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Mini-review and Review

Organization of Non-centrosomal Microtubules in Epithelial Cells

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ABSTRACT. **Polarized epithelial cells contain a characteristic array of microtubules in which non-centrosomal microtubules are aligned along the apical-to-basal axis of the cell with their minus ends oriented towards the apical pole. Although this unique orientation of microtubules was discovered in the late 1980s, how this orientation is established remains unresolved partly because of limited information about molecular factors that regulate the minus ends of non-centrosomal microtubules. Recent studies, however, identified novel minus end–associated proteins, revealing mechanisms by which the polarized arrays of microtubules are established in epithelial cells. These studies have also demonstrated the importance of apico-basally orientated microtubules in intra-structural organization of cells. This review focuses on recent progress of our understanding of the molecular basis for epithelium-specific microtubule assembly and function.**

Key words: non-centrosomal microtubule, epithelial cell, microtubule minus end, CAMSAP, Patronin

Introduction

Microtubules play pivotal roles in fundamental cellular processes such as chromosome segregation, intracellular transport, directional migration, and cell morphogenesis. An individual microtubule is a 25-nm-diameter hollow tube consisting of 13 tubulin filaments in most cases (Pierson *et al.*, 1978; Tilney *et al.*, 1973). Each filament is built from heterodimers of α and β-tubulin, giving rise to distinct structures at the minus and plus ends of the microtubule. Microtubules are dynamic, i.e., they grow and shrink, an attribute called 'dynamic instability' (reviewed in Desai and Mitchison, 1997). Plus and minus ends behave differently, that is, the plus end grows faster and more dynamically than the minus end. *In vivo*, microtubule polarity and dynamics depend on cell type or cell cycle stage. For example, during directional migration of cells, the plus ends of microtubules are oriented towards the leading edges. In axons of neurons, the plus ends of microtubules face distally, whereas in dendrites microtubule alignment shows mixed polarity. In polarized epithelial cells, microtubules are aligned along the apical-to-basal axis, with the minus ends oriented towards the apical side (Fig. 1). In dividing cells, spindle microtubules assemble with their minus ends gathered toward the two poles and plus ends attached to kinetochores or facing the overlapping region in the middle of the spindle. A number of proteins that control plus-end dynamics have been identified, and their functions have been extensively studied (reviewed in Akhmanova and Steinmetz, 2008, 2015; Howard and Hyman, 2003; Mimori-Kiyosue, 2011; Willige *et al.*, 2016). In contrast, our knowledge of minus-end organization is limited, although it is expanding (reviewed in Akhmanova and Hoogenraad, 2015; Kollman *et al.*, 2011).

The centrosome is traditionally considered as the major microtubule organizing center where microtubules nucleate and their minus ends are tethered, resulting in a radial extension of microtubules from this subcellular structure (Mogensen *et al.*, 2000) (Fig. 1). However, in certain cell types such as neurons, epithelial cells, and myoblasts, the centrosome does not serve as the microtubule organizing center; instead, other mechanisms or structures promote microtubule growth (reviewed in Conde and Cáceres, 2009; Guerin and Kramer, 2009; Keating and Borisy, 1999; Müsch, 2004). Non-centrosomal organization of microtubules is also observed in the unicellular fission yeast (reviewed in Hagan, 1998; Sawin and Tran, 2006) as well as in plant cells that do not have centrosome (reviewed in Ehrhardt and Shaw, 2006; Hamada, 2014). Even during spindle organization, meiotic spindles in the oocyte form without the centrosome (reviewed in Compton, 1998). Thus, in many cell types, formation of microtubule arrays

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Abbreviations: γTuC, gamma tubulin complex; γTuRC, gamma tubulin ring complex; CAMSAP, calmodulin-regulated spectrin-associated protein; PTRN-1, patronin-1; NOCA-1, non-centrosomal array 1; PLEKHA7, pleckstrin homology domain containing family A member 7.

