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Cellular Logistics: Unraveling the Interplay Between Microtubule Organization and Intracellular Transport

Mithila Burute and Lukas C. Kapitein

Department of Biology, Utrecht University, 3584 CH Utrecht, The Netherlands; email: l.kapitein@uu.nl

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Keywords

microtubules, cytoskeleton, intracellular transport, motor proteins, posttranslational modifications, polarity

Abstract

Microtubules are core components of the cytoskeleton and serve as tracks for motor protein–based intracellular transport. Microtubule networks are highly diverse across different cell types and are believed to adapt to cell type–specific transport demands. Here we review how the spatial organization of different subsets of microtubules into higher-order networks determines the traffic rules for motor-based transport in different animal cell types. We describe the interplay between microtubule network organization and motor-based transport within epithelial cells, oocytes, neurons, cilia, and the spindle apparatus.

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Contents

1. INTRODUCTION

Many cell types have specialized morphologies that enable their specific function. The cytoskeleton and its associated motor proteins are key players in shaping cells and establishing spatial organization within a cell (Barlan & Gelfand 2017, Vale 2003). The spatially regulated polymerization of microtubules and actin polymers can drive morphological transitions, such as global or local protrusion of the cellular membrane. Such transitions drive, for instance, cell migration and the development of specialized extensions, such as cilia, microvilli, axons, and dendrites. In addition, the structural asymmetry of microtubules and actin enables cytoskeletal motor proteins to take ATP-dependent steps toward either the plus or the minus end of actin or microtubules. Myosins can move over actin, while microtubule-based transport is driven by members of the kinesin superfamily or dyneins. Whereas dyneins move toward the microtubule minus end, most kinesins are plus end directed (**Figure 1*a***). Besides moving cargoes over microtubules and actin, several motor proteins also contribute to the formation of large-scale cytoskeletal assemblies with specialized functions, such as contractile actin bundles (sarcomeres and stress fibers) and the microtubule-based spindle apparatus used to segregate chromosomes during cell division (Sweeney & Holzbaur 2018). Thus, understanding cellular organization requires understanding the dynamic interplay between motor protein activity and cytoskeletal organization.

Over the past decades, the use of purified components to reconstitute cytoskeletal dynamics and motor-based transport has often been the method of choice to achieve mechanistic insights into these processes. Such well-controlled experiments allow for careful dissection of the basic properties of different proteins and have revealed how cytoskeletal dynamics and motor-based

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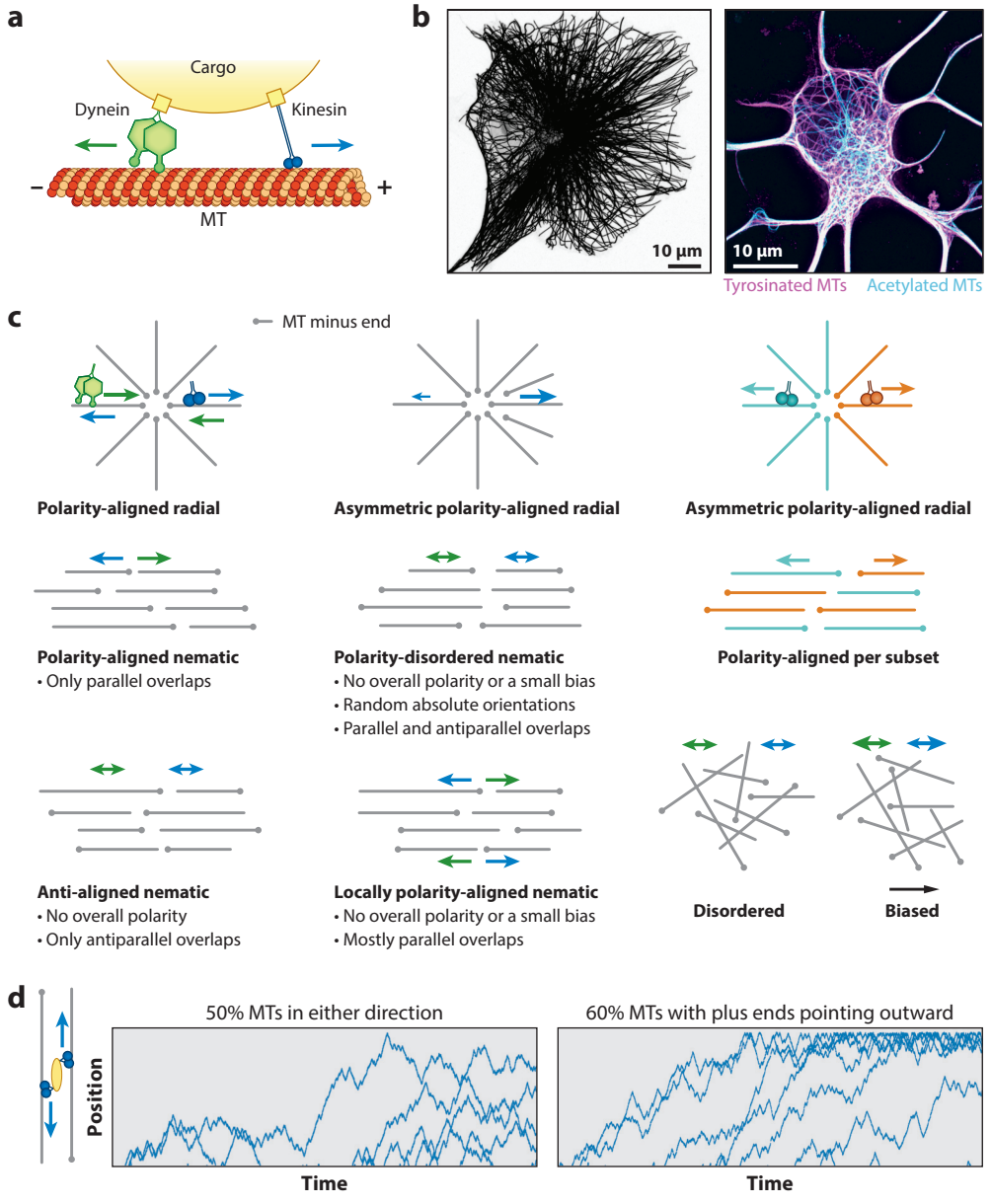

Figure 1

Microtubule (MT) organization and motor-based transport. (*a*) Diagram illustrating microtubule structure and motor protein directionality. (*b*) Example images of microtubules in a COS7 cell and a hippocampal neuron. (*c*) Different types of microtubule organization (see Section 3.1 for discussion) and the expected directionality of dynein (*green arrows*) and plus end–directed kinesin (*blue arrows*). The top right diagram indicates the directionality of kinesins with a preference for different microtubule subsets (*teal* and *orange arrows*). (*d*) Simulations showing the effect of microtubule orientations on cargo redistribution. Figure adapted with permission from Tas et al. (2017).

transport can be modulated by regulatory proteins, chemical compounds, specific cytoskeletal geometries, and other factors. In addition, such assays are increasingly providing detailed structural insights into the mechanisms of motility and microtubule growth. Nonetheless, complementary approaches are needed to achieve an understanding of cellular transport systems as a whole. For example, certain phenomena observed in simplified systems might not operate within cells, while many aspects of cellular transport have so far evaded reconstitution because the relevant molecular players have not yet been identified. Moreover, exploring how cellular context controls cellular transport is perhaps best studied within cells. Thus, understanding the interplay between cytoskeletal organization and intracellular transport also requires careful analysis of transport inside cells in combination with a detailed mapping of the cellular cytoskeleton.

