Cold Spring Harbor Perspectives in Biology
www.cshperspectives.org

Microtubule Motors in Establishment of Epithelial Cell Polarity

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Epithelial cells play a key role in insuring physiological homeostasis by acting as a barrier between the outside environment and internal organs. They are also responsible for the vectorial transport of ions and fluid essential to the function of many organs. To accomplish these tasks, epithelial cells must generate an asymmetrically organized plasma membrane comprised of structurally and functionally distinct apical and basolateral membranes. Adherent and occluding junctions, respectively, anchor cells within a layer and prevent lateral diffusion of proteins in the outer leaflet of the plasma membrane and restrict passage of proteins and solutes through intercellular spaces. At a fundamental level, the establishment and maintenance of epithelial polarity requires that signals initiated at cell–substratum and cell–cell adhesions are transmitted appropriately and dynamically to the cytoskeleton, to the membrane-trafficking machinery, and to the regulation of occluding and adherent junctions. Rigorous descriptive and mechanistic studies published over the last 50 years have provided great detail to our understanding of epithelial polarization. Yet still, critical early steps in morphogenesis are not yet fully appreciated. In this review, we discuss how cytoskeletal motor proteins, primarily kinesins, contribute to coordinated modification of microtubule and actin arrays, formation and remodeling of cell adhesions to targeted membrane trafficking, and to initiating the formation and expansion of an apical lumen.

Development of morphological and functional polarity underlies many important cellular activities, including directed cell migration, differentiation of numerous cell types, organogenesis, and injury repair. In both single and multicellular animals, the establishment and maintenance of cell polarity requires that microtubule (MT) and actin dynamics, and remodeling of their respective networks, are coordinated locally in cells. In large part, localized regulation of Rho-family GTPase activity mediates this coordination of MTs and actin, and accommodates formation of specialized subcellular domains (Gundersen et al. 2004; Siegrist and Doe 2007; Li and Gundersen 2008; Wojnacki et al. 2014). As an accompaniment to reg-

Editor: Keith E. Mostov
Additional Perspectives on Cell Polarity available at www.cshperspectives.org

 doi: 10.1101/cshperspect.a027896
Cite this article as *Cold Spring Harb Perspect Biol* 2018;10:a027896

ulation of MT dynamics, subsets of MTs are locally stabilized along the developing polarity axis and tubulin subunits in these stabilized MTs accumulate biochemically distinct post-translational modifications (Bre et al. 1987, 1991; Piperno et al. 1987; Bacallao et al. 1989; Pepperkok et al. 1990; Bulinski and Gundersen 1991). These biochemically distinguishable MTs are preferred substrates for the binding and motility of a subset of MT-associated motor proteins (Liao and Gundersen 1998; Kreitzer et al. 1999; Ikegami et al. 2007; Verhey and Gaertig 2007; Dunn et al. 2008; Cai et al. 2009; Konishi and Setou 2009; Hammond et al. 2010; Sirajuddin et al. 2014). In turn, MT remodeling and stabilization repositions many organelles and establishes distinct trafficking routes used by MT motors to direct transport of cytosolic and membrane/secreted proteins to specific domains inside and on the surface of cells (Gundersen 2002).

Epithelial cells line all body surfaces facing the outside world and maintain physiological homeostasis through their characteristic ability to perform vectorial transport of ions and fluid essential to the function of many organs. To do so, apical and basolateral membranes must be structurally and functionally distinct, a feature maintained by tight junctions (TJs) that prevent lateral diffusion of membrane proteins in the outer leaflet of these plasma membrane domains and restrict passage of proteins and solutes through intercellular spaces (Rodriguez-Boulan and Nelson 1989). Attenuation, inhibition, or reversal of epithelial polarity by either direct or indirect means characterize diverse human pathologies, including cancers, cystic diseases, cardiovascular disease, and absorptive and secretory disorders (Wilson 2011; Barrera et al. 2013; Hinck and Nathke 2014; Gandalovicova et al. 2016; Overeem et al. 2016). Although it is known that signals initiated at cell–matrix and cell–cell adhesions trigger local actin and MT reorganization, and that this informs polarized membrane trafficking along MTs, key molecules and regulatory mechanisms orchestrating the structural adaptations that direct functional polarization have not been elucidated fully.