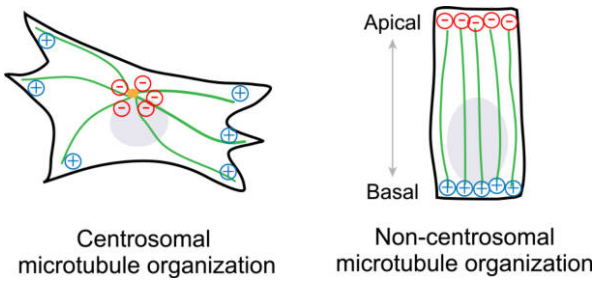

Fig. 1. Centrosomal and non-centrosomal organization of microtubules in mammalian cells. In many cell types like fibroblasts (left), microtubules (green) emanate from the centrosome (orange dot). Their minus ends (red, –) are anchored to the centrosome, and plus ends (blue, +) spread towards cell edges. In differentiated epithelial cells (right), the minus ends do not associate with the centrosome but rather accumulate around apical cortices of the cell, with the plus ends being oriented towards the basal side. These longitudinal microtubules align along the apical-to-basal axis of the cell. Nuclei are shown in gray.

does not depend on the centrosome. Although the mechanisms for the centrosome-dependent organization of microtubules have been studied extensively, those for non-centrosomal microtubule growth remain poorly understood. For example, not much is known about the subcellular sites from which microtubules grow, where their minus ends localize, how their orientation is controlled, or which cellular functions they carry out. Here we review recent findings concerning the mechanisms of non-centrosomal microtubule assembly, focusing on epithelial cells.

Proteins that regulate minus-end dynamics in non-centrosomal microtubules

Minus-end regulators that work with γ-tubulin complex: In the absence of a centrosome as microtubule organizing center, it is expected that other molecules or structures interact with the minus ends of microtubules to regulate their dynamics and/or localization. γ-Tubulin is a component of the γ-tubulin complex (γTuC), which includes γTuSC, γTuRC, and the extended γTuRC (reviewed in Petry and Vale, 2015), and this is the best known protein that directly associates with minus ends. γTuC works to nucleate the polymerization of microtubules, which results in the capping of minus ends (Moritz *et al.*, 2000). Many other proteins such as ninein, CG-NAP, and augmin control the localization of microtubule minus ends or the nucleation of microtubules in combination with γTuRC (Kamasaki *et al.*, 2013; Liu *et al.*, 2014; Takahashi *et al.*, 2002).

Ninein was originally discovered as a centrosomal protein that anchors the minus ends of microtubules to the mother centriole (Bouckson-Castaing *et al.*, 1996; Mogensen *et al.*, 2000). Its N-terminus directly associates with γTuRC (Delgehyr *et al.*, 2005). Ninein also helps organize non-centrosomal microtubules. During the differentiation of mouse cochlear supporting cells, microtubules are released from the centrosome, together with ninein, and their minus ends become anchored to the apical sites of these cells, suggesting ninein involvement in this anchoring process (Keating *et al.*, 1997; Moss *et al.*, 2007). In the mouse epidermis, ninein relocalizes from the centrosome to cell junctions depending on the presence of desmoplakin, and it tethers microtubules to these sites (Lechler and Fuchs, 2007). In *Caenorhabditis elegans*, NOCA-1, a ninein homolog, also organizes non-centrosomal microtubules together with γ-tubulin (Wang *et al.*, 2015). There is no clear evidence that ninein directly associates with the minus ends of microtubules.

CG-NAP (centrosome and Golgi localized PKN-associated protein, also known as AKAP350 or AKAP450) is a large coiled-coil protein, and their N-terminal region interacts with γTuRC (Schmidt *et al.*, 1999; Takahashi *et al.*, 1999, 2002; Witczak *et al.*, 1999). CG-NAP recruits γTuRC either to the centrosome or Golgi for facilitating microtubule nucleation. Although the centrosome is traditionally considered as the major microtubule organizing center, the Golgi has also been shown as a microtubule nucleation site both in proliferating cells (Chabin-Brion *et al.*, 2001; Efimov *et al.*, 2007) and differentiated cells (Oddoux *et al.*, 2013; Ori-McKenney *et al.*, 2012), as reviewed elsewhere (Zhu and Kaverina, 2013). CG-NAP and the microtubule plus-end tracking proteins CLASPs (CLASP1 and CLASP2) are essential for Golgi-derived non-centrosomal microtubule formation in the human retinal pigment epithelium-derived cell line PRE1 cells (Efimov *et al.*, 2007; Rivero *et al.*, 2009).

Augmin is a conserved, eight-subunit protein complex that facilitates microtubule nucleation in association with γTuRC at the existing microtubules (Goshima *et al.*, 2008; Uehara *et al.*, 2009; also reviewed in Sánchez-Huertas and Lüders, 2015). During cell division, augmin localizes to the spindle microtubules and promotes γTuRC-mediated microtubule nucleation within the spindle independently of centrosomes. The augmin-γTuRC module organizes non-centrosomal microtubules also in interphase plant cells (Liu *et al.*, 2014) or neurons (Sánchez-Huertas *et al.*, 2016) by controlling the geometry of minus-ends of non-centrosomal microtubules.

