Microtubule-induced cortical cell polarity

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Most cells are polarized. Embryonic and stem cells can use their polarity to generate cell diversity by asymmetric cell division, whereas differentiated cells use their polarity to execute specific functions. For example, fibroblasts form an actin-rich leading edge required for cell migration, neurons form distinctive axonal and dendritic compartments important for directional signaling, and epithelial cells have apical and basolateral cortical domains necessary for maintaining tissue impermeability. It is well established that actin and actin-associated proteins are essential for generating molecular and morphological cell polarity, but only recently has it become accepted that microtubules can induce and/or maintain polarity. One common feature among different cell types is that microtubules can establish the position of cortical polarity, but are not required for cortical polarity per se. In this review, we discuss how different cell types utilize microtubules and microtubule-associated signaling pathways to generate cortical cell polarity, highlight common mechanisms, and discuss open questions for directing future research.

Cell polarity is essential for the proper function of many if not all cell types. Polarity can be due to subcellular asymmetric compartmentalization of proteins, mRNAs, and/or organelles, ultimately leading to cell type-specific morphological polarity. Failure to establish or maintain cell polarity can have dire consequences. For example, loss of apical/basal polarity in an epithelial monolayer frequently results in multilayering of cells coupled with uncontrolled cell proliferation (for review, see Bilder 2004).

It has long been recognized that extrinsic cues can induce cell polarity. In this review, we will focus on recent work showing that cell-intrinsic cues from the microtubule cytoskeleton can induce and/or maintain cortical polarity in cells ranging from fission yeast to human fibroblasts. We will not discuss the role of microtubules in plant cell polarity (for review, see Sieberer et al. 2005), nor will we discuss microtubule-dependent cortical events in nonpolarized cells such as specification of the contractile ring position during cytokinesis (for review, see D’Avino et al. 2005).

Microtubules are highly dynamic polar filaments composed of 13 protofilaments, each consisting of a linear array of \( \alpha/\beta \)-tubulin dimers. Interphase microtubules are organized radially with their slow-growing minus ends anchored at the centrosome (or spindle pole body in yeast) typically near the nucleus and the faster growing plus ends close to the cell cortex. Growing microtubule plus ends are composed of GTP-tubulin, which helps stabilize them (more mature sections of microtubules typically bind microtubule-associated proteins [MAPs], which provide stability). The constant rate of GTP hydrolysis means that microtubules must keep growing to maintain their “GTP-cap,” however, and thus stalled microtubules rapidly lose their GTP-tubulin cap and are susceptible to collapse. The cycles of microtubule growth and collapse are termed “dynamic instability.” An ever-increasing number of proteins have been identified that localize exclusively to the plus ends of microtubules. A short list of microtubule plus end-binding proteins (also known as +TIPs for microtubule plus end-tracking proteins) includes CLIP-170 (cytoplasmic linker protein), APC (adenomatous polyposis coli), and EB1 (end-binding protein 1), as well as the dynactin subunit p150. Functions of +TIPs include regulation of microtubule plus end dynamics as well as providing microtubule-to-cortex attachment necessary for vesicle delivery, force generation, and signaling for cortical cell polarity. Localization of proteins to microtubule plus ends occurs through one of three ways, including kinesin-mediated plus end transport, direct binding to the microtubule plus end itself, and by “hitchhiking” on other proteins already localized at the plus end. Several recent excellent reviews provide more detail (Carvalho et al. 2003; Galjart and Perez 2003; Vaughan 2004; Akhmanova and Hoogenraad 2005, Wu et al. 2006).

Among the different cell types we discuss, one common role for microtubules in generating polarity appears to be their requirement for delivering positional information necessary to establish the proper site of cortical polarity. Once microtubules and their associated proteins determine the polarity site, a positive feedback loop likely initiates acting between the actin-rich cortex and the microtubule plus ends, to reinforce and maintain this polarity site (Fig. 1). While positive feedback loops may initially provide cells with the ability to sense and
Cells lacking mod5p fail to anchor tea1p at the cortex and cells lacking tea1p fail to recruit mod5p from around the cell cortex to the tip (Snait and Sawin 2003), revealing a positive feedback loop that reinforces the microtubule-cortical polarity linkage [Fig. 2B]. Mutation in any of these five genes leads to a delay in NETO and occasional bent/branched cell phenotypes, similar to loss of microtubules. As all of these proteins except mod5p are microtubule-associated, it raises the possibility that they act indirectly by promoting microtubule length or dynamics. At least for tea1p, this is not the case. Expression of tea1p N-terminal domain in cells lacking endogenous tea1p rescues microtubule dynamics but tea1p remains delocalized from the cortex and cells are still bent or T-shaped, showing that tea1p has polarity functions independent of regulating microtubule dynamics (Bretscher and Nurse 2002). Thus, microtubules induce tea1p/tea4p cortical polarity via plus end-to-cortex contact.

