



Generation and regulation of microtubule network asymmetry to drive cell polarity

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Abstract

Microtubules control cell architecture by serving as a scaffold for intracellular transport, signaling, and organelle positioning. Microtubules are intrinsically polarized, and their orientation, density, and post-translational modifications both respond and contribute to cell polarity. Animal cells that can rapidly reorient their polarity axis, such as fibroblasts, immune cells, and cancer cells, contain radially organized microtubule arrays anchored at the centrosome and the Golgi apparatus, whereas stably polarized cells often acquire non-centrosomal microtubule networks attached to the cell cortex, nucleus, or other structures. Microtubule density, longevity, and post-translational modifications strongly depend on the dynamics of their plus ends. Factors controlling microtubule plus-end dynamics are often part of cortical assemblies that integrate cytoskeletal organization, cell adhesion, and secretion and are subject to microtubule-dependent feedback regulation. Finally, microtubules can mechanically contribute to cell asymmetry by promoting cell elongation, a property that might be important for cells with dense microtubule arrays growing in soft environments.

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Introduction

The microtubule cytoskeleton is a major scaffold that mediates transport and positioning of organelles and macromolecular complexes, controls signaling and, in some cell types, also mechanically supports cell shape. Diverse cell functions, such as cell division, migration, tissue formation, secretion, and transmission of signals,

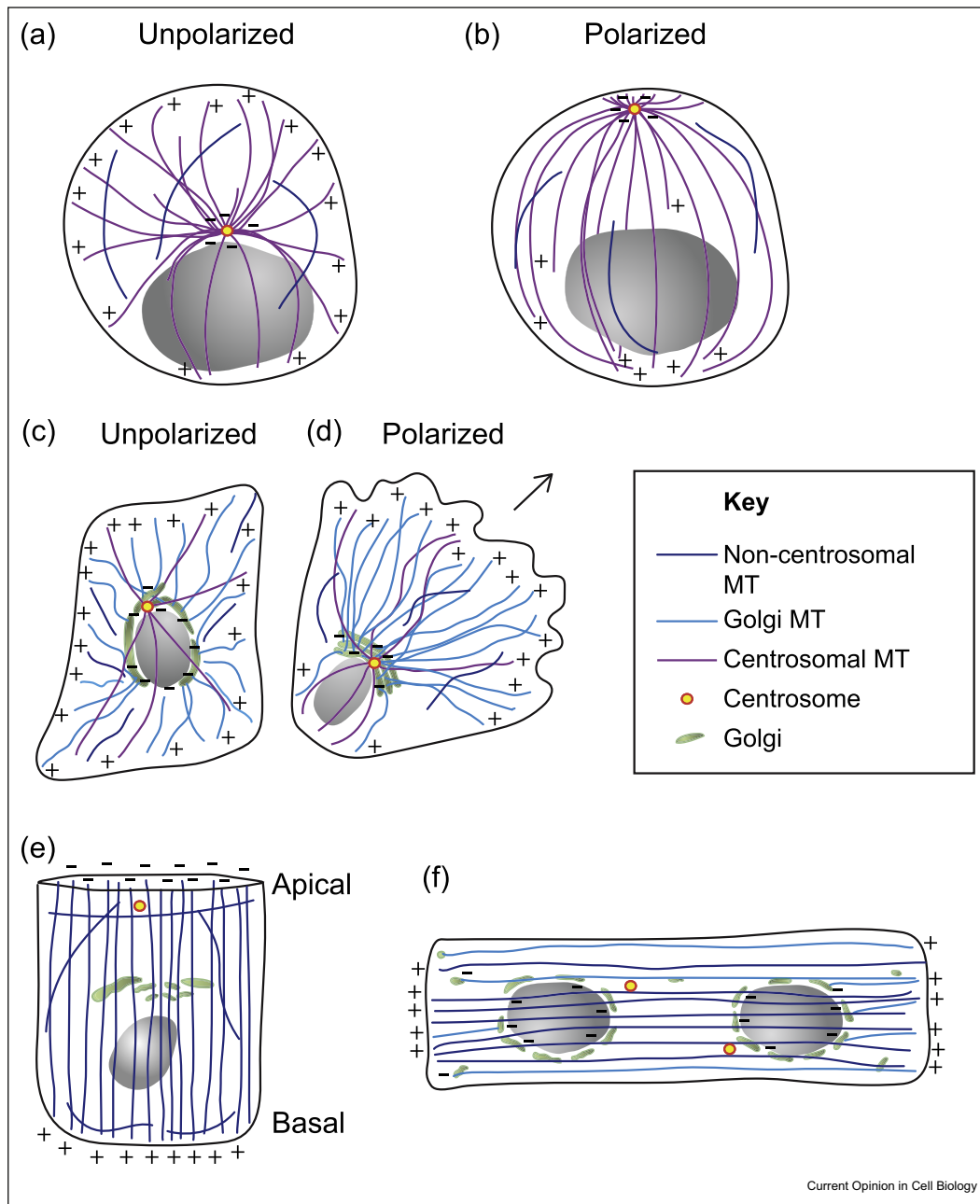
are associated with the generation of cellular asymmetries. Microtubule networks participate in these processes by responding and contributing to cell polarity. Microtubules themselves are intrinsically polarized because they are built of directionally aligned polarized subunits, $\alpha\beta$ -tubulin dimers. Microtubules thus have two distinct ends, the minus end and the plus end. The minus ends exhibit slow dynamics and can be stably anchored at a variety of microtubule-organizing centers (MTOCs), whereas the plus ends can grow and shrink rapidly and interact with different intracellular structures [1–3]. Motors that carry cargos along microtubules, kinesins, and dyneins can typically only move in one direction [4], and microtubule orientation thus strongly determines the logistics of intracellular transport.