In recent years, new types of cell-based experiments have enabled detailed exploration of cellular transport systems and their logistics. Advanced microscopy approaches have provided new insights into cytoskeletal organization, while assays that explore the activity of specific motors inside cells have revealed that different motors can move toward specific destinations, often by moving on distinct microtubule subsets. This supports the tubulin code hypothesis, which proposes that the genetic and chemical diversity of tubulin regulates microtubule properties and functioning (Janke 2014, Verhey & Gaertig 2007, Yu et al. 2015). In this review, we mostly concentrate on the microtubule cytoskeleton of animal cells and describe how different cells use specialized microtubule networks to ensure proper intracellular logistics. After highlighting the basic principles underlying microtubule organization and motor-based transport, we describe how the diversification of microtubule properties, such as microtubule orientation and surface modifications, can be used to guide different motors to distinct locations in a variety of cellular systems.

2. BASICS OF MICROTUBULES AND MICROTUBULE-BASED TRANSPORT

2.1. Microtubules

Microtubules assemble from heterodimers of α- and β-tubulin, which interact in a head-to-tail fashion to form linear, structurally polarized protofilaments and also associate laterally, resulting in a hollow tube with an outer diameter of ∼25 nm (**Figure 1*a***) (Akhmanova & Steinmetz 2015, Brouhard & Rice 2018, Manka & Moores 2018).

2.1.1. Microtubule dynamics and diversity. Microtubules switch between phases of growth and disassembly in a process termed dynamic instability (Mitchison & Kirschner 1984). These dynamics can be observed in the absence of any regulatory proteins and thus appear coupled to the intrinsic properties of tubulin dimers within the lattice. Free tubulin binds GTP, which (for β-tubulin) is hydrolyzed to GDP shortly after incorporation into the microtubule lattice. Because GDP tubulin lattices are unstable, persistent growth is believed to depend on the presence of a stabilizing cap of GTP tubulin at the microtubule plus end. In this GTP-cap model, loss of the cap will result in rapid disassembly of the microtubule, termed a catastrophe, which can be followed by a transition to a growing state, termed rescue. While cryo-EM approaches have resulted in rapid progress in dissecting the structural variations in different tubulin states (Manka & Moores 2018, Nogales & Zhang 2016, Zhang et al. 2015), how the interplay and transitions between these states lead to dynamic instability remains unclear.

The human genome encodes 17 tubulin genes (Fojo 2009, Roll-Mecak 2019). The structured core within them is highly conserved, and the majority of variations lie within the unstructured C terminus. The C-terminal amino acid tail of tubulin protrudes out from the microtubule surface and is subjected to various posttranslational modifications (PTMs), including detyrosination,

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acetylation, glutamylation, glycylation, and phosphorylation (Janke 2014, Song & Brady 2015, Verhey & Gaertig 2007, Wloga et al. 2017, Yu et al. 2015). While acetylation occurs on the luminal side of the microtubule, most other PTMs take place on the outer surface. Different PTMs are enriched in certain tissue or cellular compartments (**Figure 1*b***). For example, tubulin glutamylation is abundant in neurons, flagellar axonemes, and centriolar microtubules, while detyrosinated microtubules are enriched at the leading side of a migrating cell and in the mitotic spindle. Similarly, certain β-tubulin isoforms are enriched in axonemes, neurons, and platelets (Roll-Mecak 2019).

Many microtubule-associated proteins (MAPs) interact with microtubules and alter their properties (Akhmanova & Steinmetz 2015, Goodson & Jonasson 2018, Kapitein & Hoogenraad 2015). Some interacting proteins can directly modulate microtubule numbers, for instance, by regulating nucleation [e.g., γ-tubulin (Kollman et al. 2011), augmin/human augmin complex HAUS (Goshima et al. 2008)] or by severing preexisting microtubules [e.g., katanin, spastin, fidgetin (McNally & Roll-Mecak 2018)]. Other MAPs associate with microtubule ends and regulate their dynamics, such as plus end–tracking proteins and minus end–targeting proteins (Akhmanova & Steinmetz 2015). MAPs of yet another class interact with the microtubule lattice to stabilize or crosslink microtubules. Finally, tubulin-modifying enzymes are responsible for the various PTMs mentioned above or the reversal thereof (Janke 2014, Yu et al. 2015). Importantly, these different proteins can influence each other's actions, which can lead to the generation of distinct microtubule subtypes. For example, because certain modifications appear slowly after microtubule polymerization and will not accumulate before depolymerization occurs, only microtubules that are stabilized by certain MAPs will accumulate more of these modifications (Kirschner & Mitchison 1986). In turn, these modifications can impede the action of microtubule-destabilizing proteins, further increasing the lifetime of these microtubules. Certain levels of microtubule polyglutamylation inhibit the microtubule-severing action of spastin (Valenstein & Roll-Mecak 2016), while detyrosination of tubulin impedes the activity of the microtubule depolymerase MCAK (Peris et al. 2009). The activity of severing enzymes is regulated by different MAPs in a similar manner (Qiang et al. 2006).

2.1.2. Microtubule organization. How do all these processes contribute to the formation of specialized microtubule arrays? First, by controlling the location and orientation of microtubule nucleation, ordered networks can be built (Sanchez & Feldman 2017, Wu & Akhmanova 2017). In nondifferentiated, dividing animal cells, most new microtubules emerge at the centrosome. Because the centrosome is typically located close to the nucleus, the result is a radial array of microtubules whose plus ends point toward the cell membrane (**Figure 1*b***; see **Figure 2** for methods of polarity detection). In addition to microtubules nucleated at the centrosome, the Golgi apparatus often nucleates a considerable number of microtubules (Efimov et al. 2007). One function of these microtubules is bringing Golgi stacks together into the Golgi ribbon after mitosis. In addition, Golgi-derived microtubules are believed to contribute to cellular polarization because they increase microtubule density in the part of the cell where the Golgi apparatus resides (Miller et al. 2009). Importantly, given the perinuclear location of the Golgi apparatus, the overall orientation of microtubules in the cells will still be radial, with the plus ends pointing outward. In contrast, the γ-tubulin ring complex (γ-TuRC) relocalizes to the apical surface of many columnar epithelial cells, resulting in a polarized microtubule network in which the plus ends point toward the basolateral surface while most minus ends are located apically (Sanchez & Feldman 2017, Toya & Takeichi 2016). Depending on the exact cell type, such cells could also have centrosome-derived microtubules. Thus, in different cell types, distinct organelles or structures can serve as sites for nucleation. In addition, microtubules themselves can aid in nucleation of microtubules with the

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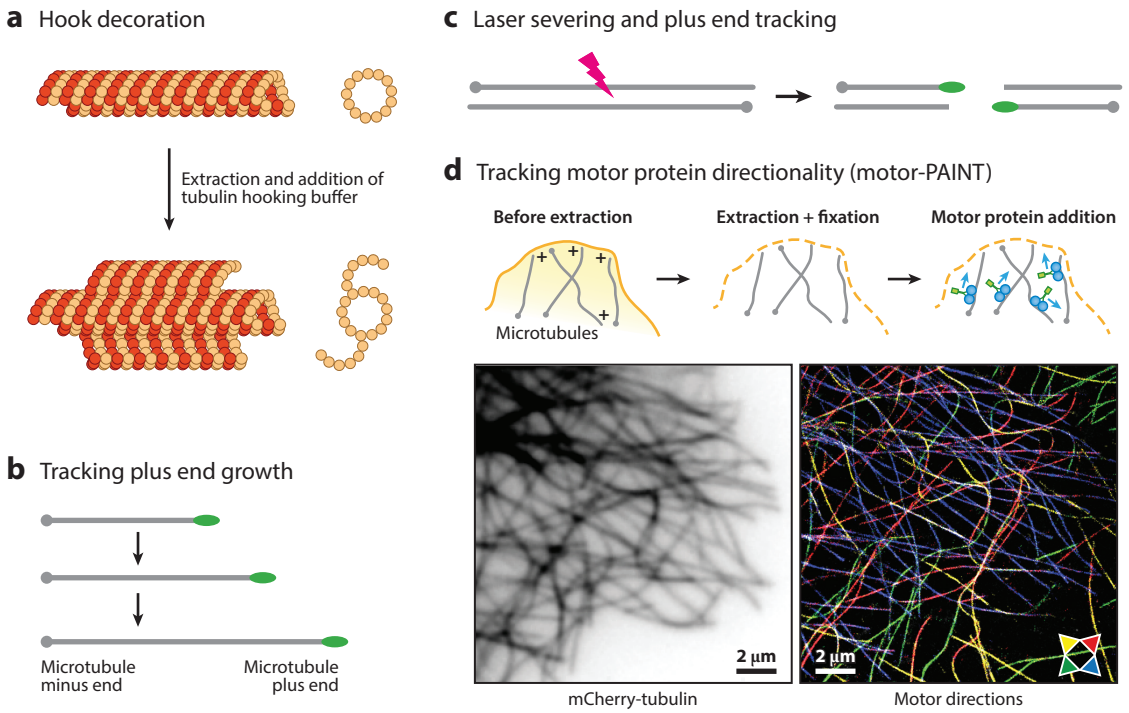