MICROTUBULE MOTORS IN INITIATION OF EPITHELIAL POLARIZATION

Cell–substratum and cell–cell adhesions are thought to provide spatial landmarks that direct cytoskeletal reorganization, set up secretory targeting patches (e.g., the exocyst), and promote development of distinct transport routes through the cytoplasm leading to apicobasal membrane polarity (Drubin and Nelson 1996; O'Brien et al. 2002; Nelson 2003; Yu et al. 2005; Stehbens et al. 2009; Lee and Streuli 2014). It is not precisely known how these adhesion sites are determined de novo, but the components of these adhesions must be delivered to the plasma membrane by MT-dependent transport, and retained there by establishing interactions with the underlying cytoskeletal network. Failure to establish or loss of functional adhesions is an early hallmark of disease transformation and contributes to attenuation of membrane polarity by obscuring topological cues normally present in intact epithelial tissues. Studies in flies and cultured mammalian epithelia have shown, however, that neither a solid substratum nor adherens junctions (AJs) are necessary for de novo epithelial polarization (Dow and Humbert 2007). Instead, MT-dependent transport of membrane-associated polarity proteins and signaling molecules that promote selective stabilization of MTs is sufficient to polarize even single epithelial cells (Spicer et al. 2003; Baas et al. 2004; Brajenovic et al. 2004; Harris and Peifer 2004, 2005; Trinczek et al. 2004; Theard et al. 2007). Together, these data suggest that nontopological cues for polarity exist and, in fact, may precede adhesion-based, topological cues as stimuli in establishing epithelial polarity.

The nature of putative nontopological polarity cues is unclear as relatively few components of the cytoplasm possess inherent asymmetry. MTs (and actin), however, fall into this class being that they are polarized by their mechanism of assembly into filaments. MT-associated motors, kinesins, and dynein (as well as myosins), are also polarized functionally with respect to their movement toward one end of the filament, and their activity depends on interaction with the polarized cytoskeletal sub-

strate. Thus, as mediators of targeted protein transport, MT motors can easily be envisioned as fundamental participants in construction of morphological and functional polarity. Indeed, at least for cell–cell contacts, it is known that MT motors are responsible for delivery of transmembrane proteins that form these adhesive contacts through both the biosynthetic and recycling pathways (Mary et al. 2002; Chen et al. 2003; Ivanov et al. 2006; Nekrasova et al. 2011). These newly formed adhesions likely relay signals back to MTs resulting in changes in their organization and the formation of noncentrosomal arrays associated with polarized epithelia. How adhesion molecules are retained at the plasma membrane before establishment of cell adhesions that induce cortical cytoskeletal remodeling is not clear. Most likely, it involves a combination of stochastic or outside-in signaling events leading to capture and stabilization of a subset of MTs. In turn, this reorients membrane-trafficking machineries and directs the delivery of adhesive molecules to the plasma membrane where they form immature contact zones. Signals from these immature contacts subsequently direct more robust cytoskeletal rearrangements, which then stabilize the nascent cell adhesions. This idea that MT motors are key to establishing polarity is supported by studies in Fungi in which MT plus-end-directed kinesins (Tea2, Kip2, and KipA) are necessary for polarized cell growth. Mechanistically, these kinesins localize at growing MT plus ends and at cell ends (Browning et al. 2000; Wedlich-Soldner et al. 2002; Konzack et al. 2005), carry or organize proteins required for local MT capture and stabilization at the cell cortex (Browning et al. 2003; Busch et al. 2004), proteins needed for local actin remodeling (Feierbach et al. 2004; Martin et al. 2005; Snaith et al. 2005), and proteins needed for polarized cell growth (Wedlich-Soldner et al. 2002; Browning et al. 2003; Snaith and Sawin 2003; Steinberg and Fuchs 2004; Takeshita et al. 2008). The MT minus-end-directed motor dynein contributes to polarized cell growth by regulating the position of MT organizing centers, transporting free MTs, tethering MT plus ends to the cortex, and by regulating membrane recycling at the growing ends of cells (Table 1) (Fink and Steinberg 2006).