CAMSAP/Patronin family proteins: The CAMSAP/Patronin family is a group of recently identified proteins that directly associate with the minus ends of microtubules (Baines *et al.*, 2009; Goodwin and Vale, 2010; Hendershott and Vale, 2014; Jiang *et al.*, 2014; Meng *et al.*, 2008). Unlike the above-mentioned molecules, they regulate microtubule minus ends independently of γ-tubulin. Initially, 'Nezha (KIAA1543)' was identified as a protein that localizes along apical cell-cell junctions, using the human colon carcinoma-derived cell line Caco-2 (Meng *et al.*, 2008). Later, Nezha was found to bind the minus ends

of non-centrosomal microtubules (Meng *et al.*, 2008). Two other proteins related to Nezha were subsequently reported, namely calmodulin-regulated spectrin-associated protein 1 (CAMSAP1) and 2 (CAMSAP2, KIAA1078/CAMSAP1L1) (Baines *et al.*, 2009), and Nezha was called CAMSAP3 in that study. Nezha/CAMSAP3 was called Marshalin in the organ of Corti where CAMSAP3 is abundantly expressed (Zheng *et al.*, 2013). Meanwhile, using *Drosophila* S2 cells, *Ssp4* was identified as a gene whose mutation causes short spindles during mitosis and unstable microtubules during interphase (Goshima *et al.*, 2007). Further studies found that the *Ssp4* gene product, 'Patronin', is a homolog of CAMSAPs and that it protects microtubule minus ends from depolymerization by KLP10A, a *Drosophila* kinesin-13 (Goodwin and Vale, 2010; Hendershott and Vale, 2014; Wang *et al.*, 2013). Both CAMSAPs and Patronin associate with and stabilize the growing minus ends of microtubules (Hendershott and Vale, 2014; Jiang *et al.*, 2014; Tanaka *et al.*, 2012). A similar protein called PTRN-1 was also identified in *C. elegans*. Unlike the γTuC that caps minus ends, CAMSAPs decorate a restricted region of the microtubule lattice close to the minus ends, and this is governed by the number of CAMSAPs; CAMSAP2 tend to decorate a larger region than CAMSAP3 (Jiang *et al.*, 2014; Tanaka *et al.*, 2012). Katanin, a microtubule-severing ATPase, limits the extent of CAMSAP2 coverage (Jiang *et al.*, 2014). Unlike in *Drosophila* cells, the localization of CAMSAPs to mitotic spindles has not been reported in vertebrate cells.

CAMSAPs bind microtubules via a proline-rich region within the microtubule-binding domain and the C-terminal globular CKK, which stands for 'C-terminal domain common to CAMSAP1, KIAA1078 and KIAA1543', although binding properties differ slightly among the three CAMSAPs (Hendershott and Vale, 2014; Jiang *et al.*, 2014; Meng *et al.*, 2008). Although CAMSAP3, CAMSAP2, and PTRN-1 appear to organize microtubules independently of γ-tubulin (Tanaka *et al.*, 2012; Wang *et al.*, 2015), there is no *in vitro* evidence that CAMSAPs nucleate microtubules. Thus, it remains to be investigated how CAMSAPs control microtubule polymerization, except for the observation that CAMSAPs tether microtubules to particular sites via their minus ends, allowing microtubule plus-end growth at these sites. In *C. elegans*, PTRN-1 organizes circumferentially oriented microtubules in the epidermis, in parallel with NOCA-1 acting with γTuRC (Wang *et al.*, 2015).

To summarize, the stabilization and localization of the microtubule minus ends appear to be controlled by two distinct modules: 1) γTuC and γTuC-associated proteins such as ninein, CG-NAP or augmin, and 2) CAMSAP/Patronin family proteins.

Microtubule-severing proteins: Microtubule-severing proteins such as spastin and katanin contribute to non-centrosomal microtubule organization. In *Drosophila*, γTuRC is released from the centrosome by spastin and then relocalizes to the apical domain of cells via association with the transmembrane protein Piopio during trachea development (Brodu *et al.*, 2010). This process is thought to rearrange microtubules into a non-centrosomal form. In neurons, katanin releases microtubules from the centrosome to generate non-centrosomal populations, and katanin also regulates the length of non-centrosomal microtubules (Ahmad *et al.*, 1999). In plant cells, katanin and γTuC cooperate to assemble non-centrosomal cortical microtubule arrays by controlling the creation of new microtubules from existing microtubules (Nakamura *et al.*, 2010). Katanin increases microtubule density in meiotic spindles of *C. elegans* and controls spindle length (McNally *et al.*, 2006; Srayko *et al.*, 2006). Newly created minus ends depolymerize unless otherwise stabilized by CAMSAPs (Jiang *et al.*, 2014). In mammalian cells, katanin binds to CAMSAP2 and CAMSAP3, but it does not sever CAMSAP-decorated microtubules; instead, it shortens the CAMSAP-decorated region of minus ends (Jiang *et al.*, 2014). How microtubule-severing factors contribute to the assembly of non-centrosomal microtubules remains to be clarified.