Microtubule networks can be polarized in terms of overall geometry, orientation, density, post-translational modifications (PTMs), and microtubule-associated proteins (MAPs). In this review, we focus on recent advances in our understanding of how microtubule asymmetries are generated and used in interphase animal cells. Similar mechanisms also drive the formation and functioning of mitotic spindles, a topic that was recently reviewed [5,6] and will not be discussed here.

Geometry and orientation of microtubule arrays: centrosomal and non-centrosomal microtubules

Microtubules are rigid polymers [7,8] that can easily reach a length spanning the dimensions of an average animal cell; therefore, the localization of microtubule nucleation and minus-end–anchoring sites can determine the geometry of microtubule arrays [1,3,9] (Figure 1). In dividing animal cells, centrosomes are the major MTOCs responsible for the formation of a radial, aster-like microtubule network. Although these arrays are intrinsically symmetric, displacement of the network by microtubule motors exerting pulling forces on microtubule shafts or ends, or pushing forces generated by growing microtubule tips, can rapidly create asymmetry in such systems (reviewed in Ref. [10]; see Ref. [11] for a detailed model-based analysis). A classic example of such a process is MTOC displacement toward the immune synapse in T cells, which is driven by dynein-mediated pulling forces and is crucial for

Figure 1



Examples of microtubule geometries with and without the centrosome. **(a)** An unpolarized T cell with a radial microtubule system anchored at the centrally located centrosome. **(b)** After a T cell forms an immune synapse, the centrosome is relocated to the cell cortex, creating a polarized microtubule geometry. **(c)** An unpolarized mesenchymal cell containing both Golgi- and centrosome-anchored microtubules. **(d)** In a polarized mesenchymal cell migrating on a 2D substrate, the Golgi complex and the centrosome are positioned on one side of the nucleus and promote the formation of a polarized microtubule array oriented in the direction of migration (arrow). **(e)** A mature epithelial cell, with the apical cortex serving as the non-centrosomal MTOC, forms a polarized microtubule network that runs mostly in the apico-basal direction, although additional microtubules with different orientations are also present. **(f)** A myotube, with the nuclear envelope and Golgi membranes serving as the non-centrosomal MTOCs, forms a longitudinal antiparallel microtubule network.

proper organelle positioning, signaling, and immune synapse function [12,13] (Figure 1a and b). Furthermore, many types of migratory animal cells have a radial microtubule system with a centrally located centrosome; such cells can rapidly change their polarity by

altering the relative position of the centrosome and the nucleus (reviewed in (Ref. [14])). MTOC localization during cell motility varies depending on the cell type and the geometrical constraints of the cell's environment [15,16]. For example, in leukocytes, which use an

amoeboid migration mode, the centrosome is positioned behind the nucleus [17], and recent work shows that dynamic microtubules populating retracting protrusions coordinate their coherence and prevent cell fragmentation [18]. By contrast, in mesenchymal cells and in migrating neurons, the centrosome often leads the way [14], although this is not the case when a cell is restricted to one dimension using micropatterned lines [19,20].