In metazoa, a role for MT motors in generating cell polarity is less well characterized. Studies in cultured hippocampal neurons show that KIF2A regulates pruning of collateral axon branches by depolymerizing MTs (Homma et al. 2003; Maor-Nof et al. 2013). In contrast, a truncated, constitutively active kinesin-1 accumulates in the neurite that becomes an axon and may play a role in axon specification (Jacobson et al. 2006). In cultured epithelial cells, kinesin-2 family motors KIF3A/B and KIF17 contribute to regulation of MT dynamics and stabilization, to cytoskeletal remodeling, and to positioning sites of apical membrane insertion and lumen formation (Jaulin and Kreitzer 2010; Acharya et al. 2013, 2016; Boehlke et al. 2013; Li et al. 2014a). Both motors localize to MT plus ends and regulate MT plus-end dynamics, but appear to affect epithelial polarity by distinct mechanisms, as highlighted below. Dynein may contribute to establishment of polarity by orienting MTs and tethering MT plus ends at the cortex, most notably at cell–cell adhesions (Ligon and Holzbaur 2007). In this capacity, it may function in a manner similar to its role in capture of astral MTs during mitosis, and in orientation of planar cell polarity (Siegrist and Doe 2005). It is important to note, however, that a role for dynein may not be formative in this mode of polarization. More clear is the role of dynein in animal development, in which it determines oocyte polarity by regulating the delivery of bicoid, and gurken RNAs to the anterior pole and by asymmetric positioning of the nucleus (Brendza et al. 2002; Januschke et al. 2002). Interestingly, kinesin-1 is required for anterior dynein localization and function in oocyte polarization.

KINESINS IN TARGETING CORTICAL CAPTURE SITES FOR MTs

Remodeling of MT arrays in experimental models of polarity proceeds by a conserved mechanism known as MT search and capture (Goode et al. 2000; Gundersen 2002; Siegrist and Doe 2007). The inherent dynamic instability of MTs

Table 1. Kinesin motors in cell polarity

	Polarity phenotype	Transport of polarity factors, cytoskeletal effectors, and vesicles	MT+TIP interactions	Effects on MTs	Effects on actin	AJC remodeling and stabilization	References
Kinesin-1 family							
KIF5	Axon specification and outgrowth	Transport of APC, rab10-associated vesicles to axon Transport of vesicles to supply axon tip growth		MT sliding, generates mechanical force for axon extension			Brendza et al. 2000, 2002; Januschke et al. 2002; Palacios and St Johnston 2002; Jacobsen et al. 2006; Lu et al. 2013; Deng et al. 2014; Ruane et al. 2016
	Embryonic anterior–posterior polarity	Transport of oskar mRNA to posterior pole					
NKin (*Neurospora crassa*)	Branched and misshapen hyphae Reduced mycelial extension	Transport of vesicles to Spitznkorper					Seiler et al. 1997
Kin2 (*Ustilago maydis*)	Decreased hyphal tip growth	Transport of vesicles to hyphal tip					Lehmler and Bolker 1997
Kinesin-2 family							
KIF3A/B	Epithelia with no lumen (in collagen) or multiple lumens (in Matrigel)	Delivery of TJ proteins		Stimulates MT dynamicity			Jimbo et al. 2002; Boehkle et al. 2013; Li et al.2014
	Failure to localize apical membrane proteins coming from rab11 endosome	Transport of APC, interaction with β-catenin		MT orientation toward leading edge of migrating cells			
KIF17	Epithelia with no lumen or multiple lumens	APC, Rho regulator* (*inferred)	EB1, APC, IQGAP1* (*unpublished)	Dampens MT plus-end dynamics	Concentration/assembly of apical actin network in 3D	Accumulation of branched actin at cell–cell adhesions	Jaulin and Kreitzer 2010; Acharya et al. 2013, 2016; Espenel et al. 2013
	Failure to direct apical membrne proteins to lumen			Stabilizes MTs	Activation of RhoA, inhibition of cofilin	Strengthens/stabilizes cell–cell adhesion	
				Increases posttranslational modification of MTs			

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Table 1. *Continued*

	Polarity phenotype	Transport of polarity factors, cytoskeletal effectors, and vesicles	MT+TIP interactions	Effects on MTs	Effects on actin	AJC remodeling and stabilization	References
Kinesin-3 family							
KinA (*Aspergillus nidulans*)	Reduced hyphal growth, branched, and misshapen hyphae	Transport of chitin synthases to hyphal tip		MT destabilization			Requena et al. 2001; Takeshita et al. 2015
NKin2 (*N. crassa*)		Transport of vesicles to Spitznkorper		Preferrential interaction with posttranslationally detyrosinated MTs			Seidel et al. 2013
Kin3 (*U. maydis*)				No preferrential interaction with MT subpopulations			Weldich-Soldner 2002; Steinberg 2015
Kinesin-4 family							
KIF4	Inhibition of stable MT orientation		EB1	Stabilizes MTs oriented in direction of migration Increases posttranslational modification of MTs			Morris et al. 2014
Kinesin-6 family							
ZEN4/MKLP1 (*Caenorhabditis elegans*)	Lost polarity/failure to epithelialize arcade cells	Apicolateral localization of Par3, aPKC		Apical MT accumulation	Apical actin accumulation	Localization of cadherin and AJM-1 at apicolateral junctions	Portereiko et al. 2004
Kinesin-7 family							
Tea2 (*Schizosaccharomyces pombe*)	Bent and T-shaped cells	Tip1, Tea1 (ezrin/radixin/moesin-like), Tea4	Mal3; Tip1(EB1; CLIP170)	MT capture and stabilization Stable MTs only on one side of cell, may reflect NETO	Localization of Rho1 at cell ends Interaction with Mod5 and For3 Actin localization in cortical patches and cell ends		Arellana et al. 1997; Browning et al. 2000, 2003; Busch et al. 2004; Feierbach et al. 2004; Kume et al. 2011