Figure 2

Tools to map the orientation of microtubules. (*a*) Hook decoration. (*b*) Microtubule plus end tracking. (*c*) Laser severing and plus end tracking. (*d*) Tracking motor protein directionality by motor-PAINT. Panel *d* adapted with permission from Tas et al. (2017).

same absolute orientation by recruiting and orienting the γ-TuRC through the augmin/HAUS complex (Goshima et al. 2008, Petry et al. 2013). This mechanism facilitates the formation of large radial arrays in which microtubule density does not decrease when the distance from the focal point increases. This mechanism has also been proposed to contribute to the formation of the parallel microtubule network in axons, because knockdown of augmin subunits results in the appearance of oppositely oriented microtubules (Cunha-Ferreira et al. 2018, Sánchez-Huertas et al. 2016).

Controlling microtubule growth properties is another mechanism of shaping networks. While plus ends typically alternate between growing and shrinking, most microtubule minus ends are relatively stable. This stability results from complexes that anchor these ends to specific sites (Sanchez & Feldman 2017, Wu & Akhmanova 2017) or from the minus end–binding proteins of the CAMSAP/Nezha family; these proteins can rapidly stabilize freshly generated minus ends (Akhmanova & Hoogenraad 2015, Meng et al. 2008). Capture of microtubule plus ends, for example, at kinetochores (Foley & Kapoor 2013) or the cortex (Lansbergen et al. 2006), can also prevent depolymerization and result in long-lived microtubules. The lattice of such microtubules could subsequently accumulate different modifications and MAPs to further stabilize and differentiate these microtubules relative to the dynamic microtubules (Kirschner & Mitchison 1986).

Finally, microtubules can be organized by displacing them. This could occur by coupling them to moving structures, such as an actin network that displays retrograde flow. In addition, motor proteins can directly move microtubules in multiple ways (Kapitein & Hoogenraad 2015). Several motors can crosslink microtubules and move them relative to each other (Fink et al. 2009,

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Jolly et al. 2010, Kapitein et al. 2005, Oladipo et al. 2007). In the spindle, dynein and Kinesin-14 focus minus ends into spindle poles, while Kinesin-5 motors are believed to push apart antiparallel microtubules (Prosser & Pelletier 2017). In *Drosophila* neurons, Kinesin-1 and Kinesin-2 contribute to microtubule transport and orientation (Mattie et al. 2010, Winding et al. 2016). For example, Kinesin-2 guides polymerizing plus ends along existing microtubules to ensure proper incorporation of new microtubules into parallel arrays (Mattie et al. 2010). Thus, by controlling the nucleation, dynamics, stability, and displacement of different microtubules, complex networks with distinct microtubule subsets can be established.

2.2. Microtubule-Based Transport

Motor proteins of the kinesin superfamily and dyneins drive microtubule-based intracellular transport. Most kinesins move toward the microtubule plus end, whereas dyneins move toward the minus end. These motors use the energy derived from ATP hydrolysis to transport cargoes (e.g., Kinesin-1, Kinesin-2, Kinesin-3), to organize microtubules (e.g., Kinesin-1, Kinesin-5, Kinesin-6, Kinesin-14), and to alter microtubule dynamics (e.g., Kinesin-4, Kinesin-8, Kinesin-13) (Hirokawa & Noda 2008, Sweeney & Holzbaur 2018).

2.2.1. Kinesin and dynein. The human genome encodes 45 different kinesins that can be categorized into 14 families on the basis of phylogenetic analysis of their motor heads (Lawrence et al. 2004). Most kinesin motors have a head region (including the motor domain), a stalk, and a tail region. The motor domains undergo ATP-dependent conformational changes that mediate force generation, and the stalk domain often determines the oligomeric state of the motor, while the tail mediates cargo interaction and can serve autoregulatory roles. Robust processive motion (i.e., taking multiple steps per microtubule encounter) depends on the presence of (at least) two motor domains whose chemomechanical cycles are kept out of phase to prevent detachment from the microtubule. Indeed, truncated dimeric Kinesin-1 constructs can move processively in vitro and in cells. In contrast, full-length Kinesin-1 is largely inactive in the absence of cargo because of an autoinhibitory interaction between the tail domain and a region in the motor domains (Kaan et al. 2011). Relief from this autoinhibition depends on the interaction with specific adaptor proteins that link the motor to different cargoes. Other motors, such as the Kinesin-3 KIF1A, are thought to be only weakly dimeric and hence weakly processive, unless cargo adaptors induce stable dimerization and thereby trigger efficient transport (Sweeney & Holzbaur 2018).

Dynein motors move toward the minus end of microtubules. There are two main members of dynein superfamily: axonemal (also known as ciliary or flagellar) dyneins and cytoplasmic dyneins. Axonemal dyneins drive the motility of axonemes (microtubule-based structures found in cilia and sperm tails) by inducing the relative sliding of adjacent microtubule doublets, while cargo transport along these structures [i.e., intraflagellar transport (IFT)] is driven by kinesins and cytoplasmic dynein 2. In contrast, cytoplasmic dynein 1 is the only dynein isoform that functions in the cytoplasm of eukaryotic cells, where it contributes to formation of the spindle apparatus and is believed to drive most minus end–directed cargo transport. These motors are large multiprotein complexes, and only recently have structural work and in vitro reconstitutions begun to reveal the spatial organization, mechanism of motility, and modes of regulation of this motor (for an excellent recent review on dynein structure and function, see Reck-Peterson et al. 2018). In recent years, the role of adaptor proteins in activating dynein motility has emerged as a key concept in understanding dynein's activity and regulation. This role was initially proposed for the dynein adaptor BICD (Splinter et al. 2012), and subsequent in vitro reconstitutions have revealed that multiple adaptors can independently promote the interaction between dynein and

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the multisubunit cofactor dynactin and hence trigger processive motility (McKenney et al. 2014, Reck-Peterson et al. 2018, Schlager et al. 2014).

2.2.2. Microtubule diversity and motor selectivity. Given the existence of different subsets of microtubules, the question arises whether different motors preferentially interact with different microtubules. Indeed, several members of the kinesin superfamily prefer microtubule subsets that carry specific chemical modifications or associated proteins. Initial work found that antibodies against detyrosinated microtubules were more effective in blocking Kinesin-1 binding than were antibodies against tyrosinated tubulin (Liao & Gundersen 1998). Later, single-molecule-imaging experiments in cells combined with immunocytochemistry to identify microtubule subsets revealed that Kinesin-1 prefers stable microtubules enriched in acetylation and detyrosination (Cai et al. 2009, Dunn et al. 2008). More recent work has used rigor mutants of different kinesins, i.e., constructs with a mutated ATP pocket that traps them in the strong microtubule-bound state (Farías et al. 2015, Guardia et al. 2016, Tas et al. 2017). Again, this approach revealed a striking selectivity of Kinesin-1 for highly modified microtubules, while Kinesin-3 prefers the more dynamic tyrosinated microtubules.