Various forms of non-centrosomal microtubules in epithelial cells

Epithelial cells are the major structural and functional components of various organs. They show apical-basal polarity that is linked with their functions, such as absorption and secretion and serving as a barrier. In fully differentiated epithelial cells, microtubules, in general, do not radially emanate from the centrosome; instead they are aligned along the apical-to-basal axis of the cell. This microtubule orientation was first observed by electron microscopy using a hook decoration method (Heidemann and McIntosh, 1980) in teleost retinal pigment epithelial cells (Troutt and Burnside, 1988), *Drosophila* wing epidermal cells (Mogensen *et al.*, 1989), and MDCK cells (Bacallao *et al.*, 1989). Electron micrographs of horizontal sections showed that most of the cytoplasmic microtubules have counter-clockwise hooks, indicating that they are oriented in the same directions with the minus ends towards the apical side of cells. Such polarized assembly of microtubules is well conserved among the various epithelial cell types as well as across species; however, the mechanisms for positioning the minus ends appear to vary depending on cell type (Fig. 2) and developmental stage.

Microtubule assembly at apical cortical regions: Mechanisms that govern minus-end organization at the apical region of cells have been well studied in inner ear cells (Antonellis *et al.*, 2014; Bellett *et al.*, 2009; Goldspink *et al.*, 2013; Mogensen *et al.*, 1997; Moss *et al.*, 2007). As we noted above, in cochlear supporting cells of the inner ear, microtubules are released from the centrosome, and their minus ends translocate to the adherens junction located at a

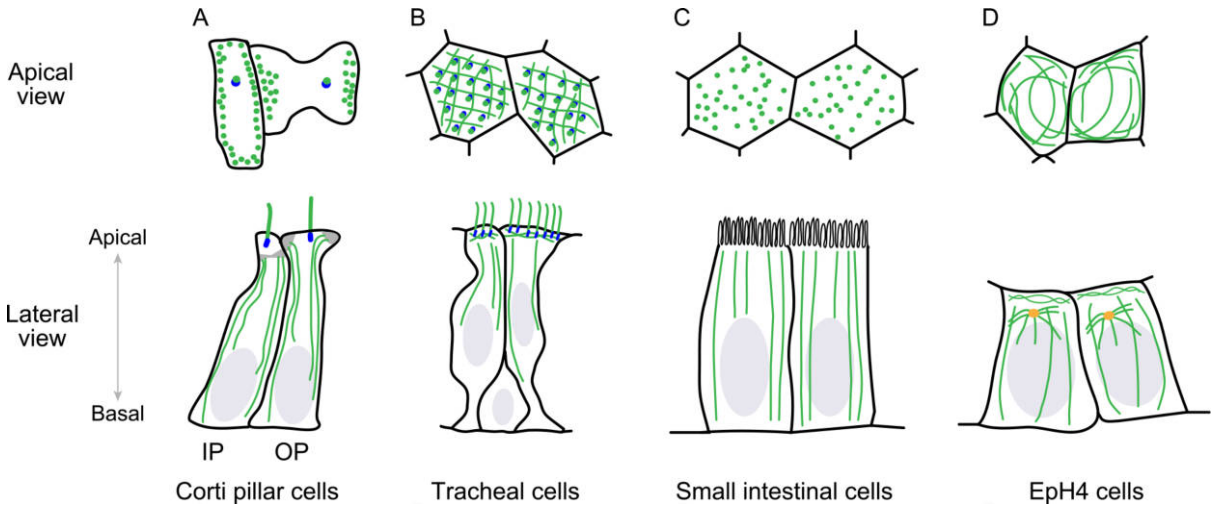

Fig. 2. Schematic drawings of various types of microtubule organization in different epithelial cell types in mouse. (A) Cochlear supporting cells of the inner ear (Mogensen *et al.*, 2000). IP, inner pillar cell; OP, outer pillar cell. (B) Trachea cells (Kunimoto *et al.*, 2012). (C) Small intestine cells (Toya *et al.*, 2016). (D) Mammary gland–derived EpH4 cells (Yano *et al.*, 2013). Note that all epithelial cells show the apical-to-basal orientation of microtubules, although the pattern of microtubule architecture varies among cell types. Basal bodies and centrosomes are indicated in blue and orange, respectively. Nuclei are shown in gray and microtubules in green.