Importantly, the number of microtubules that can be directly attached to the centrosome is limited, and in cells with dense microtubule arrays, such as epithelial or endothelial cells, a significant proportion of microtubules is not linked to the centrosome but is instead attached to Golgi membranes that lie in the vicinity of the centrosome ([21,22]; reviewed in (Refs. [23,24])). Unlike the centrosome, the Golgi complex is an asymmetric MTOC with unevenly distributed microtubule-nucleating sites (see Ref. [25] and references therein), and its positioning on one side of the nucleus leads to asymmetry in microtubule density (Figure 1c and d, Figure 2). Recent work showed that 3D-cultured endothelial cells that were depleted of non-centrosomal microtubules and as a result had a perfectly radial, centrosomal microtubule array could not concentrate their microtubules in one protrusion or elongate along a single axis and were unable to form sprouts in a collagen matrix [22]. Such cells could still move on 2D substrates but exhibited polarity defects, likely because Golgi-anchored non-centrosomal microtubules can reorient more rapidly than a centrosome-anchored microtubule aster [22,26]. These data demonstrate that the requirements for microtubule organization and polarity differ depending on the geometry and the stiffness of the environment in which a cell migrates, and that orienting the nucleo-centrosomal axis in a certain direction is insufficient for cell polarization and mesenchymal-type motility in soft 3D environments.

Although non-centrosomal microtubules are critical for endothelial cell polarity, complete centrosome loss was found to have little impact on endothelial cell migration in both 2D and 3D because the Golgi complex alone was sufficient to nucleate a radially organized, polarized microtubule network [22]. However, similar analysis in 2D-migrating RPE1 cells revealed significant defects in cell polarity, associated with increased activity of Rac1 GTPase [27]. The difference between these two systems may be attributed to the fact that centrosome loss strongly upregulated the overall microtubule density in RPE1, but not in endothelial cells [22,27,28]. Migration defects in RPE1 cells were thus likely the consequence of increased microtubule abundance rather than defects in microtubule network geometry. Furthermore, in other systems, such as axonogenesis in developing neurons and neuronal migration, the presence and correct positioning of the centrosome are also not necessary for the

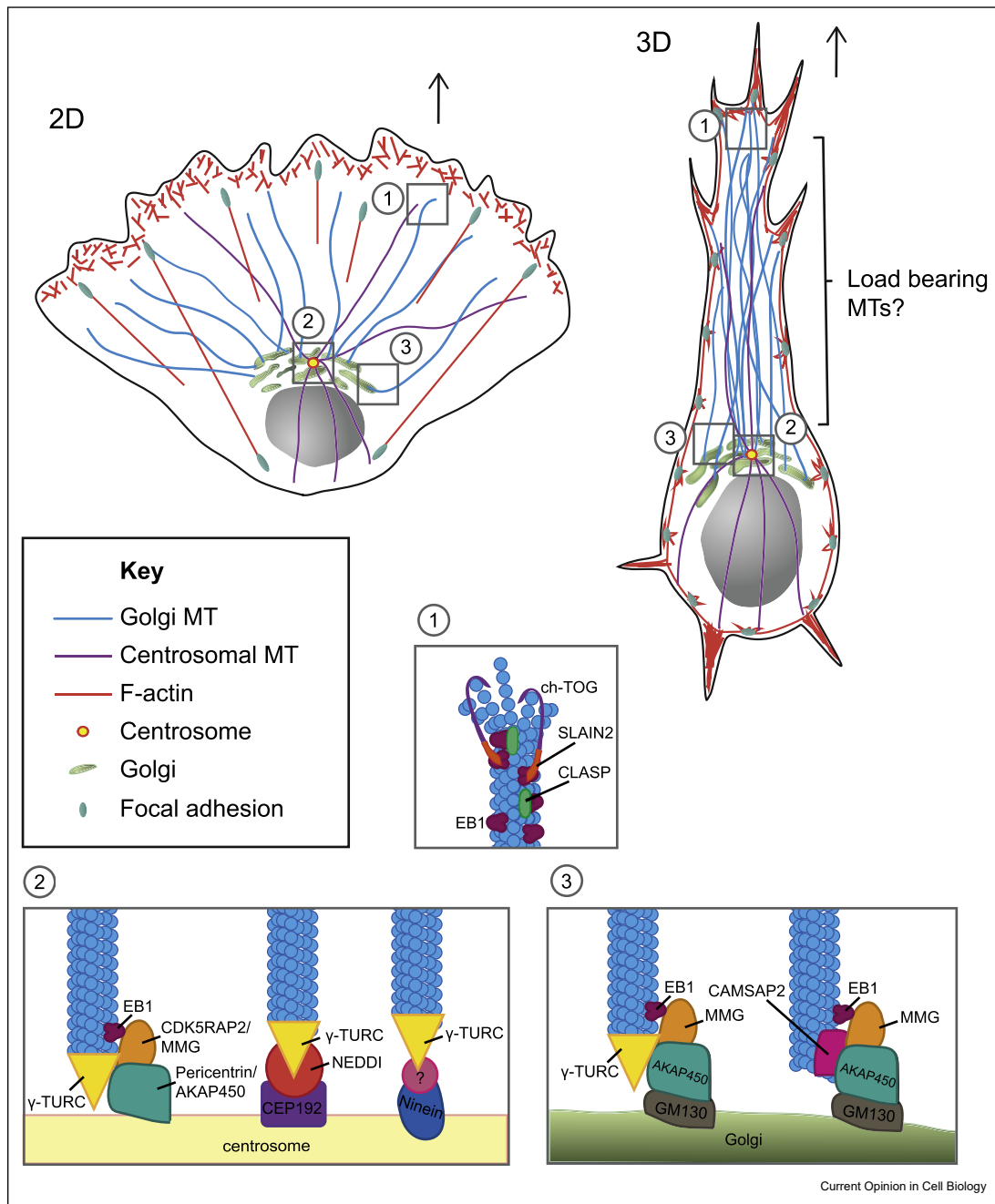
establishment of cell polarity [29,30]. This is in line with the observation that intact centrosomes are dispensable for many steps of animal development [31,32].