Nonetheless, it is not clear to what extent preferential motor binding directly results from PTMs. Various in vitro assays in which kinesins were tested on microtubules carrying various modifications revealed interesting differential effects of certain modifications on different motors (Kaul et al. 2014, Sirajuddin et al. 2014). However, some of the effects seem insufficient to explain the strong preferences of Kinesin-1 for a specific microtubule subset observed in cells (i.e., an up-to-twofold increase or decrease in speed and processivity in most cases). An alternative hypothesis may be that motors are influenced by other features that are downstream, or perhaps even upstream, of these modifications. For example, tubulin modifications may modulate the binding of different MAPs that in turn influence motor activity. Alternatively, certain MAPs may modulate motor proteins and promote the accumulation of specific PTMs, for example, by increasing microtubule lifetime. In the latter case, the accumulation of motors would be correlated with certain modifications but would not functionally depend on them.

The effects of MAPs on motor activity are currently gaining renewed attention. Several early studies reported strong opposing effects of both tau and MAP2 on Kinesin-1 binding and/or motility, whereas dynein was much less affected (Chaudhary et al. 2018; Dixit et al. 2008; McVicker et al. 2011; Vershinin et al. 2007, 2008; Xu et al. 2013). More recently, cellular experiments showed that MAP2 has stronger inhibitory effects on Kinesin-1 than on Kinesin-3 (Gumy et al. 2017). In addition, MAP7 was identified as an important activator of Kinesin-1 activity, while Doublecortin and DCLK1 were shown to promote Kinesin-3 binding (Lipka et al. 2016, Liu et al. 2012). In vitro reconstitution revealed that MAP7 promotes binding of Kinesin-1 but opposes binding of the Kinesin-3 KIF1A (Hooikaas et al. 2019, Monroy et al. 2018). Likewise, other in vitro work revealed that Septin-9 promotes microtubule binding of Kinesin-3 while opposing binding of Kinesin-1 (Karasmanis et al. 2018). The positive effects of Septin-9 appear dependent on the lysine-rich domain in the L12 loop of KIF1A because, upon grafting this loop onto Kinesin-1, Septin-9 promotes microtubule interactions of Kinesin-1 in in vitro assays. The mechanisms underlying these effects are unclear but will hopefully be revealed using structural methods that can map the exact binding interfaces of all these proteins on the microtubules (Al-Bassam et al. 2007, Kellogg et al. 2018, Shigematsu et al. 2018).

In addition to a role for PTMs and MAPs, structural changes in the microtubule lattice may contribute to motor selectivity. Several articles have reported that Kinesin-1 can alter the structure of microtubules in subtle ways, resulting in a slightly elongated lattice (Peet et al. 2018, Shima et al. 2018). Because global changes are observed with substoichiometric decoration of kinesin, its

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binding appears to trigger structural changes that can propagate through the microtubule lattice over length scales of multiple tubulin dimers. These changes may enhance the subsequent binding of Kinesin-1 and thereby cause cooperative binding (Muto et al. 2005, Shima et al. 2018). In addition, these changes may in some cases persist for some time upon motor detachment. Similar suggestions of long-range structural changes that arise upon protein binding through mechanical coupling in the lattice have been made for the binding of other MAPs (Brouhard & Rice 2018).

Finally, one might assume that dynein should not be too selective for a specific subset, given that it is implicated in most minus end–directed transport processes. Nonetheless, in vitro experiments have shown that initiation of dynein runs is promoted by tyrosinated tubulin through interaction with the Cap-Gly domain of dynactin, which may help in biasing transport initiation to the dynamic plus end of microtubules (McKenney et al. 2016, Nirschl et al. 2016). After transport initiation, tyrosination is no longer required to sustain motility.

2.2.3. Cargoes with multiple motors. It is well known that multiple cargoes recruit more than one type of motor (Barlan & Gelfand 2017, Bonifacino & Neefjes 2017, Guardia et al. 2016, Hancock 2014, Prevo et al. 2017). Moreover, some adaptors that can activate dynein also interact with kinesins (Redwine et al. 2017, Schlager et al. 2010, Splinter et al. 2010). How the activity of different motors on the same cargo is coordinated remains an open question, but several scenarios can be envisioned, and all such scenarios are likely at play in different situations. Because many excellent reviews have covered different aspects of this problem in the past years (Barlan & Gelfand 2017, Bonifacino & Neefjes 2017, Guardia et al. 2016, Hancock 2014, Prevo et al. 2017), we do not focus on it here and note only that cells have various ways to control in space and time which motors are dominant on specific cargoes. However, even if one type of motor is active on a cargo, different copies can still interact with oppositely oriented microtubules at the same time. This situation can result in directional conflicts and frequent reversals of motility (Derivery et al. 2015, Kapitein et al. 2010a). How overall microtubule organization controls motor-based transport is the topic of the remainder of this review.

3. FORM FOLLOWS FUNCTION: MICROTUBULE ORGANIZATION AND INTRACELLULAR TRANSPORT

3.1. General Considerations

There are many different ways in which microtubule organization can control motor-based transport to establish well-defined transport pathways for different cargoes. Before turning to specific cellular systems, we first discuss some of the general principles. In cells with a radial microtubule array focused near the cell center, minus end–directed dynein is required for retrograde transport toward the cell center, whereas plus end–directed kinesins drive outward, anterograde transport (**Figure 1*c***). If such an array is symmetric, the radial distribution established by plus end–directed transport will be isotropic, meaning that there will be no preference for one side of the cell. Nonetheless, during interphase the centrosome is often off-centered, resulting in an asymmetric microtubule distribution (Mimori-Kiyosue 2011). This effect is further enhanced by the presence of the nucleus, which acts as a barrier for microtubule growth (Luxton & Gundersen 2011). In addition, the Golgi apparatus is located close to the centrosome, and therefore Golgi-derived microtubules further increase microtubule density on one side of the cell while preserving the overall plus end out orientation of the network (Efimov et al. 2007). Such asymmetric microtubule density may lead to increased cargo flux on the high-density side of the cell (Miller et al. 2009). This asymmetric cargo flux can be caused by the increased probability of

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vesicles leaving the Golgi apparatus to interact with Golgi-derived microtubules. In addition, if cargoes switch between non-microtubule-bound, diffusive states and episodes of directional movement on microtubules, increased capture in high-density regions will also result in an asymmetric distribution.

Other mechanisms may further contribute to asymmetric (or polarized) transport on radial arrays. If microtubules are selectively stabilized or modified on one side of the array, for example, through plus end stabilization at cortical sites, these microtubules may form a subset of tracks for motors that prefer stable microtubules. This organization will strongly bias the transport driven by these motors to one side of the cell (**Figure 1*c***). Finally, different types of pathways may locally regulate motor activity and thereby promote motility on one side of a radial array. In this case, asymmetric targeting is not necessarily encoded by the microtubule network. Nonetheless, if such regulatory factors themselves depend on motor activity for their distribution, feedback may amplify a small initial bias and result in asymmetric transport. Such feedback may, for example, occur if motor binding promotes the subsequent binding of more motors through subtle alterations to the microtubule lattice (Muto et al. 2005, Shima et al. 2018).

On arrays where most microtubules are bundled or otherwise aligned, different situations can be envisioned (**Figure 1*c***). First, all the microtubules may have the same absolute orientations, resulting in uniform polarity, or parallel alignment. In this case, it is trivial how motors with different directionalities contribute to transport to either side of the array. In contrast, microtubule polarity may also be mixed. In a mixed array, microtubules may be randomly oriented, irrespective of the polarity of neighboring microtubules. Alternatively, if such an array is formed by MAPs that selectively crosslink two microtubules with opposite (i.e., antiparallel) orientations, it will be ordered and display alternating polarity between neighboring microtubules (Gaillard et al. 2008). In addition, mixed microtubule arrays may display local polarity alignment, featuring distinct subarrays with (mostly) uniform polarity that are oriented antiparallel to neighboring subarrays (Tas et al. 2017).

