact role of microtubules in ncMTOC formation is unclear. Residual centrosomal microtubules may serve as a site for nascent ncMTOC formation and its subsequent transport (Figure 3B). Consistently, microtubule-based microtubule nucleation has been observed in the mitotic spindle, *in vitro*, and in plant epidermal cells, and is postulated to occur in axons and dendrites of cultured mature neurons [39,42,72,73]. In these contexts, the protein complex augmin is thought to mediate nucleation from existing microtubules. Alternatively, microtubule nucleating factors might be directly recruited to non-centrosomal sites, nucleating microtubules, which in turn help transport additional nucleating, stabilizing, and/or anchoring factors (Figure 3C).

Concluding remarks

Although ncMTOCs are likely found in the majority of differentiated cells *in vivo*, we are just beginning to understand their composition and assembly. Indeed, we still have much to learn about how cells select and activate non-centrosomal sites as MTOCs, and about ncMTOC composition and function. As non-centrosomal microtubules are critically important for cell function, further studies of ncMTOCs will enhance our basic understanding of cell differentiation. Furthermore, MTOC activity is implicated in human disease. Hyperactive MTOC function at the centrosome has been linked to several types of epithelial cancers and is a hallmark of breast tumors [74-76]. Additionally, enhanced microtubule nucleation at the centrosome is linked to invasive cell behavior [77]. Thus, understanding how cells control their microtubule organization might give us new insight into diseases such as cancer.

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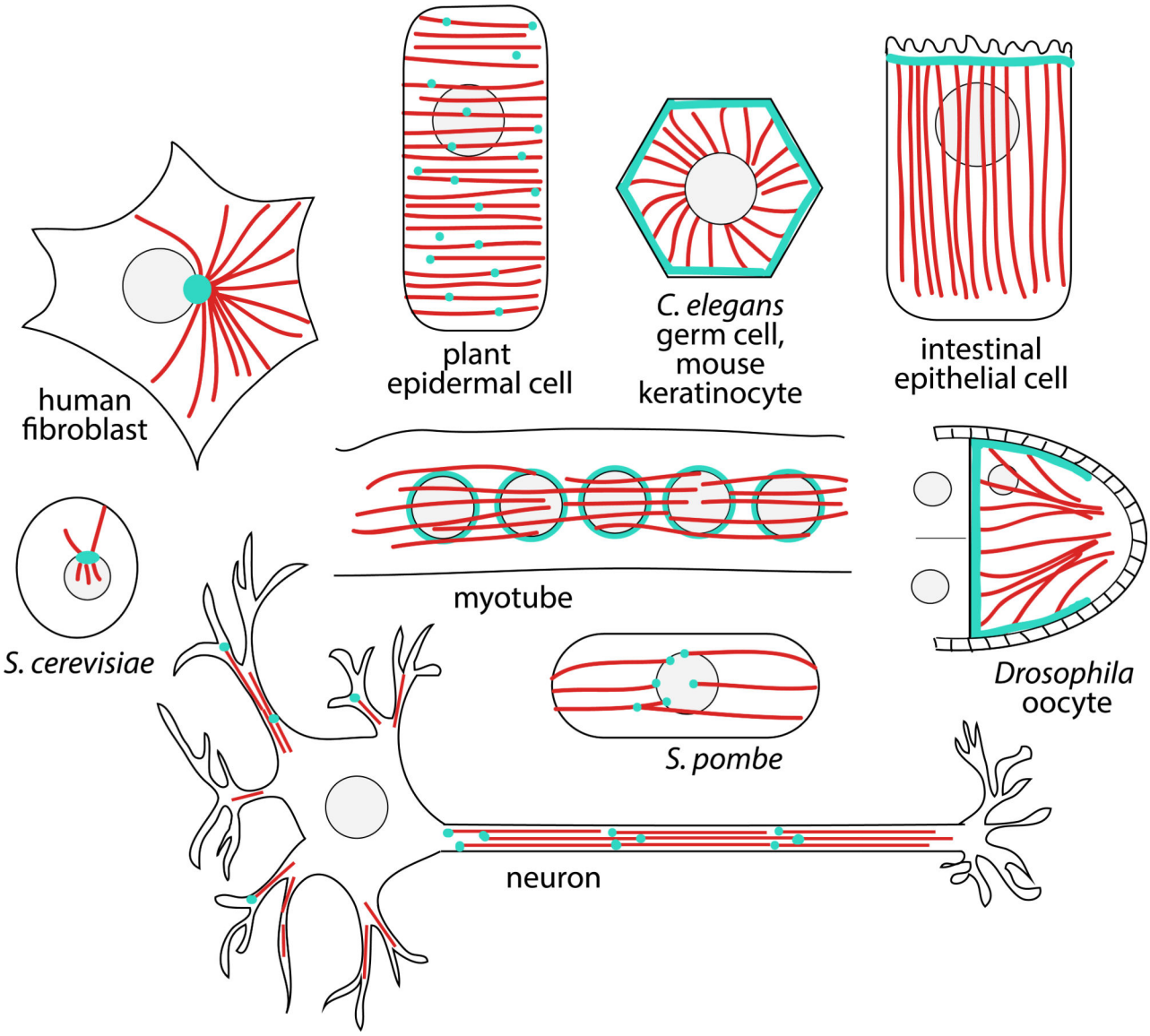