Indeed, during differentiation, many cell types acquire a stable, dense, and polarized microtubule array, and this is often accompanied by centrosome inactivation and repositioning of the microtubule nucleation and minus-end—anchoring sites from the centrosome to other locations, such as the apical actin cell cortex in epithelial cells, or the nuclear envelope and the Golgi apparatus in muscle cells (reviewed in (Refs. [1,3,9]; Figure 1e and f). Some of these centrosomal factors are reused, whereas others are unique to non-centrosomal MTOCs. For example, γ -tubulin ring complex (γ -TURC) and its tethering adaptors such as AKAP450 can be deployed at the centrosome, Golgi, and nuclear envelope [33,34]. On the other hand, mammalian calmodulin regulated spectrin-associated protein (CAMSAP) family proteins, which stabilize free, uncapped minus ends, are not found at the centrosome but are key mediators of the microtubule-organizing activity of the Golgi membrane and the cell cortex [26,35–39] (Figure 2).

The complexity of non-centrosomal MTOCs is only starting to emerge, and the common themes in the organization of these structures are the cooperation and redundancy between γ -TURC-dependent and γ -TURC-independent pathways, as well as participation of multiple and diverse MAPs ([40,41], reviewed in Refs. [9,42]). An additional mechanism that amplifies microtubule arrays of uniform polarity is branching microtubule nucleation [43]. This mechanism is important for the formation of mitotic spindles [43] and for the generation of linear microtubule arrays in neurons [44,45].

Interestingly, retention of centrosome activity in a polarizing cell can alter the positioning of membrane organelles and perturb polarity [37]. Increased centrosome number and activity has been associated with invasive cell behavior and cancer ([46], reviewed in Ref. [47]). The switch from centrosomal to non-centrosomal microtubule systems, thus, often accompanies stable cell polarization within tissues. The mechanisms of centrosome inactivation in differentiating cells, which involves a progressive cell cycle—regulated loss of γ -TURC-tethering adaptors from the centrosome, are only beginning to be understood ([30,48,49], reviewed in Refs. [3,50]). The relative abundance of centrosomal versus non-centrosomal microtubules can be regulated by microtubule release from the centrosome and their stabilization at non-centrosomal sites. For example, Golgi-anchored microtubules can be generated by release from centrosome, stabilization by CAMSAP2, and capture at the Golgi membranes [26]. In *Drosophila* tracheal cells, a microtubule-