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Table 1. *Continued*

	Polarity phenotype	Transport of polarity factors, cytoskeletal effectors, and vesicles	MT+TIP interactions	Effects on MTs	Effects on actin	AJC remodeling and stabilization	References
KipA (*A. nidulans*)	Curved hyphae, mislocalization of the Spitznkorper vesicle supply center	TeaA, TeaR (Tea1, mod5)	TeaA (CLIP170, Tip1; EBA-inferred by genetics)	MT focusing at cell tips, MT "straightness"			Konzack et al. 2005; Takeshita 2008; Zeng et al. 2014
Kip2 (*Saccharomyces cerevisiae*)	Nuclear and mitotic spindle positioning	Transport of dynein and Kar9 to schmoo tip	Bik1 (CLIP170), Kar9, Bim1 (EB1)	MT polymerization/ elongation MT stabilization			Cottingham and Hoyt 1997; Huyett et al. 1998; Miller et al. 1998; Maekawa et al. 2003; Carvalho et al. 2004; Drechsler et al. 2015; Hibbel et al. 2015
Kinesin-8 family							
Kip3 (*S. cerevisiae*)	Nuclear positioning defect			MT plus-end depolymerization MT orientation and extension into the bud			Miller et al. 1998; Varga et al. 2006
Kinesin-13 family							
KIF2A	Inhibition of collateral axon pruning			Depolymerizes MTs in collateral branches			Homma et al. 2003; Maor-Nof et al. 2013
Kinesin-14 family							
Kar3 (*S. cerevisiae*)	Nuclear migration and positioning defect			MT attachment to schmoo tip			Maddox et al. 2003

allows for MT plus ends (and proteins associated with them) to search and detect localized signals at the cortex. In response to such cues, a subset of MTs is captured, selectively, and stabilized (Kirschner and Mitchison 1986). The resulting stable, and now polarized, MT arrays accumulate tubulin posttranslational modifications and define an axis along which signals are transduced and provide tracks for the polarized transport of exocytic and endocytic vesicles, proteins complexes, mRNA, and organelles by MT-associated motors.

MT stabilization occurs by a plus-end capping/capture mechanism that prevents addition and loss of tubulin subunits from the polymer (Infante et al. 2000). This capping is dependent on an ATPase activity consistent with that of kinesins. In migrating fibroblasts, MTs oriented in the direction of migration are stabilized selectively in a process mediated by localized signaling through RhoA, mDia1, and integrins (Cook et al. 1998; Palazzo et al. 2001, 2004). In these cells, the kinesin-4 family motor KIF4 (XKLP-1 in *Xenopus laevis*) interacts with EB1 at MT plus ends, stabilizes a subset of MTs, and is required downstream from lysophosphatidic acid–induced activation of RhoA for MT capture (Morris et al. 2014). In vitro, addition of XKLP-1 to polymerized tubulin inhibits both MT growth and shrinkage, but affects MT dynamics by modifying the MT lattice rather than plus ends (Bringmann et al. 2004). Local activation of Rac and Cdc42 has also been implicated in MT capture at the leading edge of fibroblasts via interactions with the cortical scaffolding protein IQGAP1 and the MT plus-end-associated protein CLIP170 (Fukata et al. 2002). Interestingly, mDia1 in complex with the MT plus-end-associated protein EB1, interacts with the tumor suppressor adenomatous polyposis coli (APC) (Wen et al. 2004) and APC interacts with IQGAP1 (Watanabe et al. 2004). APC localizes to MT plus ends in fibroblasts and epithelial cells and contributes to MT stabilization in both cell types (Mogensen et al. 2002; Kita et al. 2006; Kroboth et al. 2007).