Mixed microtubule networks are found in several of the systems discussed below, for example, *Drosophila* oocytes, the spindle apparatus, and the dendrites of mammalian neurons. To understand how mixed microtubule networks modulate motor-driven transport, several research groups have used mathematical modeling or numerical simulations (Ciocanel et al. 2018, Derivery et al. 2015, Kapitein et al. 2010a, Khuc Trong et al. 2015). These approaches have revealed that even a small asymmetry in microtubule orientations is sufficient to create a strong bias in directional transport (**Figure 1*c,d***).

3.2. Transport in Cycling Cells: Role of Microtubule Density and Microtubule Subsets

Cells with a radial microtubule array can also (transiently) require polarized transport. For example, during cell migration, specific cargoes are moved to the leading edge of cells to, among other actions, regulate focal adhesion dynamics or to degrade the extracellular matrix (Schmoranzer et al. 2003, Stehbens & Wittmann 2012). In addition, cytotoxic T lymphocytes form immunological synapses with target cells, where the former cells secrete lytic granules that concentrate at this site (Stinchcombe et al. 2006). One way to achieve such polarized transport is to increase local microtubule density by positioning the centrosome and Golgi apparatus in front of the nucleus. Indeed, active orientation of the nucleus-centrosome-Golgi axis in the direction of migration has been observed in various cell types and often results in biased transport of secretory vesicles toward the leading edge (Burute et al. 2017, Kaverina & Straube 2011, Luxton & Gundersen 2011, Schmoranzer et al. 2003). Interestingly, cytotoxic T lymphocytes use an alternative mechanism to

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enrich cargoes at the immunological synapse. In this case, the centrosome relocates to the plasma membrane upon contacting a target cell, which mediates dynein-driven clustering and subsequent secretion of lytic granules (Stinchcombe et al. 2006).

In several systems, biased transport to the leading edge is further promoted by the stabilization and preferential use of microtubules that are anchored near focal adhesions (Bouchet et al. 2016, Gundersen & Bulinski 1988, Kaverina & Straube 2011, Lansbergen et al. 2006, Stehbens et al. 2014). Such use of specific microtubule subsets for transport also contributes to the proper organization of organelles in many interphase cells. For example, Kinesin-1 preferentially moves lysosomes over perinuclear acetylated microtubules, whereas Kinesin-3 transports lysosomes over more peripheral and tyrosinated microtubules (Guardia et al. 2016). The subcellular localization of lysosomes not only determines their maturation and lytic capacity (Guardia et al. 2016) but also contributes to metabolic signaling (Korolchuk et al. 2011, Pu et al. 2016). In addition, the fusion between autophagosomes and lysosomes occurs predominantly on detyrosinated microtubules (Mohan et al. 2018). Similarly, a subset of acetylated microtubules are also used for endoplasmic reticulum (ER) sliding and are preferred sites of contact between mitochondria and the ER (Friedman et al. 2010).

3.3. Columnar Epithelia: Linear Arrays Facilitate Transport Along the Apicobasal Axis

Multicellular organisms use epithelial cells to demarcate between the inside and the outside (Blasky et al. 2015). These cells have a highly polarized organization with distinct architecture toward the outside (apical side) and inside (basolateral side). In a fully developed epithelium, organelles and other cargoes are positioned at or are transported to specific subcellular sites along the apico-basolateral axis. To facilitate polarized transport, columnar epithelial cells establish a polarized microtubule network in which most plus ends are pointing toward the basolateral surface, while most minus ends are located apically (**Figure 3*a***). Noncentrosomal nucleation and CAMSAP3-dependent minus end stabilization are required for this organization (Noordstra et al. 2016, Toya & Takeichi 2016, Toya et al. 2016). Depending on the exact cell type, such cells can also have centrosome-derived microtubules (Sanchez & Feldman 2017, Toya & Takeichi 2016). Polarized apical-to-basal organization was first observed in teleost retinal pigment epithelial cells (Troutt & Burnside 1988), *Drosophila* wing epidermal cells (Mogensen et al. 1989), and cultured canine kidney (MDCK) cells (Bacallao et al. 1989), followed by similar observations in many other epithelial cells (Sanchez & Feldman 2017, Toya & Takeichi 2016). To assess microtubule polarity, the hook decoration method was used (Heidemann & McIntosh 1980). This method relies on the ability of exogenous tubulin to form lateral curved sheets along the walls of existing microtubules in certain conditions. In cross sections imaged with electron microscopy, these sheets appear as clockwise or anticlockwise hooks, depending on their orientation (**Figure 2*a***).

Polarized apical-to-basal microtubule organization is believed to facilitate polarized transport between the apical and basal surfaces, with a clear division of labor between opposite polarity motors. Indeed, loss of microtubules disrupted transport and resulted in missorting of certain apical and basolateral markers in MDCK cells (Gilbert et al. 1991). Nonetheless, most markers were still enriched on the proper side of the cell, indicating that polarity is largely preserved and that microtubules have a facilitating, but nonessential, role in polarity maintenance in these cells. Likewise, only in compromised conditions do *Caenorhabditis elegans* epidermal cells require microtubules for the proper distribution of junctional components (Quintin et al. 2016). The strongest indication that the specific apical-to-basal organization of microtubules is important for polarized targeting of cargoes comes from studies demonstrating a role for minus end–directed dynein in the apical

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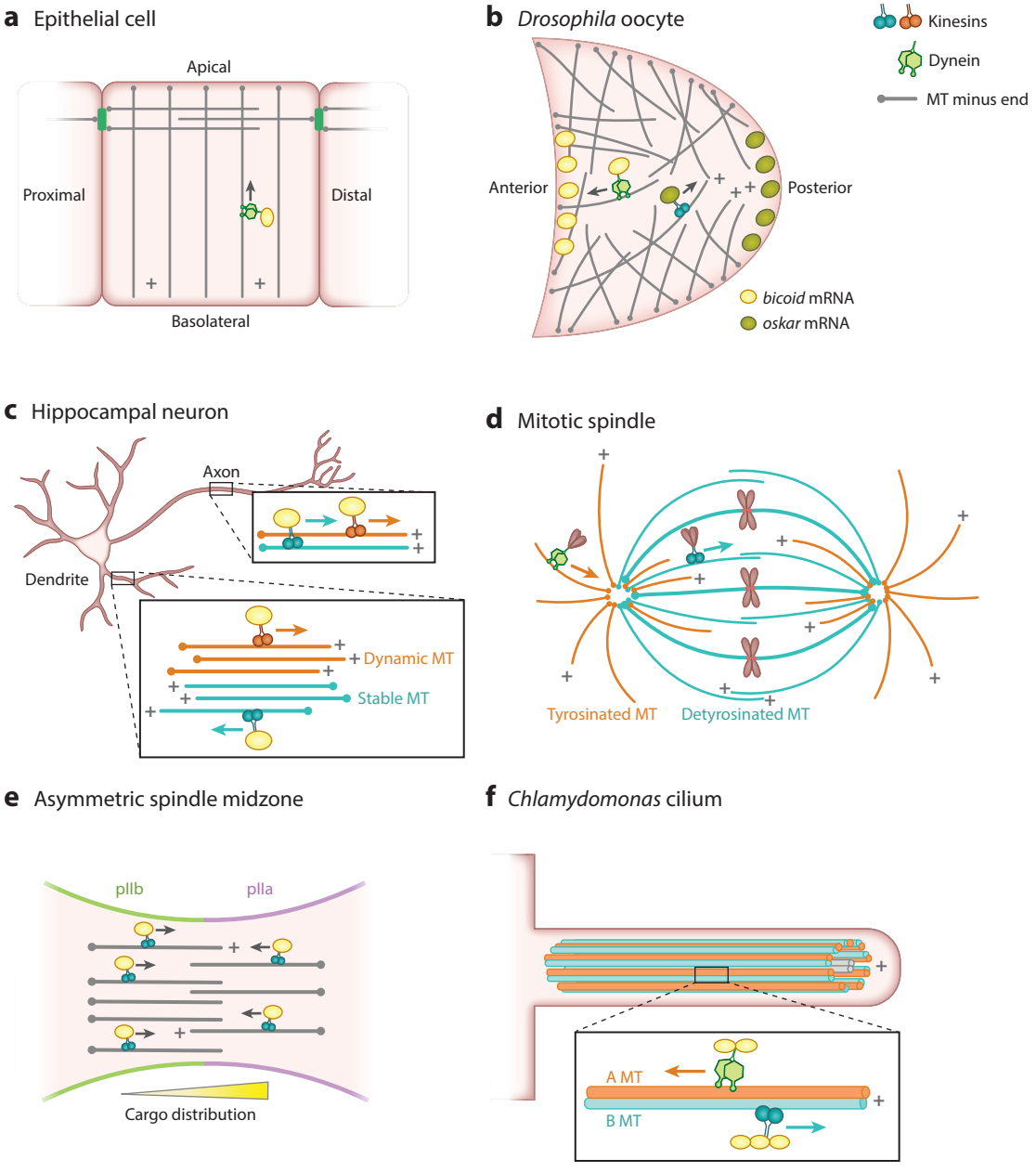