sub-apical region of the cells; there, they are captured by ninein, which also translocates to this site from the centrosome (Bellett *et al.*, 2009; Mogensen *et al.*, 1997; Moss *et al.*, 2007). In cochlear hair cells that have an actin-based cuticular plate at their apical regions, the minus ends of non-centrosomal microtubules are connected to this plate through ACF7/MACF1, a protein that cross-links F-actin and microtubules (Antonellis *et al.*, 2014), suggesting its role in the apical anchoring of non-centrosomal microtubules.

Besides the inner ear cells in which microtubule minus ends are anchored to apical sites, various types of apical microtubule organization have also been observed in other cells. In mouse trachea cells that have multiple cilia at their apical cortex, the non-centrosomal microtubule lattice runs at the sub-apical regions between the basal feet of the basal body (Kunimoto *et al.*, 2012). The mouse mammary epithelial cell line, Eph4 cell, contains a planar apical network of non-centrosomal microtubules, and these microtubules associate with tight junctions via Cingulin; this is dependent on AMPK-mediated phosphorylation of Cingulin (Yano *et al.*, 2013).

In *Drosophila*, the pupal wing epithelium has non-centrosomal microtubules that align along the proximal-distal axis, perpendicular to the apical-basal axis, prior to the onset of hair growth (Harumoto *et al.*, 2010; Matis *et al.*, 2014; Shimada *et al.*, 2006). At the apical region, microtubules first appear as dense bundles anchored to the proximal junction and then extend across the adherens junctions towards the distal part of the cell (Matis *et al.*, 2014). The planar cell polarity mediator Ft/Ds/Fj directs the orientation of these microtubules, and this process provides signals for orienting core planar cell polarity proteins using microtubules (Matis *et al.*, 2014). The polarity of apical microtubules that are organized in this manner is not uniform but rather is biased towards a plus-end-distal orientation (Harumoto *et al.*, 2010; Shimada *et al.*, 2006). However, the details of the molecular mechanisms that regulate this microtubule organisation remain unknown.

CAMSAP/Patronin-mediated microtubule arrays: Recent studies addressed the question of how the apicobasal array of microtubules forms in epithelial cells. In mouse small intestine cells, CAMSAP3 accumulates at the apical cortex that contains the actin-based terminal web associated with microvilli, and it anchors the minus-ends of microtubules to this site (Toya *et al.*, 2016). When *Camsap3* is mutated, the minus ends of microtubules are no longer tethered to the apical cortex, and many of the microtubules exhibit a wavy appearance with a loss of specific directionality (Fig. 3) (Toya *et al.*, 2016). These results have demonstrated that CAMSAP3 plays a key role in the longitudinal orientation of non-centrosomal microtubules in mammalian intestinal epithelial cells.

The apical localization of CAMSAP3 depends on F-actin. CAMSAP3 proteins that carry mutations in a conserved region crucial for binding to the β-spectrin variant, spectrin βIIΣ1, which was initially determined using CAMSAP1 (King *et al.*, 2014), dissociate from the apical region —although they maintain association with microtubules (Toya *et al.*, 2016), suggesting that spectrin might be involved in the apical localization of CAMSAP3. Recent studies demonstrated that, in *Drosophila* follicle cells,

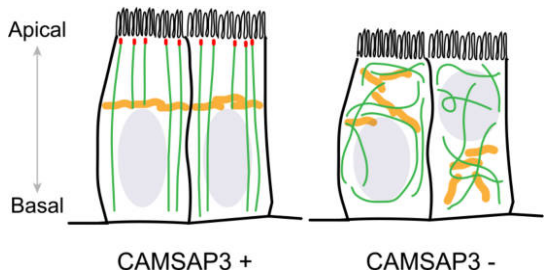

Fig. 3. Schematic drawings of microtubules and organelle assembly in small intestinal cells of wild-type and *Camsap3*-mutant mice. In wild-type (CAMSAP3 +) cells, CAMSAP3 (red) localizes right under the apical membrane, associating with the minus ends of microtubules that extend longitudinally. In *Camsap3*-mutant (CAMSAP3 –) cells, the minus ends of microtubules no longer face the apical cortex, and the apical-to-basal orientation of microtubules is perturbed. In these cells, positioning of organelles, such as nucleus (gray) and Golgi apparatus (orange), is disorganized as well. Microtubules are shown in green.