During epithelial polarization, MTs are further reorganized from centrosomally nucleated, radial arrays into longitudinal bundles (minus ends oriented apically) and arrays of mixed polarity underlying the apical pole and overlying the basal membrane (Bacallao et al. 1989; Gilbert et al. 1991). These noncentrosomal MTs are stabilized and enriched in posttranslationally modified tubulin (Bre et al. 1987, 1991; Pepperkok et al. 1990). In addition, a subset of dynamic MTs is still nucleated at the centrosome and emanates toward the apical and apicolateral membrane domains and serve as tracks for transport and apical delivery of membrane and secreted proteins (Jaulin et al. 2007). The kinase Par1 regulates MT stabilization and reorganization in epithelial cells (Cox et al. 2001; Doerflinger et al. 2003; Cohen et al. 2004), in part by regulating cadherin association with the cortical actin cytoskeleton (Elbert et al. 2006), and thus potentially establishing zones of immature cell–cell adhesion. Although it is clear that cadherin engagement and formation of cell–cell junctions triggers changes in MT dynamics in epithelial cells (Chausovsky et al. 2000; Waterman-Storer et al. 2000; Shtutman et al. 2008), downstream signaling pathways leading to MT reorganization remain obscure. One can easily envision, however, that MT plus ends are selectively captured and stabilized at or near the cell cortex in response to signals transmitted by integrins, cadherins, or membrane-associated signaling complexes.

COORDINATING CYTOSKELETAL REMODELING WITH REMODELING OF CELL–CELL ADHESIONS

Coordinated remodeling of the cytoskeleton and cell adhesions is key to morphogenetic changes associated with epithelial polarization and function of polarized epithelial cells. Known collectively as the apical junctional complex (AJC), apical junctions couple adjacent cells physically, whereas tight junctions set boundaries between apical and basolateral membranes and control paracellular permeability (Guillot and Lecuit 2013). Components of the AJC are delivered to the membrane by transport along MTs (Mary et al. 2002; Chen et al. 2003; Portereiko et al. 2004; Yanagisawa et al. 2004; Ivanov et al. 2006; Nekrasova et al. 2011)

and are anchored at adhesive sites by their association with actin and MT adaptors. As cell–cell adhesions mature, signaling molecules that also associate with the AJC induce changes in actin and MT arrays by modifying polymer dynamics and stability (Chausovsky et al. 2000; Mege et al. 2006; Bellett et al. 2009; Ratheesh et al. 2012; Brieher and Yap 2013). Thus, the cytoskeleton affects AJC formation and maturation, and signaling at the AJC reciprocally affects actin and MTs; together, these processes direct morphogenetic responses to numerous cues (Brieher and Yap 2013; Mack and Georgiou 2014). Although many of the molecular components that control formation, dynamics, and remodeling of AJCs and the cytoskeleton are well described, we know little about how these events are coordinated in response to intrinsic and extrinsic cues that affect cell polarity. A logical hypothesis is that molecules that can affect both the cytoskeleton and cell adhesions are key to coordinating cellular remodeling during polarization.

In metazoa, sites of cell–cell contact may be equivalent to growing cell ends in Fungi and, indeed, MT plus ends target to these regions of the plasma membrane where they regulate delivery and turnover of components of AJs by MT motors, as well as cortical actin organization (Pokutta and Weis 2007; Stehbens et al. 2009). The molecular effectors of MT capture and stabilization in epithelia are not yet clearly defined. However, it is interesting to note that the kinesin-2 family motor KIF17, which participates in development of apicobasolateral polarity and lumen formation in MDCK and Caco2 epithelial cells, interacts with both EB1 and APC (Jaulin and Kreitzer 2010). In cells, KIF17 suppresses MT plus-end dynamics and stabilizes MTs. The localization of KIF17 at MT plus ends depends on EB1, which activates KIF17 by relieving its autoinhibited conformation (Espenel et al. 2013). In vitro, EB1 enhances the binding and ATPase activity of KIF17, but is not required to stabilize MTs (Acharya et al. 2013). Importantly, unlike KIF4-mediated MT stabilization in fibroblasts, KIF17, at least in part, appears to act upstream of RhoA in epithelial cells (Acharya et al. 2016). Because KIF17 also localizes at sites of cell–cell adhesion (Acharya et al. 2016), these data suggest KIF17 can act on both MT dynamics and on cortical capture. This idea is consistent with the KIF17-dependent localization of APC to membrane protrusions in epithelial cells (Jaulin and Kreitzer 2010). Precisely how the interaction of KIF17 with these proteins at MT plus ends contributes to downstream polarization events is unknown. RhoA activation by KIF17 appears to occur at sites of cell–cell adhesion, and this induces actin remodeling and stabilization of E-cadherin at these sites by inhibiting the activity of the actin-severing protein cofilin (Acharya et al. 2016). As such, it may act in a manner similar to Tea2 kinesin in *Schizosaccharomyces pombe*, which interacts with EB1 through its motor domain like KIF17, targets MTs for capture at growing cell ends, and carries kinesin-tail associated cargos that induce actin remodeling and are required for polarized cell growth. It is likely that a cargo carried by KIF17 to sites of cell–cell adhesion similarly modifies the activity of KIF17 toward MT stabilization, actin remodeling, and AJ stability, although this putative cargo has not yet been identified.