Figure 1. Organization of MTOCs and microtubules in a variety of cell types

Microtubules (red) are organized by MTOCs (blue), the arrangement and localization of which varies with cell type. Drawings are not to scale.

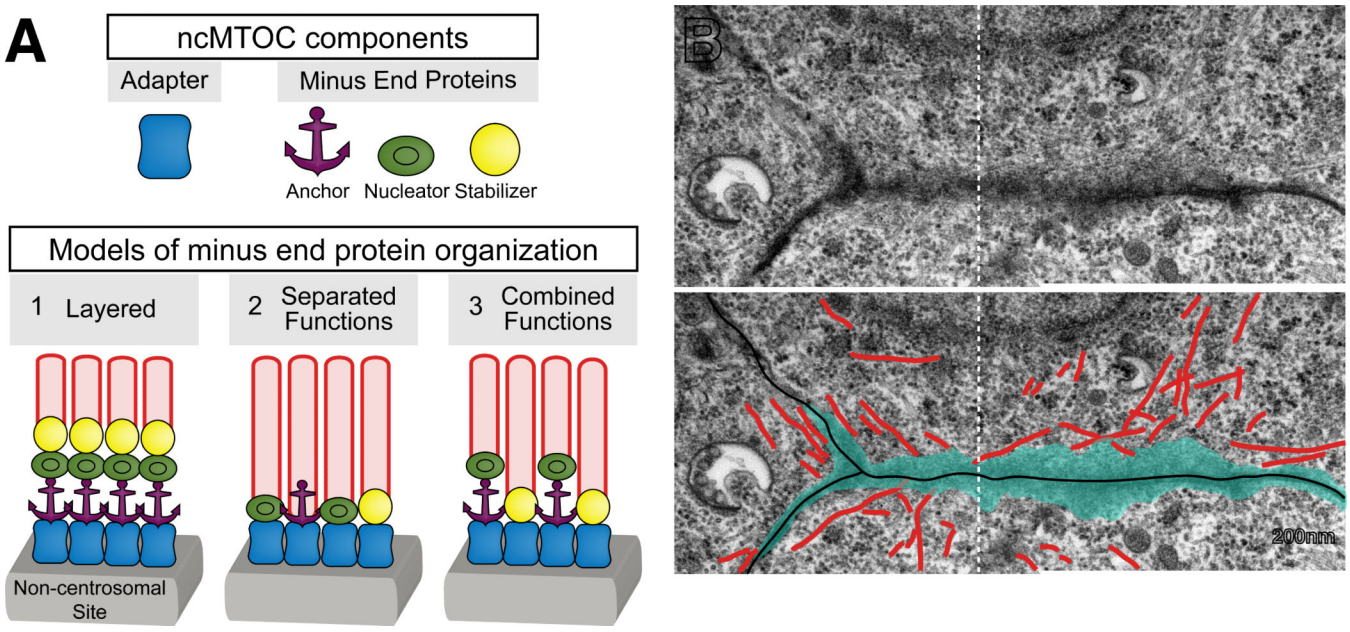

Figure 2. ncMTOC structure and composition

(A) Cartoons depicting ncMTOC components and models for their arrangement at non-centrosomal sites. Cell-type specific adaptors (blue) bound to non-centrosomal sites (grey) interact with microtubule minus end proteins that anchor (purple), nucleate (green) and/or stabilize (yellow) microtubules (red). (1) Minus end proteins might be layered on top of adapters, which function together to sustain microtubules. (2) Alternatively, different minus end proteins could localize to independent minus ends, distributing their function between different populations of microtubules. (3) Finally, minus end proteins might colocalize at the same microtubule ends, functioning together to promote microtubule nucleation, anchoring, and/or stabilization. For example, NOCA-1 and γ-tubulin colocalize on microtubules in the *C. elegans* larval epidermis, but PTRN-1 does not and functions in a parallel pathway [25]. (B) An electron microscopy image of ncMTOCs (blue) at