Patronin localizes to apical regions, together with Shot, the *Drosophila* homolog of mammalian ACF7/MACF1, and the Patronin-Shot complex binds a spectrin complex (Khanal *et al.*, 2016; Nashchekin *et al.*, 2016), supporting the idea that CAMSAP3 may interact with spectrin for apical localization. However, the role of the Shot-Patronin interaction in this system remains mysterious, as loss of Shot does not affect Patronin localization. On the other hand, in the *Drosophila* oocyte, Shot is essential for cortical localization of Patronin (Nashchekin *et al.*, 2016), although whether spectrin is involved in this process remains to be determined. Thus, further studies are necessary for determining precise molecular mechanisms by which CAMSAP/Patronin is recruited to apical cell cortices, which seem to vary depending on cell type.

Microtubules anchored to cell junctions: Although F-actin is a well-known junction component, microtubules are also thought to associate with cell junctions (Reviewed in Harris and Tepass, 2010; Meng and Takeichi, 2009; Mège *et al.*, 2006). At adherens junctions, both plus and minus ends have been observed (Bellett *et al.*, 2009), and the plus-end localization seems to switch to the minus end depending on developmental stage (Moss *et al.*, 2007), and the final destination of minus ends appears to depend on cell type as mentioned above.

CAMSAP3 was originally identified via its interaction with PLEKHA7, which also binds p120-catenin, an E-cadherin-associating protein (Meng *et al.*, 2008). PLEKHA7 is a WW, pleckstrin homology, and coiled-coil domain–containing protein that localizes to zonula adherens, an apically located adherens junction specialized for epithelial cells, and the coiled-coil domain of PLEKA7 binds to the C-terminal region of CAMSAP3. In semi-confluent Caco-2 cells, CAMSAP3 is detected not only in the apical cytoplasm but also along cell junctions, and it appears to tether microtubules to the junctions (Fig. 4). In these cells, CAMSAP3 can also be detected at centrosomes, although its function there remains unknown (Tanaka *et al.*, 2012). Depletion of CAMSAP3 impairs junction architecture in these cells (Meng *et al.*, 2008). On the other hand, in mouse small intestinal epithelial cells *in vivo*, CAMSAP3 exclusively accumulates at non-junctional cortical regions but does not localize to cell junctions (Toya *et al.*, 2016). Functional loss of CAMSAP3 also does not affect junction architecture in these cells. This apparent discrepancy observed between Caco-2 cells and intestinal cells *in vivo* can be explained by the observation of relocalization of CAMSAP3 during maturation of epithelial sheets (Fig. 4). When Caco-2 cells were cultured on an artificial membrane that allows the cell layer to fully mature, CAMSAP3 lost its junctional localization; instead, it was predominantly distributed at apical cortical regions (Toya *et al.*, 2016). In this type of culture, PLEKHA7 was always detectable at cell junctions, suggesting that the ability of CAMSAP3 to interact with PLEKHA7 may change during epithelial sheet maturation, although the full story concerning such CAMSAP3 relocalization remains to be written.

The centrosomal Cap-Gly protein CAP350 also localizes at cell junctions via its binding to α-catenin, a component of cadherin-associated junctions (Maria P Gavilan, 2015; Yan *et al.*, 2006). Similar to the phenotypes of CAMSAP3-depleted Caco-2 cells, CAP350-depleted MDCKII cells had disorganized cell junctions, defective polarized growth, and multiple lumen formation together with a perturbation of microtubule organization during the genesis of cysts (Maria P Gavilan, 2015; Meng *et al.*, 2008; Toya *et al.*, 2016). CAP350 directly binds to microtubules through its N-terminal basic region. Unlike CAMSAP3, however, its interaction with microtubules does not seem to be restricted to the minus ends (Hoppeler-Lebel *et al.*, 2007; Maria P Gavilan, 2015; Yan *et al.*, 2006). In addition, CAP350 localizes throughout cell-cell contacts via α-catenin. Thus, α-catenin-bound CAP350 may support the interaction between microtubules and the lateral membranes of polarized epithelial cells, contrasted with the role of CAMSAP3 in tethering them to the apical sides, and both are likely required for the observed microtubule-dependent polarized cell architecture. In cochlear supporting cells, CAP350 does not localize to cell junctions but instead concentrates at the centrosome and also is present diffusely in the cytoplasm surrounding microtubules (Hoppeler-Lebel *et al.*, 2007). This apparent discrepancy in CAP350 subcellular localization among different cell types remains to be clarified.