Kinesins are additionally used by an alternative pathway to facilitate MT remodeling as epithelial cells become polarized. In this mode, the MT minus-end-binding protein Nezha/CAMSAP (calmodulin-regulated spectrin-associated protein 3) and MT minus-end-directed KIFC3 localize at AJs (Meng et al. 2008). CAMSAP3 interacts with pleckstrin homology domain protein PLEKHA7 and β-catenin at AJ and stabilizes noncentrosomal MT minus ends. KIFC3 recruits deubiquitinating enzymes to AJ protecting E-cadherin from degradation (Meng et al. 2008; Brieher and Yap 2013). CAMSAP3 also regulates local RhoA activity, potentially by recruiting GEF-H1 to noncentrosomal MTs at AJ; this may function in regulating the balance of Rho family GTPases at AJs leading to changes in both MT and actin organization (Nagae et al. 2013). Similarly, the centralspindlin complex containing kinesin MKLP1 interacts with α-catenin at AJs and recruits ECT2 RhoGEF to and excludes p190-RhoGAP from these sites to orchestrate a balance of RhoA activity that controls AJ architecture (Ratheesh et al. 2012; Priya et al. 2013). These examples lend

additional support to the idea that KIF17 carries a cargo involved in coordinating RhoA activities toward cytoskeletal and AJ remodeling.

MICROTUBULE MOTORS IN DIRECTED DELIVERY OF POLARITY PROTEINS

Kinesins also likely participate in epithelial polarization through their well-established role as cargo transporters (Wozniak et al. 2004; Hirokawa et al. 2009). In addition to maintaining membrane polarity through targeted delivery of apical and basolateral membrane protein cargoes, several kinesins have been implicated in initiation of epithelial morphogenesis based on their interactions with key polarity proteins, including APC, Par3, discs large, and components of AJs and focal adhesions (Cui et al. 2002; Jimbo et al. 2002; Krylyshkina et al. 2002; Asaba et al. 2003; Chen et al. 2003; Nishimura et al. 2004; Shi et al. 2004; Siegrist and Doe 2005; Teng et al. 2005). These studies support the idea that kinesin-mediated transport of polarity proteins may contribute to aspects of cell polarization. Transport of polarity proteins is not limited to kinesins, as dynein is also known to regulate the cortical delivery and localization of polarity proteins, including crumbs, scribble, and pins (Siegrist and Doe 2005, 2007).