Figure 3

Interplay between microtubule (MT) organization and transport in different systems. (*a*) Apical-basal polarity and planar cell polarity in epithelial cells. (*b*) Polarized transport of mRNA on a disorganized microtubule network in *Drosophila* oocytes. (*c*) Polarized transport and transport over mixed microtubule arrays in hippocampal neurons. (*d*) Congression of chromosomes (*brown*) in the mitotic spindle using dynein-mediated transport to the spindle pole over tyrosinated microtubules, followed by CENP-E-driven motility over detyrosinated interpolar microtubules. (*e*) Robust asymmetric positioning of endosomes resulting from a small asymmetry in microtubule orientations in the spindle midzone. (*f*) Selective use of microtubules in the *Chlamydomonas* cilium. Motor motility on different microtubule subsets is indicated by arrows in the same color as the microtubule used for transport (*teal* and *orange arrows*).

accumulation of various cargoes. For example, the apical localization of mRNA in the follicle cell epithelium of *Drosophila* depends on dynein (Horne-Badovinac & Bilder 2008). Likewise, dynein knockdown in *Drosophila* epithelial cells disrupted the apical accumulation of Rab11-positive vesicles required to transport Cadherin 99C and to ensure proper microvilli formation (Khanal et al. 2016).

Disruption of the polarized microtubule organization without losing microtubule mass was recently achieved using depletion or mislocalization of the minus end–stabilizing protein CAMSAP3/Patronin (Khanal et al. 2016, Noordstra et al. 2016, Toya et al. 2016). The results were mispositioning of the Golgi apparatus and the nucleus (Toya et al. 2016), misplaced RAB11-positive endosomes (Noordstra et al. 2016), and polarity defects (Noordstra et al. 2016). While these findings demonstrate a clear function of the polarized, noncentrosomal microtubule network, these effects were not lethal to animals, since most CAMSAP3 mutant mice survived (Toya et al. 2016). The stronger defects observed in cultured Caco2 cells compared to intestinal enterocytes most likely resulted from the residual centrosomal array that remained upon CAMSAP3 knockdown in these cells and that promoted dynein-dependent mislocalization of RAB11-positive vesicles (Noordstra et al. 2016).

Microtubule minus ends are also anchored at the zonula adherens, the cadherin-based junction between epithelial cells. This organization promotes the transport driven by dynein and the minus end–directed kinesin KIFC3 to maintain cell junction integrity (Meng et al. 2008). In addition, microtubule organization at cell junctions also contributes to planar cell polarity (PCP).

3.4. Planar Cell Polarity: Biased Transport on the Apical Microtubule Network

Many animal tissues display PCP, whereby cells establish uniform polarization within the plane of a cell sheet. This organization is important to properly establish different types of tissues, for example, during the formation of the lungs and kidney. For excellent recent reviews, see Aw & Devenport (2017), Butler & Wallingford (2017), Goodrich & Strutt (2011), and Henderson et al. (2018).

PCP is mediated by the segregation of different key PCP proteins to different sides of the cell, where these proteins coordinate both polarization within the cell and PCP between cells by forming junctional signaling complexes. The *Drosophila* wing has emerged as an important model system to study PCP. Here, live-cell imaging revealed that different PCP proteins, such as Frizzled and Dishevelled, preferentially move toward distal cell boundaries (Matis et al. 2014, Shimada et al. 2006) over apical microtubules that align parallel to the tissue surface in the direction of the proximal-distal (P-D) axis. Consistently, quantifying the number of microtubule plus ends moving along the P-D axis revealed that a slightly higher percentage of plus ends (54%) are oriented distally (**Figures 2*b*** and **3*a***) (Harumoto et al. 2010, Olofsson et al. 2014, Shimada et al. 2006).

The small bias in microtubule orientations observed by tracking dynamic plus ends may be sufficient to drive polarized transport through a biased random walk. Nonetheless, several cargoes display remarkably long unidirectional runs from proximal-to-basal junctions (Matis et al. 2014), suggesting a much more ordered microtubule network. Indeed, recent work has proposed that microtubules form supracellular cables in the *Drosophila* wing; such cables are connected at adherent junctions and may serve mechanical roles in addition to facilitating polarized transport (Singh et al. 2018). These microtubules may form a subset of stable microtubules that are not visible when microtubule end-binding proteins are imaged. This may also explain the robust PCP observed in distal wing cells, where microtubule growth does not have a directional bias (Harumoto et al. 2010).

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3.5. mRNA Positioning in *Drosophila* Oocytes: Biased Random Walks on Disorganized Microtubules

In female fruit flies, *bicoid* and *oskar* mRNAs are positioned to the anterior end and the posterior end of the developing egg cell, respectively. These mRNAs instruct formation of the head and the abdomen once the egg becomes fertilized (St. Johnston 2005). In these large cells, the presence of Par-1 at the posterior cortex opposes the positioning of Shot/Patronin-dependent noncentrosomal nucleation sites, which therefore accumulate along the anterior/lateral cortex (Nashchekin et al. 2016, Parton et al. 2011). The result is a relatively disorganized microtubule network without a clear overall polarity. Therefore, it has long remained unclear how robust positioning of *bicoid* and *oskar* to opposite sides of the cell is achieved (Cha et al. 2001). Live-cell imaging of microtubule growth revealed that *oskar* takes Kinesin-1-driven runs and displays the dynamics of a biased random walk, with approximately 56% of the runs going toward the posterior pole (Zimyanin et al. 2008). This finding is consistent with the subsequent analysis of microtubule orientations using fluorescent end-binding proteins; the latter analysis also revealed a bias in the microtubule network (Parton et al. 2011). In the anterior region, 55% of microtubules grew toward the posterior pole, and this percentage increased to almost 70% in the posterior region. Thus, while these cells lack a polarized microtubule array that runs from one pole to the other, preventing nucleation on the posterior pole generates sufficient asymmetry to ensure the proper accumulation of kinesin-driven cargoes at this pole (**Figure 3*b***). Indeed, numerical simulations have confirmed that asymmetric cortical nucleation is sufficient to polarize the microtubule network and to define the anteroposterior axis (Khuc Trong et al. 2015).

At the anterior side of the oocyte, microtubules are more disorganized. The small asymmetry in orientations is insufficient for robust dynein-dependent delivery of *bicoid* in stage 9 oocytes, which move without bias in this region. Therefore, proper positioning also requires the cortical anchoring of *bicoid* at this side of the cell. Thus, in this part of the cell, dynein-driven transport can best be described as an active random walk that ensures that mRNAs are mobile enough to occasionally reach the anterior cortex and get anchored. Why thermal diffusion followed by capture would not be sufficient in this system is not entirely clear, but an active random walk may result in better diffusive behavior in this system, which also features cytoplasmic streaming (Quinlan 2016).