What is the function of cortical tea1p/tea4p in direct-
Microtubule-induced cortical polarity

Microtubules induce cortical Rac1 activation and lamellipodium formation during cell migration

Here we focus on the polarized migration of fibroblasts, for which the most detailed information about microtubule-induced cortical polarity is known. Excellent reviews of leukocyte chemotaxis and astrocyte migration provide coverage of these closely related areas, where microtubules also regulate aspects of cell polarity [Afzolter and Weijer 2005; Etienne-Manneville 2006].

Migrating cells have a stereotypical polarized morphology that predicts their direction of migration. They have a leading edge rich in microfilaments and a trailing membrane domain that must dissolve actin stress fibers and integrin-mediated attachments to the substrate (for review, see Wittmann and Waterman-Storer 2001; Watanabe et al. 2005). During cell migration the microtubule cytoskeleton is polarized: Minus ends are concentrated at the centrosome in the middle of the cell, and plus ends contact the actin-rich leading edge as well as targeting focal adhesions in the rear of the cell (for review, see Small and Kaverina 2003). The first evidence that microtubules were required for directional cell migration, and thus candidates for regulating cortical polarity, came from treatment of fibroblasts with microtubule-depolymerizing drugs, which resulted in reduced, unpolarized membrane ruffling and lack of directional migration [Vasiliev et al. 1970; Goldman 1971; Bershadsky et al. 1991; Grigoriev et al. 1999]. Thus, microtubules are essential for proper positioning of the leading edge, but not for migration per se [Fig. 3A].

How do microtubules induce leading-edge cortical polarity? It is not solely by acting as tracks to deliver cellular components to the leading edge. Low concentrations of nocodazole or Taxol can block cell migration by inhibiting microtubule dynamic instability [such as growth, shortening] without affecting microtubule polarity or abundance [Liao et al. 1995; Mikhailov and Gundersen 1998]. In addition, adding Taxol to nocodazole-treated cells can induce the formation of short stable growing microtubules and transient activation of polarized protrusive activity [Waterman-Storer et al. 1999]. These results suggest that microtubules promote cell polarity by repeated cortical “touching” rather than by forming “tracks” for polarized transport.

How do microtubules plus ends alter cortical polarity? All signs point toward the local activation of the small GTPase Rac1, which is known to stimulate the formation of microfilaments and lamellipodial growth [Ridley et al. 1992; Waterman-Storer et al. 1999, for review, see Hall 2005]. Small G proteins like Rac1 can be activated by GEFs that stimulate exchange of GDP for GTP, and inhibited by GTPase-activating proteins (GAPs) that simulate the intrinsic GTPase activity of the G protein leading to a GDP-bound protein (for review, see Rossman et al. 2005). Increased levels of Rac1 •GTP correlate with increased rates of microtubule polymerization after nocodazole washout, and Rac1 •GTP levels are elevated following addition of Taxol to nocodazole-treated cells, which promotes transient microtubule growth [Waterman-Storer et al. 1999].

How do microtubules induce Rac1 activation? Recent work has shown that microtubules can stimulate cortical Rac1 activity and cell migration through IQGAP1. Despite its name, IQGAP1 is not a GAP that inhibits G protein activity, but rather a large protein that stabilizes and increases Rac1 •GTP/Cdc42 •GTP levels as well as...
edge cortex, and this cortical localization requires both microtubules and cortical APC (Fukata et al. 2002; Watanabe et al. 2004). One simple model is that small amounts of IQGAP1 are transported by plus end-associated CLIP-170 to the leading edge cortex, where IQGAP1 activates Rac1 (Fig. 3B); alternatively, microtubules/CLIP-170 could stabilize or activate cortical IQGAP1. Interestingly, activated Rac1 promotes microtubule/CLIP-170/IQGAP1 complex formation in vitro, and a longer microtubule–cortex dwell time in vivo (Fukata et al. 2002; Watanabe et al. 2004). This is consistent with a positive feedback loop in which cortical IQGAP1 stimulates Rac1, and Rac1 then promotes microtubule/CLIP-170-dependent stabilization of cortical IQGAP1.