MICROTUBULE MOTOR PROTEINS AND LUMEN FORMATION

A long history documents the importance of MTs and MT remodeling in delivery of apical membrane proteins and in apical lumen formation (Bacallao et al. 1989; Gilbert and Rodriguez-Boulan 1991; Gilbert et al. 1991; Lafont et al. 1994; Ojakian et al. 1997; Noda et al. 2001; Yap and Manley 2001; Jacob et al. 2003; Kreitzer et al. 2003; Cohen et al. 2004; Gervais and Casanova 2010; Jaulin and Kreitzer 2010; Quinones et al. 2011). During the formation of an apical lumen in 3D cell culture models (seeding single cells in extracellular matrix), apical membrane proteins are retrieved from the exterior, matrix-facing surface by endocytosis and delivered to a rab11-positive vacuolar/endocytic compartment (Schluter et al. 2009; Li et al. 2014b). As early as at the two-cell stage, recycling of apical markers to a central location at cell–cell contact zones, termed the apical membrane initiation site (AMIS, also known as the preapical patch), specifies the position of a nascent lumen. Coincidentally, basolateral membrane proteins, including cell–cell adhesion components, are excluded from this region allowing expansion of the nascent lumen (Ferrari et al. 2008). Formation of the AMIS can be recapitulated in 2D when cells are cultured in reduced calcium media (Vega-Salas et al. 1987; Gilbert and Rodriguez-Boulan 1991; Ojakian et al. 1997). Under these conditions, cadherin-dependent cell–cell adhesions dissociate and apical membrane proteins mobilize from the cell surface to a large interior structure known as the vacuolar apical compartment (VAC). Actin-rich microvilli are contained in the VAC, but basolateral membrane proteins are excluded, suggesting that this structure is equivalent to an apical endosome. On adding normal calcium media, VACs relocalize to sites of reforming cell–cell contacts in which they first form an AMIS at intercellular contact zones. This is followed by expansion of an intercellular lumen that is subsequently mobilized by an unknown mechanism to the "free" apical surface of monolayer cells (Vega-Salas et al. 1988). Redistribution of VAC membranes to form the intercellular lumen is MT-dependent (Ojakian et al. 1997) and may occur en masse, or by vesiculation and directed transport of vesicles containing these apical membranes. Although it is known that MT depolymerization attenuates or randomizes apical membrane targeting in 2D cultures and inhibits apical lumen formation in 3D cultures (Gilbert and Rodriguez-Boulan 1991; Ojakian et al. 1997; Kreitzer et al. 2003), the mechanism through which MTs contribute to formation of an apical lumen is not known. Two, nonexclusive mechanisms come to mind: (1) MTs are remodeled as integrins engage the extracellular matrix (Akhtar and Streuli 2013) to position the VAC for apical exocytosis and lumen formation, and (2) MTs are remodeled to generate specialized tracks for MT motor-driven delivery of VAC components to the nascent lumen site, as suggested by Ojakian et al. (1997).

Although evidence for kinesin and dynein motor proteins in establishing epithelial polarity is accumulating, their role in apical lumen formation during morphogenesis is less well understood. Two recent studies show a role for heterodimeric kinesin-2, KIF3A/B, in lumen formation. Depletion of KIF3A from MDCK cells grown in 3D matrices prevents lumen formation and apical membrane targeting of Kim1. It also inhibits formation of HGF-induced tubular extensions. In two-dimensional culture, KIF3A depletion also stimulates MT dynamics and orients MTs toward the leading edge of migrating cells in wound-healing assays (Boehlke et al. 2013). Although these findings point to a role for KIF3A in lumen formation through its effects on MTs, studies by Li et al. (2014a) suggest that Kinesin-2 regulates lumen formation by controlling apical endosome transport. In support of this, expression of dominant negative KIF3A in MDCK cells prevented the transport of FIP-5, a Rab11-interacting protein, along central spindle MTs. Considered with the selective binding of FIP-5 to KIF3A/B, these data suggest that KIF3A/B contributes to initial stages of lumen formation by transporting apical endosomal vesicles to the correct location between cells.

The homodimeric kinesin-2, KIF17, also plays a role in lumen formation in MDCK cells cultured in 3D matrices (Jaulin and Kreitzer 2010). When KIF17 is depleted from these cells, they fail to form a single lumen and apical markers GP135 and GP114 remain randomly localized on the plasma membrane. KIF17 depletion and overexpression experiments in 2D cultures revealed that KIF17 attenuates MT dynamics, stabilizes MTs, induces cortical MT and actin remodeling by activating RhoA locally, and stabilizes E-cadherin at AJ (Acharya et al. 2013, 2016; Jaulin and Kreitzer 2010). Despite the similar failure of KIF3A- and KIF17-depleted cells to position apical membrane markers and form apical lumens, differences in how these kinesins affect MTs suggest they do not act by entirely equivalent mechanisms. The effects of KIF17 perturbations on MTs are consistent with observed changes in MT dynamics and organization associated with establishment of apicobasolateral polarity; this could directly or indirectly impact recycling and targeting of apical proteins to the nascent lumen. The effects of KIF3A perturbation on MTs and on epithelial sprouting suggest this motor is important for the acquisition of mesenchymal features (Boehlke et al. 2013), and that it participates in lumen formation in its capacity as a motor for transport of Rab11-positive recycling endosomes to the AMIS (Li et al. 2014a).

MICROTUBULE ORGANIZATION AND MOTOR PROTEINS IN ANIMAL TISSUE MORPHOGENESIS

Although a role for kinesins in lumen formation in animal models is not documented, evidence does exist for dynein in lumen formation in the *Drosophila* trachea, a network of interconnected tubes that transport oxygen and other gases to target tissues. Larval terminal cells mutant for dynein motor complex genes, Dynein heavy chain, Dynein light intermediate chain, and Glued (encoding Dynactin p150) have thin cytoplasmic branches that fail to fill with air (Schottenfeld-Roames and Ghabrial 2012). Embryo and larval terminal cells mutant for Lissencephaly-1 (Lis-1), encoding a Dynein-associated protein also show defects in lumen formation (Gervais and Casanova 2010; Schottenfeld-Roames and Ghabrial 2012). Dynein in *Drosophila* tracheal lumen formation likely functions to transport intracellular membrane components to the site of lumen formation.