Figure 2



Comparison of microtubule architecture during mesenchymal-type cell migration in 2D and 3D. Cells displaying mesenchymal-type migration typically have rather dense microtubule arrays that are organized by the centrosome and the Golgi apparatus. Microtubule plus ends are present throughout the cell but are enriched in the direction of migration (arrow) in both 2D and 3D cultured cells. Plus-end-stabilizing proteins CLASP1/2, SLAIN2, and ch-TOG ensure processive microtubule growth (inset Panel 1); the role of these proteins in protrusion formation and migration is more important in soft 3D environments than on hard 2D substrates. The underlying mechanism is still not clear; however, one possibility is that microtubules have a mechanical load-bearing role. Microtubule minus ends are mostly anchored at the centrosome or the Golgi apparatus. At the centrosome, multiple, possibly partially redundant protein complexes nucleate and anchor microtubules through the activities of γ -TURC, its receptors CDK5RAP5, myomegalin (MMG), pericentrin, AKAP450, and NEDD1, as well as additional adaptors such as CEP192 and ninein (inset Panel 2). At the Golgi, microtubules are nucleated and tethered by the complexes consisting of γ -TURC, MMG, AKAP450, and GM130, or CAMSAP2, MMG, AKAP450, and GM130 (inset Panel 3). The +TIP EB1 interacts with MMG and CDK5RAP2 and is also involved; CLASP1/2 promotes microtubule nucleation (not depicted). Golgi-tethered microtubules, but not centrosomal microtubules, are essential for polarization of cells migrating on hard 2D substrates and even more so in soft 3D matrices.

severing protein spastin is essential for the release of MTOC components, which are subsequently anchored apically through the transmembrane protein piopio [51]. Microtubule release from the centrosome can be counteracted by centrosomal isoform of the microtubule minus-end-anchoring protein ninein [52], and in differentiating neurons, which acquire a non-centrosomal microtubule organization, ninein expression switches from a centrosomal splice form to a non-centrosomal form [53]. Relocalization of ninein from the centrosome to non-centrosomal sites has also been reported in mammalian epithelial cells [54,55]. Ninein and the members of CAMSAP/patronin family may control two distinct, cell type-specific pathways of non-centrosomal microtubule minus-end-anchoring proteins ([56], reviewed in Refs. [1,42]), but additional pathways are likely to be involved (see, for example, Ref [40]).

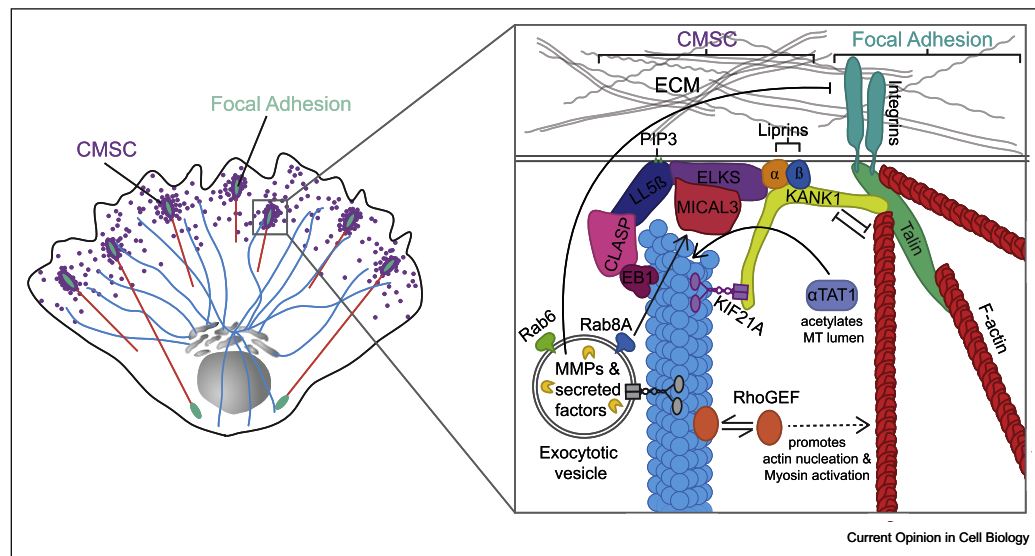
Polarized regulation of microtubule density and stability at the plus ends

Microtubule organization is also regulated at the plus ends: various proteins can interact with the polymerizing microtubule ends to stabilize or destabilize them or to guide the direction of their growth. Major players in such regulation are microtubule plus-end-tracking proteins (+TIPs) and their partners, which can control

microtubule dynamics and connect microtubules to the cell cortex or the actin cytoskeleton [2]. The core of +TIP complexes is formed by End Binding (EB) proteins, which recognize growing microtubule ends and recruit a plethora of different factors [2]. When EB-mediated +TIP recruitment was transiently and locally disrupted using an optically controlled EB construct, this provoked destabilization of cortically aimed microtubules and robust aversive cell turning, indicating that localized +TIP-mediated microtubule stabilization or destabilization can dictate cell polarity [57].