In addition to IQGAP1-dependent Rac1 activation, microtubules may be able to stimulate cortical Rac1 via local delivery of GEFS to the leading edge. Here we will discuss GEF-H1, Trio, and ASEF. GEF-H1 can activate both Rac1 and Rho but not Cdc42 (Ren et al. 1998; Gao et al. 2001; Krendel et al. 2002; Zenke et al. 2004; Callow et al. 2005). When associated with the p21-activated kinase PAK4, GEF-H1 promotes Rac1 activation and lamellipodium formation; in the absence of PAK4 or microtubules, GEF-H1 activates Rho and promotes stress fiber formation (Ren et al. 1998; Gao et al. 2001; Krendel et al. 2002; Callow et al. 2005). GEF-H1 is strikingly microtubule-associated in migrating cells, but it is not enriched at the leading edge cortex (Ren et al. 1998; Glaven et al. 1999; Krendel et al. 2002; Benais-Pont et al. 2003; Callow et al. 2005). Thus, GEF-H1 promotes migration by activating Rac1 at the leading edge and/or by inhibiting stress fiber formation. Overexpression of the Trio GEF induces lamellipodia formation by activating RhoG, which in turn, activates Rac1 (Blangy et al. 2000). Trio is also required for cell migration in Caenorhabditis elegans (Honigberg and Kenyon 2000). Trio shows microtubule-dependent localization to the leading edge of migrating fibroblasts, although it has not been detected on microtubules (Blangy et al. 2000). The third GEF, ASEF, is required for cell motility in a colorectal tumor cell line, and ASEF overexpression in an epithelial cell line induces Rac1-dependent polarized membrane ruffles (Kawasaki et al. 2000, 2003). Like Trio, ASEF is detected at the leading edge cortex but not on microtubules, although it can directly interact with the microtubule-binding protein APC (Kawasaki et al. 2000). Thus, at least three GEFS are implicated in microtubule-dependent polarized migration, but there are gaps in each mechanism. Most importantly, it is unknown precisely how dynamic microtubules induce or stabilize Trio and ASEF at the leading edge cortex.

A final twist is that microtubules track along actin cables to “home in” on focal adhesions (Kaverina et al. 1999) and disassemble them by a Rho/Rac-independent pathway that requires Focal adhesion kinase (Fak) and the endocytosis pathway component dynamin (Ezratty et al. 2005). Dynamin is known to bind microtubules (Shpetner and Vallee 1992), raising the interesting possibility that microtubules could deliver dynamin to the focal adhesion, which triggers its removal via endocytosis.

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**Figure 3.** Microtubules determine the site of leading edge cortical polarity during fibroblast migration. (A) Microtubules position the site of cortical polarity. Fibroblast microtubule plus ends contact the leading edge cortex [red] as well as target trailing edge focal adhesions; fibroblasts lacking microtubules can polarize at random cortical sites [red] but do not show directional migration. (B) Mechanism of microtubule-induced cortical polarity. IQGAP1 can induce cortical Rac1 activation [Rac1 • GTP, Rac1*]. It might be delivered by microtubule plus ends [dotted-outlined IQGAP1], or it might be activated at the cortex by CLIP-170 at growing microtubule plus ends. See the text for details.
s. Microtubules could also inhibit focal adhesions by binding and inactivating the RhoGEF activity of GEF-H1 (Krendel et al. 2002; Zenk et al. 2004; Callow et al. 2005). Thus, microtubules may coordinate cell migration in two ways: first, by delivering Rac1 GEFs to the leading edge to promote actin polymerization and lamellipodia formation, and second, by targeting and destroying focal adhesions in the rear of the cell.

Polarity establishment mechanisms often have positive feedback loops to stabilize the polarity axis, and cell migration is no exception. Microtubules promote Rac1-mediated lamellipodium formation, and recent work shows that cortical Rac1 can stabilize microtubule plus ends. Rac1 activation of Pak1 kinase results in phosphorylation and inactivation of stathmin in multiple cell types including migrating cells (daub et al. 2001; Bannierj ee et al. 2002; Wittmann et al. 2004); stathmin is a potent microtubule destabilizer (Andersen 2000). Thus, high Rac1 activity promotes microtubule stability. In addition, active Cdc42 at the leading edge leads to the phosphorylation and inactivation of glycogen synthase kinase 3 (GSK-3). GSK-3 phosphorylation requires Par-6 and PKCζ kinase activity and leads to recruitment of APC to microtubule plus ends, an important event required for centrosome reorientation (Etienne-Manneville and Hall 2003). In addition, microtubule plus ends can be stabilized by association of the plus end proteins EB1/APC with the cortical formin Diaphanous (mDia) (Gundersen et al. 2005). EB1/APC/mDia can all be detected at the plus ends of microtubules, and both APC and mDia can be detected at the leading edge cortex (Gundersen et al. 2005). This suggests that migrating cells may use cortical formins to capture and stabilize microtubule plus ends, a nice reversal of the situation in fission yeast where microtubules induce activation of the formin for3p (see above). It will be interesting to determine if for3p can stabilize microtubules in yeast, and whether mDia is activated to promote actin polymerization in the leading edge of migrating cells, which would add two more positive feedback loops linking microtubules and the cell cortex.