3.6. Neuronal Transport: Axon-Dendrite Selectivity and Transport on Mixed Polarity Arrays

The ability of neurons to receive, process, and transmit information depends on their polarized organization into axons and dendrites (Bentley & Banker 2016). Axons propagate signals from the cell body to other target cells, whereas dendrites receive signals from axons at specialized junctions termed synapses, where the axonal, presynaptic release of neurotransmitters triggers the activation of postsynaptic receptors. To build such a highly polarized cell, many building blocks need to be differentially transported to either axons or dendrites. Failure to properly distribute cellular components contributes to the pathology of different diseases. Various mechanisms contribute to this selective targeting, but we focus here on how microtubule organization controls neuronal transport (for more general reviews, see Bentley & Banker 2016, Gumy & Hoogenraad 2018, Nirschl et al. 2017).

In lower organisms, such as *Drosophila* and *C. elegans*, microtubules in axons and dendrites are of uniform polarity but are oriented oppositely (Maniar et al. 2012, Rolls 2011, Stone et al. 2008). These features ensure that plus end–directed kinesins drive anterograde transport in the axons, whereas dynein is required for transport into dendrites over the minus end out–oriented

network (Harterink et al. 2016, Rolls 2011). A recent study exploited the low microtubule density in *C. elegans* axons to directly measure microtubule numbers and length by using clearly detectable growth events for single-microtubule calibration and renormalization of microtubule intensities along the length of the axon (Yogev et al. 2016). This study revealed that axonal cargoes often stall at microtubule ends and that microtubule length limits cargo run length.

Remarkably, dendrites in mammalian neurons have a mixed polarity network, with both orientations being roughly equally abundant, while axons have a uniform plus end out–oriented network. This organization was first revealed using the hook decoration technique (Baas & Lin 2011, Baas et al. 1988, Burton 1988, Burton & Paige 1981, Heidemann et al. 1981) and was later confirmed by imaging proteins that decorate growing microtubule plus ends, both in cultured neurons (**Figure 2*a–c***) (Stepanova et al. 2003, Yau et al. 2016) and in vivo (Kleele et al. 2014, Yau et al. 2016). This nonuniform organization in dendrites raises intriguing questions. How do motor proteins navigate a network with mixed orientations? Can certain plus end–directed motors selectively move into axons, given the presence of plus end out–oriented microtubules in dendrites?

To answer these questions, several approaches have been used to test how different motors move in different neuronal compartments. First, expression of constitutively active and fluorescently tagged motors revealed that several kinesins accumulate only in axon tips, while other kinesins accumulate in the tips of both axons and dendrites (Huang & Banker 2012, Jacobson et al. 2006, Nakata & Hirokawa 2003, Reed et al. 2006). In addition, multiple well-controlled intracellular transport assays have been developed to directly examine how different motor proteins navigate the neuronal cytoskeleton to facilitate cargo delivery to specific sites (Ayloo et al. 2017; Duan et al. 2015; Kapitein et al. 2010a,b; van Bergeijk et al. 2015). In these assays, chemically induced or light-induced heterodimerization can trigger the recruitment of specific motors to immotile cargoes, such as peroxisomes, which from then on report the activity of the motor. These experiments revealed that dynein can selectively enter dendrites through bidirectional runs without accumulating at the tips, consistent with the mixed microtubule array (Kapitein et al. 2010a). Plus end–directed Kinesin-3 also entered dendrites but strongly accumulated in the tips of dendrites (Lipka et al. 2016). In contrast, Kinesin-1 selectively entered axons and completely failed to enter dendrites (Kapitein et al. 2010a).

How can two plus end–directed kinesins have such different behaviors? To address this question, recent work has introduced a novel technique for optical nanoscopy, termed motor-PAINT, in which motor proteins running over an extracted cytoskeleton are traced with nanometric precision to super-resolve microtubules and to determine their polarity (**Figure 2*d***) (Tas et al. 2017). In rat hippocampal neurons, this approach was combined with drug treatments and the nanoscopic imaging of tubulin modifications to explore the relation between microtubule orientations and modifications. This approach revealed that, in dendrites, different microtubule subsets are preferred by either Kinesin-1 or Kinesin-3 and are oppositely oriented (**Figure 3*c***). While the dynamic, tyrosinated microtubules preferred by Kinesin-3 are mostly oriented plus end out, the stable microtubules preferred by Kinesin-1 have the opposite orientation preference (i.e., 66% of such microtubules are minus end out). These results suggest that Kinesin-3 and Kinesin-1 drive anterograde and retrograde dendritic transport, respectively, and explain why Kinesin-1 cannot drive transport into dendrites. In addition, different microtubule subsets form polarized bundles, i.e., bundles in which most microtubules have the same orientation, which may promote unidirectional motility of cargoes driven by motors that do not discriminate between different subsets (Tas et al. 2017).

As described in Section 2.2, the mechanisms by which motors interact with different subsets are poorly understood. Likewise, how cargoes with multiple motors navigate this network is also unclear. A recent study revealed that Septin-9 is a MAP that can promote microtubule binding of

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Kinesin-3 while opposing Kinesin-1 (Karasmanis et al. 2018). Strikingly, knockdown of Septin-9 resulted in Kinesin-1-driven transport into dendrites. Thus, in normal conditions, Septin-9 may prevent Kinesin-1 interaction with tyrosinated microtubules or may contribute to the proper organization and orientation of different microtubule subsets. More work is needed to resolve the exact three-dimensional organization of the neuronal cytoskeleton and to map the distribution of different MAPs.

3.7. Spindle Apparatus: Selective Transport to the Midzone or Toward One of the Poles

In eukaryotes, a microtubule-based structure termed the spindle apparatus is formed prior to cell division to ensure the proper segregation of genetic material and organelles. The typical bipolar spindle can be viewed as two spatially separated astral arrays whose microtubules form antiparallel overlaps in the region between the asters (**Figure 3*d***). To ensure proper segregation, microtubules mediate the congression of chromosomes to the spindle midzone. In addition, microtubules from either pole form parallel bundles that connect to the sister kinetochores of each chromosome in a process termed biorientation. Once every chromosome is bioriented, the sister chromatids are separated to the opposite poles during anaphase.

By the onset of congression, the majority of chromosomes are already bioriented, and their movement is thought to be driven by forces of kinetochore-attached dynamic microtubules (Auckland & McAinsh 2015). In addition, a smaller percentage (10–20%) of chromosomes that are located more peripherally and not yet bioriented are transported by kinetochore-attached motors to the metaphase plate (Barisic et al. 2014, Kapoor et al. 2006). This motor-based congression involves dynein-dependent movement toward the closest spindle pole, followed by transport to the metaphase plate by the centromeric kinesin CENP-E (Barisic et al. 2014, Kapoor et al. 2006, Wood et al. 1997, Yang et al. 2007). But how do two oppositely oriented motors coordinate their motility to ensure proper delivery to the metaphase plate, rather than moving chromosomes away from this position?

Recent work has shown that the switch between the dominance of dynein and CENP-E movement depends on the differential preference of dynein and CENP-E for different microtubule subtypes (**Figure 3*d***) (Barisic et al. 2015). Astral microtubules are mostly tyrosinated, whereas interpolar microtubules are more detyrosinated. In addition, while initiation of dynein-mediated transport is enhanced on tyrosinated microtubules (McKenney et al. 2016), in vitro experiments revealed that CENP-E processivity and force generation were enhanced on detyrosinated microtubules. This setup may explain why dynein is dominant on astral microtubules and why CENP-E takes over once the detyrosinated microtubules are within reach. Consistently, disruption of the detyrosination pattern within the spindle by overexpression of tubulin tyrosine ligase resulted in impaired congression (Barisic et al. 2015).

Microtubule detyrosination also contributes to spindle asymmetry during meiosis. In female meiosis, only chromosomes that segregate to the egg are transmitted to offspring, while the remaining chromosomes are degraded in the polar body that is formed after cytokinesis (Akera et al. 2017). While one would expect that the two parental copies of a gene have an equal probability to end up in the egg, selfish genetic elements have an increased probability to survive. Such biased segregation can be associated with increased centromere size and larger kinetochores, but how these properties ensure biased segregation is unclear. Recent work reported that microtubule detyrosination is increased at the egg side of the spindle, i.e., the surviving side, and that chromosomes with larger kinetochores sense this modification to promote proper positioning (Akera et al. 2017). This mechanism may depend on motor-based transport or on the preferential

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destabilization of detyrosinated microtubules by the centromere-associated Kinesin-13 MCAK (Lampson & Black 2017).