At the cell cortex, multiple factors have been implicated in microtubule stabilization in certain cell regions, such as the leading edge, thus creating local differences in microtubule longevity and density (reviewed in Refs. [2,58]). Some of these factors associate specifically with focal adhesions [59]. The underlying mechanisms are diverse; for example, an adaptor protein, KANK1, directly interacts with the focal adhesion protein talin and recruits to the focal adhesion vicinity several additional cortical adaptors, which in turn associate with microtubule-stabilizing +TIPs such as CLASPs [60–62] (Figure 3). These cortical microtubule stabilization complexes (CMSCs) create long-lived microtubule ‘highways’ for directional transport [58,63]. Stable microtubules tend to accumulate PTMs such as

Figure 3



Microtubule regulation in the vicinity of focal adhesions. CMSCs (purple) are enriched at the leading cell edge and surround focal adhesions (green). The connection between focal adhesions and CMSCs is mediated by KANK1, which competes with F-actin for binding to talin. KANK1 links to ELKS via liprins and can connect to microtubules through KIF21A, a factor that promotes pausing of microtubule growth. The CMSC also stabilizes microtubules through CLASP1/2 and can promote vesicle fusion through an interaction between Rab8A and MICAL3. Microtubule acetylase α TAT1 is also enriched at focal adhesions and can promote vesicle fusion, possibly indirectly. Vesicles delivered to focal adhesions may contain matrix metalloproteases (MMPs) and other secreted factors that promote extracellular matrix (ECM) degradation and focal adhesion turnover; thus, microtubules can stimulate the turnover of the cortical complexes that stabilize their plus ends. Microtubules can also sequester Rho GEF-H1, which enhances actin nucleation and myosin II activation; microtubule depolymerization thus strengthens actin stress fibers and boosts the formation of focal adhesions.

acetylation and detyrosination [64], which can further promote microtubule longevity through indirect mechanisms. For example, detyrosination can promote microtubule stability by repelling microtubule depolymerizing enzymes of the kinesin-13 family, KIF2A, and KIF2C/MCAK [65]. Microtubule acetylation, another PTM typical of long-lived microtubules, does not confer microtubule stability, but rather enhances microtubule flexibility and therefore resistance to mechanical stresses [66,67]. Acetylation can therefore promote microtubule longevity indirectly by preventing microtubule breakage.

Long-lived microtubules serve as preferred tracks for transport by kinesin-1 [68–70]. The underlying mechanism is not yet clear; it does not involve direct recognition of PTMs such as acetylation [71], but rather appears to depend on the ability of this motor to modify the structure of the microtubule lattice and promote its own binding, thus creating a positive feedback loop [72–74]. Kinesin-1 is the major driver of transport of exocytotic vesicles [75]. Interestingly, the fusion of secretory vesicles with the plasma membrane is promoted by CMSC components, and a significant proportion of vesicle fusion events occurs in the vicinity of focal adhesions [61,75–79] (Figure 3). Exocytotic vesicles can deliver to focal adhesions proteolytic enzymes that stimulate adhesion disassembly [77]. Interestingly, in migrating astrocytes, microtubule acetylating enzyme α TAT1 is also enriched at focal adhesions and regulates their turnover by modulating exocytotic vesicle delivery and fusion [79]. Microtubules can thus indirectly modulate the lifetime of their own cortical stabilization sites by delivering molecules that promote the turnover of focal adhesions.