Lateral bundling of microtubules: Epithelial cells also have mechanisms to regulate the longitudinal microtubules at their lateral sides. MTCL1 is a microtubule-crosslinking protein that localizes intermittently along the lateral side of microtubules that longitudinally assemble in polarized epithelial cells (Sato *et al.*, 2013). MTCL1 crosslinks microtubules via its N-terminal microtubule-binding domain and

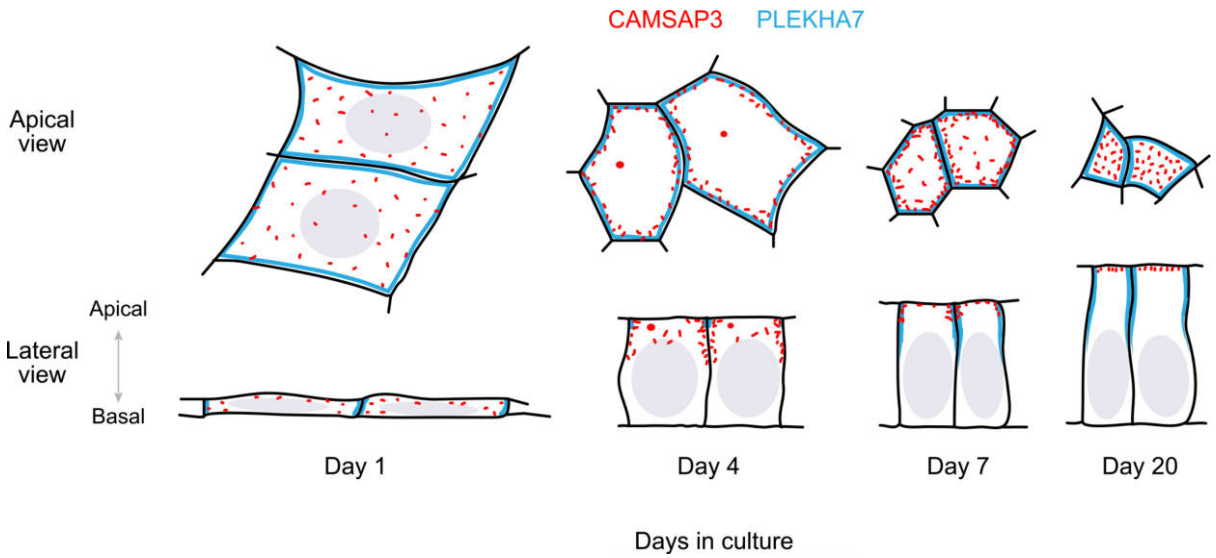

Fig. 4. Schematic depiction of the relocalization of CAMSAP3 in Caco-2 cells during growth on a membrane. Initially CAMSAP3 accumulates at cell-cell junctions as well as in the cytoplasm; at approximately day 4, it is also detectable at the centrosome (red dots near the center of the apical region). Upon cellular maturation (day 20), CAMSAP3 puncta are concentrated at the apical regions, having left cell-cell junctions. In contrast, PLKHA7, a junctional partner for CAMSAP3, localizes to cell-cell junctions throughout the culture period.

coiled-coil domains, and it appears to function to ensure the accuracy of lateral cortical organization of microtubules. Depletion of MTCL1 in MDCK cells results in decreased cell height, suggesting that, similar to CAMSAP3 and CAP350, MTCL1 also supports the action of microtubules to organize cell architecture (Sato *et al.*, 2013; Maria P Gavilan, 2015; Toya *et al.*, 2016). However, unlike CAMSAP3 or CAP350, MTCL1 does not associate with the cell junctions because they are not affected in MTCL1-depleted cells.

How non-centrosomal microtubules contribute to epithelial architecture and function

A number of studies have investigated the roles of non-centrosomal microtubules in epithelial cells, as reviewed recently for *C. elegans* (Quintin *et al.*, 2016a). However, many cells contain both centrosomal and non-centrosomal microtubules, and it is often difficult to distinguish between their roles. Because of recent advances in our understanding of minus-end regulation, we are now able to study the function of non-centrosomal microtubules more precisely than before by molecularly monitoring the minus ends. This section reviews such studies.