Asymmetric spindle architecture is also necessary for asymmetric stem cell division because it enables the unequal distribution of fate determinants to daughter cells (Coumailleau et al. 2009, Kressmann et al. 2015). One mechanism to achieve such unequal distribution is asymmetric positioning of the spindle within the cell (Li 2013). Alternatively, organelles could be actively redistributed to one side of the spindle, as demonstrated for Notch signaling components enclosed in Sara endosomes in *Drosophila* epithelial stem cells (Coumailleau et al. 2009). In this system, the biased dispatching of Sara endosomes to pIIa cells is achieved through an asymmetry in microtubule orientations in the spindle midzone (**Figure 3*e***) (Derivery et al. 2015). Here, a small asymmetry in the distribution of the minus end stabilizer Patronin causes an increase in microtubule density on the pIIb side that results in more plus ends pointing toward the pIIa side in the spindle midzone. This small asymmetry in microtubule orientations biases the transport of Sara endosomes driven by the Kinesin-3 Klp98A, resulting in a strong accumulation at the pIIa side. Graded disruption of the microtubule array by mislocalization of Patronin causes a corresponding change in endosome enrichment that nicely follows the predictions from a mathematical model of the system (Derivery et al. 2015).

3.8. Intraflagellar Transport: Selective Use of Microtubules

Cilia are microtubule-based structures that protrude from the surface of resting cells and are crucial for signaling, sensing, and motility in many organisms (Anvarian et al. 2019, Khan & Scholey 2018, Mitchison & Valente 2017). The coordinated movement of ciliary components along microtubules to and from the assembly site at the tip is essential for its morphogenesis (Prevo et al. 2017). Structurally, the cilium is composed of a crosslinked array of microtubules, termed the axoneme, that provides mechanical support, generates motility of motile cilia, and provides tracks for IFT. Axonemes of most motile cilia form a ring of nine microtubule doublets surrounding a central pair of single microtubules, known as the 9 + 2 arrangement, while nonmotile cilia lack the central pair. Each doublet is made of a full A-microtubule with 13 protofilaments and an incomplete B-microtubule of 10 protofilaments whose sides are bound to the A-microtubule. Recent work has revealed a regulatory role of the C-terminal tail of A-microtubules in the nucleation of a B-microtubule (Schmidt-Cernohorska et al. 2019). All these microtubules are uniformly oriented with their plus end pointing toward the axoneme tip, and therefore transport to and from the tips is established by plus end–directed kinesins (i.e., Kinesin-2 family members) and minus end–directed cytoplasmic dynein 2, respectively. Nonetheless, recent work has revealed several surprising features of this transport system.

Live-imaging of IFT in the single-cell ciliate *Chlamydomonas reinhardtii* revealed that most particles move very directionally, with few pauses or reversals that one might expect to result from collisions between oppositely directed particles or from the potential antagonism between oppositely directed motors on the same IFT particles (also known as IFT trains) (Stepanek & Pigino 2016). Correlative light electron microscopy demonstrated that anterogradely moving IFT trains utilize B-microtubules while retrograde IFT uses A-microtubules (**Figure 3*f***) (Stepanek & Pigino 2016). In other words, the microtubule AB doublet structure is used as a double-track highway for IFT, preventing collisions of cargoes moving in opposite directions. In addition, cryo-electron tomography of cilia revealed that dyneins are loaded with an inhibitory conformation onto the anterograde trains and thus do not engage in a tug-of-war with the Kinesin-2 during anterograde transport (Jordan et al. 2018). At the tip of the cilium, anterograde IFT trains are disassembled and reassembled into a structurally different retrograde IFT train that engages active dynein for

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processive retrograde transport. Thus, in this system, unimpeded unidirectional transport is ensured both by the use of distinct microtubule subsets for transport in opposite directions and by the controlled regulation of motor activity within particles. In *C. elegans*, IFT features spatially different anterograde motors, whose activity is spatially regulated. This regulation is related to a transition from doublet microtubules to singlet microtubules in distal cilia, where the Kinesin-2 OSM-3 takes over from Kinesin-II (Prevo et al. 2015, Silva et al. 2017, Snow et al. 2004).

How do motors recognize differences in microtubules within a doublet? Structural analysis has shown that the lattice arrangement of A- and B-microtubules is similar and cannot directly explain this differential transport (Maheshwari et al. 2015). Interestingly, earlier work revealed that detyrosination of tubulin is largely confined to the B-microtubule of *Chlamydomonas* doublets (Johnson 1998). This finding is consistent with in vitro experiments that found that detyrosination reduces dynein interactions but promotes Kinesin-2 motility (McKenney et al. 2016, Sirajuddin et al. 2014). In addition, B-microtubules are more glutamylated (Kubo et al. 2010, Suryavanshi et al. 2010), which is also beneficial for Kinesin-II motility (Sirajuddin et al. 2014).

While in *Chlamydomonas* IFT trains were found on all nine doublets, earlier structural studies using electron micrography in another flagellated protozoan, *Trypanosoma brucei*, had suggested that IFT trains may prefer only subsets of microtubule doublets (Absalon et al. 2008). Consistent with this idea, a recent study that employed structured illumination microscopy demonstrated that IFT trains bidirectionally move on only two sets of microtubule doublets (Bertiaux et al. 2018). Both the mechanisms that restrict transport to these two doublets and the function of this selectivity are still unclear.

4. PERSPECTIVE

Early work using electron microscopy revealed a rich diversity in microtubule-based structures in different organisms and cell types (Chaaban & Brouhard 2017). In addition, observations of different microtubule subsets that differ in structure, stability, and PTMs date back several decades. Nonetheless, how these different structures and microtubule subsets contribute to fulfilling specific transport demands has remained unclear.

As discussed in this review, novel approaches have recently begun to reveal how microtubule organization guides transport in different systems. New microscopy techniques, such as super-resolution microscopy and correlative electron and light microscopy, are providing new insights into microtubule organization, while controlled intracellular transport assays are revealing how different motor proteins navigate the cytoskeleton. In addition, modeling approaches can predict transport outcomes of different microtubule geometries and have revealed how small asymmetries in mixed microtubule arrays can result in robust asymmetric targeting of organelles, for example, in neuronal dendrites, in *Drosophila* oocytes, and in mitosis. Thus, while published cartoons of cytoskeletal organization often show idealized networks (see also **Figure 3**), moderate disorder in microtubule organization does not affect polarized transport as long as there is bias. The systems described here display a spectrum of overall microtubule polarization, featuring strongly polarized arrays in axons or columnar epithelia; weakly polarized arrays in *Drosophila* oocytes and cells displaying PCP; or no overall polarization, such as in dendritic networks where opposite microtubules have different modifications and recruit different motor proteins to enable polarized transport.

Although our understanding of noncentrosomal microtubule organization is rapidly growing, the mechanisms by which specialized microtubule arrays are built are unknown. Feedback between motor-based transport and microtubule organization is well established in spindle formation, in which microtubules are often the cargoes of different microtubule-based motors

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(Prosser & Pelletier 2017). More subtle feedback exists in muscle syncytium, where nuclei nucleate microtubules and are positioned by microtubule-based motors (Roman & Gomes 2018). Similar mechanisms may operate in other differentiated cell types. In addition, the strong selectivity of different motors for specific microtubule subsets remains poorly understood. Finally, it remains largely unresolved how the activity of different cargo-bound motors is coordinated to ensure that the correct transport pathway is activated. The recent discoveries of new tubulin-modifying or motor-modulating MAPs are providing exciting opportunities to address these questions. By combining the controlled perturbation of these factors in different cellular systems with high-resolution microscopy, intracellular transport assays, and modeling, a more integrated understanding of cellular logistics is likely to emerge.

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