Rho GTPases are major players in the coordination of the actin and microtubule dynamics, which regulate the local distribution of other regulatory molecules [80]. Furthermore, microtubule stability can be fine-tuned in a localized manner by various kinases, such as GSK3 β , which phosphorylates many microtubule-stabilizing MAPs and reduces their affinity to microtubules [81,82]; this kinase is typically inactivated in cell regions, such as the leading cell edge, where microtubules are preferentially stabilized. Microtubules can in turn regulate the delivery and activity of signaling molecules, including kinases and guanine nucleotide exchange factors (GEFs) for Rho GTPases (reviewed in Refs. [80,83]). For example, they can locally reduce cell contractility by sequestering GEF-H1, a RhoA activator that promotes the formation of myosin filaments and contractility, and this activity may be specifically regulated by CMSC components KANK1 and KANK2 [84,85] (Figure 3). KANK2 can also reduce force transmission at focal adhesions: it competes with actin binding to talin and induces focal adhesion sliding [83,86]. More direct cross-talk between microtubules

and actin at integrin-based adhesins has also been reported. For example, microtubule-actin crosslinking factor 1 (MACF1) guides microtubules to focal adhesions along actin fibers and also regulates focal adhesion turnover [87,88]. Microtubules thus participate in various regulatory loops that amplify biases in microtubule network organization, control actomyosin contractility, and promote cell polarity.

Mechanical role of microtubules in supporting cell shape

Bundles of dynamic microtubules can generate pushing forces [89], and recent work provided indications that microtubules may play not only a regulatory role but also a mechanical role in controlling cell shape, particularly in soft 3D environments (reviewed in Ref. [90]). For example, during mesenchymal migration of cancer cells, formation of long cell protrusions in soft substrates is dependent on persistent microtubule growth [91]. Mild perturbations of microtubule growth did not affect transport, Rho GTPase activity, or focal adhesion formation in 3D, nor did they affect migration in 2D or generation of short cell protrusions in collagen gels but prevented protrusion elongation, and this effect could not be rescued by inhibiting actomyosin-based contractility [91]. It is possible that in these conditions microtubules act as struts opposing cell retraction by bearing compressive forces; however, the precise mechanical contribution of microtubules within cancer cell protrusions is still unclear.

Microtubules were also shown to initiate but not maintain cell flattening in mouse epidermis, and the existing data point to a mechanical function rather than an indirect effect on actomyosin or adhesion [92]. Support for a mechanical role of microtubules was also found in developmental studies of fly epithelia. During gastrulation, microtubules extend from the apical side of epithelial cells and counteract actomyosin-driven constriction; weakening of the apical microtubule network by reducing minus-end stabilization and microtubule severing in a subset of cells causes cell shortening and leads to the folding of the epithelial sheet [39]. In the developing wing, epithelial cells contain microtubules oriented parallel to the apical surface, and the acute elimination of these microtubules causes cell shortening, indicating that microtubules resist compressive forces [93]. Another example is provided by shaping of red blood cells, where the marginal band of microtubules counteracts cortical contractility [94]. In cardiomyocytes, stable detyrosinated microtubules, which are coupled to intermediate filaments, are observed to deform and bear load during cell contraction [95]. Increased density and detyrosination of the microtubule network are linked to increased myocyte stiffness and reduced contractility, which are associated with heart failure [96]. Finally, a large body of work

indicates the critical role of microtubule dynamics and possibly forces generated by microtubule sliding in shaping axons and dendrites in neuronal cells (reviewed in Refs. [97,98]). An important challenge for the future is to quantify the forces generated by microtubules in different systems and determine their contribution to the overall cellular mechanics.

Conclusions and outlook

Previous work has identified numerous molecular players and pathways that connect microtubule organization and dynamics to cell polarity. However, we still lack a quantitative understanding of how these players form feedback loops that generate and amplify biases in microtubule networks, allowing them to acquire complex geometries and function in polarized cells. To develop such an understanding, we need to move away from idealized ‘textbook’ models of microtubule organization and instead quantitatively characterize real microtubule arrays in cells and tissues. Recent developments in super-resolution microscopy, for example, in combination with motor movement on extracted microtubule cytoskeleton (MotorPAINT) [70], allow for individual microtubules and their orientations within complex networks to be resolved. The application of such techniques combined with expansion microscopy [99] will enable studies of the organization of complex, dense, three-dimensional microtubule arrays. Furthermore, probing the microtubule function in space and time *in vivo* using optically controlled tools and cell type-specific manipulations will make it possible to link the functions of the microtubule cytoskeleton as a whole and its individual molecular constituents to morphogenesis and functions of cells within tissues.

Conflict of interest statement

Nothing declared.

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