In *C. elegans* embryos, epidermal cells organize circumferentially oriented non-centrosomal microtubules through the function of γTuRC and NOCA-1 (ninein) (Quintin *et al.*, 2016b; Wang *et al.*, 2015). Embryos elongate fourfold along the anterior-posterior axis within 3 hours, with a concomitant reduction in diameter, without cell division (Chisholm and Hardin, 2005). NOCA-1- and γTuRC-associated microtubules are responsible for this embryonic elongation; they ensure proper transport of the components of hemidesmosomes and adherens junctions that require reorganization during elongation (Quintin *et al.*, 2016b). In later developmental stages, microtubules in dorsal cells that align in parallel to the long axis of the cells contribute to contralateral nuclear migration after the intercalation of dorsal cells (Wang *et al.*, 2015). In larval stages, the NOCA-1–γTuRC pathway and PTRN-1 cooperatively organize non-centrosomal microtubules to promote larval development and viability (Wang *et al.*, 2015).

Ninein-dependent rearrangement of microtubules is also important for mammalian epidermal cell differentiation (Lechler and Fuchs, 2007). In the basal cells of the epidermis, microtubules emanate from the apically localized centrosome. In the suprabasal cells, however, microtubules relocate and accumulate at cell-cell junctions. In these cells, ninein, Ndel1, and Lis1 are lost from the centrosome and are recruited to desmosomes via association with desmoplakin (Lechler and Fuchs, 2007; Sumigray *et al.*, 2011). In fully differentiated epidermal cells, the centrosome no longer anchors microtubules. The Lis1-null epidermis has dramatic defects in microtubule organization at differentiated cell layers, and Lis1-null mice die perinatally owing to loss of epidermal barrier activity (Sumigray *et al.*, 2011), indicating the importance of the reorganization of microtubule assembly for epidermal functions.

Patronin and Shot cooperate to maintain the apicobasally polarized microtubules in *Drosophila* follicle cells that surround the oocyte (Khanal *et al.*, 2016; Nashchekin

et al., 2016). These microtubules contribute to the biogenesis of microvilli: they enable the apical transport of Rab11 endosomes that deliver the key microvillar determinant Cadherin 99C, the *Drosophila* homolog of mammalian PCDH15, to the apical membrane (Khanal *et al.*, 2016). Likewise, CAMSAP3-associated microtubules ensure proper positioning of organelles such as the nucleus and Golgi in epithelial cells of the small intestine, although CAMSAP3 is not essential for apical microvillar formation (Toya *et al.*, 2016). CAMSAP3 mutant mice show growth retardation, and ~15% of them die before postnatal day 30 (Toya *et al.*, 2016). Because CAMSAP3 is expressed in many other organs, it remains to be clarified what causes the observed growth retardation.

CAMSAP3 is abundantly expressed in the organ of Corti, the key sense organ for hearing in mammals, and its different isoforms are detected at different times during development (Zheng *et al.*, 2013). Cadherin 23, an adhesive protein important for stereocilia formation in the hair cells of the inner ear, interacts with CAMSAP3 through its C-terminal CKK domain (Takahashi *et al.*, 2016; Zheng *et al.*, 2009). The functional relevance of CAMSAP3-mediated microtubule organization in the organ of Corti remains to be clarified, but mutations in Cadherin 23, which correspond to mutations associated with human Usher Syndrome that causes hearing and vision impairment, decrease the interaction between Cadherin 23 and CAMSAP3 (Takahashi *et al.*, 2016).

Conclusion

Proper control of the intracellular localization of microtubule minus ends is required for the assembly of non-centrosomal microtubules in epithelial cells. The fundamental mechanisms for the assembly appear to include two steps—nucleation of microtubules, and relocation of their minus ends to the apical side or other regions. In addition to centrosomally nucleated microtubules, microtubules are also nucleated at the Golgi, and also from pre-existing, severed microtubules. These microtubules account for the formation of non-centrosomal microtubule arrays. Newly created minus ends or those released from nucleation sites need to be stabilized, and non-centrosomal γ-tubulin or CAMSAP/Patronin/PTRN-1 is likely responsible for their stabilization. The basis for the differential function of such microtubules is unknown, but it is possible that microtubules released from their nucleated sites, such as the centrosome, retain γTuC at their minus ends and subsequently associate with ninein, whereas those derived from existing microtubules bind CAMSAP/Patronin/PTRN-1 via their minus ends. They are then translocated to apical or other regions where they associate with either ninein-interacting or CAMSAP-interacting proteins. Although Shot was identified as a Patronin binding partner, current results suggest that other unidentified proteins are also involved in the anchoring this protein to apical sites in epithelial cells. Regarding mammalian CAMSAPs, no information is thus far available concerning factors that regulate their localization. Thus, the mechanisms that govern the formation and localization of non-centrosomal microtubules appear to be somewhat complex, and further studies are clearly necessary to gain deeper insight into the molecular basis for the assembly of epithelial non-centrosomal microtubules.

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