In the *Drosophila* embryo, morphogenesis of some epithelial-based organs is also accompanied by dramatic reorganization of the MT cytoskeleton. In invaginating salivary gland cells, the MT cytoskeleton is initially arranged in a dense apical array but undergoes a 90° reorientation and becomes aligned parallel to the apicobasal axis (Booth et al. 2014). Before MT reorientation, apical MTs are localized close to apical centrosomes; however, after reorientation, most apical MTs are no longer in close proximity to centrosomes. This reorientation positions the minus ends of the longitudinal MTs at the apical membrane in close contact with an apical medial actomyosin network that directs the coordinated and pulsatile con-

striction of apices during invagination. The switch from centrosomal to noncentrosomal MT growth in salivary gland cells is similar to that reported in tracheal cells. During tracheal cell invagination, MTs that are initially nucleated from γ-TURC containing centrosomes switch to being nucleated from the apical domain in a two-step process (Brodu et al. 2010). γ-TURC is released from the centriole in a spastin-dependent manner and is then anchored to the apical membrane through the transmembrane protein Piopio. As embryogenesis proceeds and salivary gland and tracheal cells migrate collectively to form tubes, MTs become progressively stabilized and extend along the apicobasal axis (Brodu et al. 2010; Myat et al. 2015).

A role for noncentrosomal MTs in tissue morphogenesis appears not to be specific to *Drosophila*. Elongation of the *Caenorhabditis elegans* embryo is dependent on noncentrosomal MTs functioning with Rho kinase to promote transport of adhesion proteins, such as E-cadherin to the AJs and myotactin to hemidesmosomes (Quintin et al. 2016a,b). Although not documented yet, it is very likely that MT motors transport cargo essential for morphogenesis along these noncentrosomal MTs.

MTs are similarly important for many of the morphogenetic movements that drive neurulation (Cearns et al. 2016). Although the apicobasal cell elongation observed in neurulation as early as the mid-1960s was thought to be MT-dependent, it was only confirmed in 2010 in studies of zebrafish neuroepithelia (Picone et al. 2010). Interkinetic nuclear migration (INM) during neurulation is dependent on MTs in the chick embryo (Spear and Erickson 2012), whereas MTs have only a minor role in INM in the zebrafish retina (Norden et al. 2009). In mice, live imaging studies of brain slices show that dynein and the kinesin-3 motor KIF1A are responsible for INM in radial glial cells, with dynein being responsible for nuclear migration apically toward the ventricle and KIF1A responsible for migration basally toward the pial surface (Tsai et al. 2010). Although there is no additional direct evidence for a role of MT motors in INM, mRNAs encoding the kinesin-1 protein KIF5C are expressed throughout neural tube closure in the chick neuroepithelium (Dathe et al. 2004). In teleosts like the zebrafish, neural progenitor cells initially form a solid rod primordium before cavitating to establish apical polarity and form a lumen at the tissue midline (Cearns et al. 2016). Recent evidence shows a novel mechanism for cell polarization during lumen formation. In the developing zebrafish, neural rod cells assemble a mirror-symmetric MT cytoskeleton around the midline to transport proteins such as Rab11a and Par3 to the site of lumen formation (Buckley et al. 2013). Previous studies suggested Rab11a to be required for apical membrane trafficking and lumen formation in MDCK cell cysts (Desclozeaux et al. 2008; Bryant et al. 2010) and recent studies in the *Drosophila* trachea show that apical enrichment of Rab11 depends on dynein-mediated transport (Le Droguen et al. 2015). These studies on *Drosophila* tracheal development and zebrafish neurulation thus implicate MT and dynein-mediated transport of Rab11 and other regulators of polarization in a key regulatory step in lumen formation. By analogy with evidence obtained in cultured epithelial cells, the importance of MT reorganization and the critical role played by the Rab11-positive apical recycling endosome in model organisms suggest it is also likely that kinesin family motors participate in lumen formation in animals. The genetic tools available in these model organisms combined with high-resolution imaging techniques are bound to provide a better understanding of how MT motor proteins regulate lumen formation in vivo in the future.

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