the apical membrane in *C. elegans* embryonic intestinal cells. Electron dense material (blue) is visible at the apical surfaces of three cells from which microtubules (red) emanate (partially reproduced from [54]). Note that two separate electron microscopy images have been overlaid (white dotted line).

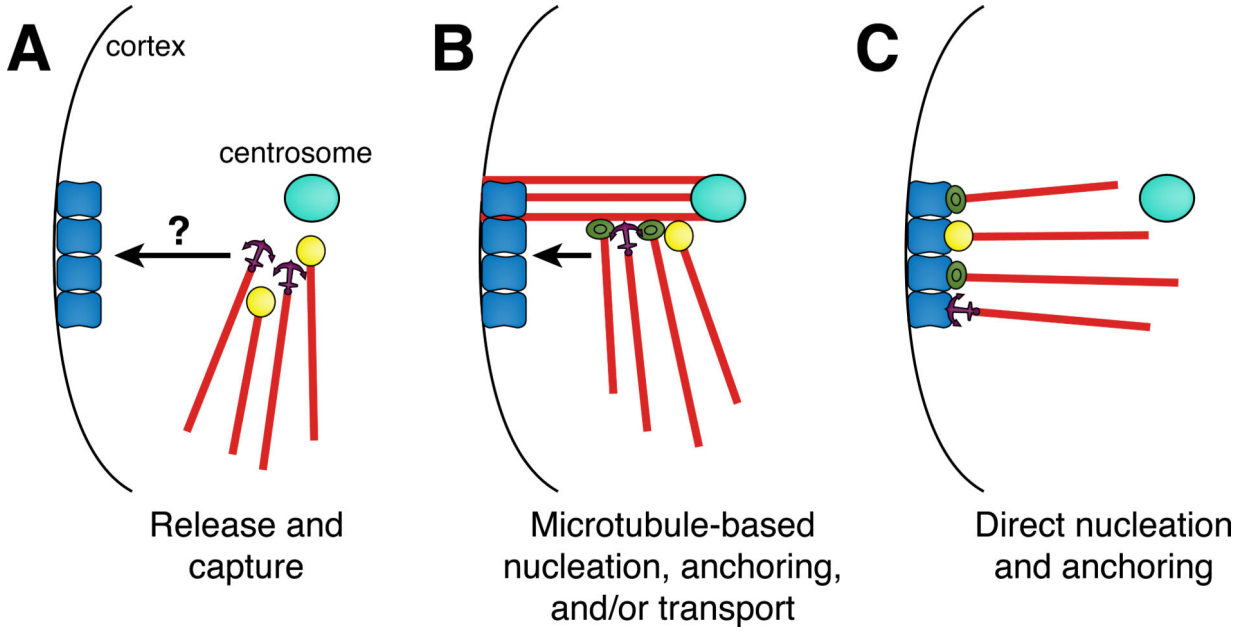

Figure 3. Potential mechanisms for ncMTOC formation

(A) A division of labor model in which microtubules are nucleated at the centrosome, released, and then captured at a non-centrosomal site. Microtubules could be released with anchoring proteins attached or free minus ends could bind to anchoring and/or stabilizing proteins following their release. Microtubules are then transported to a non-centrosomal site via an unknown mechanism and captured by site-specific adapters. (B) Non-centrosomal microtubules could be nucleated, stabilized, and/or anchored from the sides of preexisting centrosomal microtubules and then transported along microtubules to non-centrosomal sites where they would interact with site specific adapters. (C) Microtubule minus end proteins could localize directly to non-centrosomal sites without a centrosome-based intermediate, where they would nucleate, stabilize, and anchor microtubules.