**Microtubules regulate cortical polarity and actin dynamics in neuronal growth cones**

A specialized form of cell migration is neuronal growth cone guidance. Growth cones are a motile neuronal extension that is similar to the lamellipodia of a migrating fibroblast; growth cones are composed of a peripheral domain rich in actin filaments and a central domain enriched in microtubules (Suter and Forsscher 1998). However, recent work has shown that a subset of highly dynamic nonacetylated microtubules constantly probe the peripheral cortical domain, and thus could influence cortical polarity (Schaefer et al. 2002). This is supported by studies showing that exposure of one side of a growth cone to microtubule-stabilizing drugs will stimulate turning toward the drug source, whereas microtubule inhibitors promote turning away (Ruck and Zheng 2002). It is important to note that loss of microtubules does not block growth cone extension, but rather leads to a “wandering” growth cone that is blind to environmental guidance cues (Williamson et al. 1996). Thus, microtubules are essential for positioning the site of protrusion within a growth cone, but not for extension per se, similar to their role in migrating cells.

Extrinsic guidance cues could promote microtubule-induced cortical polarity in a turning growth cone either by changing the actin-rich cortex to foster microtubule–cortex anchoring or by directly affecting microtubule dynamics, if there is a positive feedback loop between microtubules and the cortex (Fig. 1), then both mechanisms would lead to microtubule-dependent growth cone guidance. The former model is supported by a number of recent studies. First, actin cables are required for protrusion of dynamic microtubules into the peripheral growth cone (Kabir et al. 2001; Schaefer et al. 2002; Zhou et al. 2002), suggesting that the actin cortex can affect the timing and number of microtubule–cortex interactions. However, there is also evidence for direct regulation of microtubules by extrinsic guidance cue signaling pathways. Growth cones exposed to the attractive cue nerve growth factor (NGF) show increased levels of dephosphorylated APC (Zhou et al. 2004), which directly stimulate microtubule plus end stability (Zumbrunn et al. 2001). This could result in an increase in microtubule–cortex interactions on the growing side of the growth cone. Additional evidence for direct regulation of microtubules by guidance cues comes from analysis of the repulsive Slit/Robo pathway. Genetic data in *Drosophila* show that growth cone repulsion from the midline source of Slit is impaired in mutants for the receptor Roundabout (Robo), the Ableson (Abl) tyrosine kinase, and the microtubule plus end-binding and stabilizing Orbit/CLASP protein (Lee et al. 2004). The authors propose a model in which Slit stabilizes microtubules (i.e., reducing dynamic microtubule–cortex interactions), resulting in growth cone migration away from the cue. In support of their model, CLASP can be detected in *Drosophila* growth cones in vivo, decorates pioneer microtubule plus ends in cultured Xenopus growth cones, and overexpression of CLASP in Xenopus neurons results in looping and decreased growth of microtubules (Lee et al. 2004). It remains unclear why stabilized microtubules promote growth cone turning toward NGF (Zumbrunn et al. 2001), whereas they lead to growth cone repulsion in response to Slit (Lee et al. 2004). Perhaps an intermediate level of stabilized microtubules are essential for growth cone turning, and too little or too much is deleterious. Resolving this question will require high temporal resolution imaging of microtubule plus ends during growth cone turning in each system.

Although evidence that microtubules can alter growth cone migration is accumulating (for review, see Rodriguez et al. 2003), little is known about how microtubule plus end-binding proteins deliver Rac activators [e.g., IQGAP1, ASEF, Trio] to one side of the growth cone resulting in actin polymerization and directed migration,
as they do in fibroblasts. In fact, several microtubule plus end-binding proteins are localized to growth cone microtubules, such as CLIP-170, EB3 [Stepanova et al. 2003], and APC [Zhou et al. 2004, Shimomura et al. 2005]. Testing these +TIP proteins for role in microtubule-regulated growth cone guidance will reveal whether growth cones and migrating fibroblasts share fundamental mechanisms for directional migration.

**Microtubules induce apical cortical polarity in Drosophila epithelia**

*Drosophila* embryos develop from a syncytial embryo with 5000 nuclei. These nuclei migrate to the cortex where membrane invaginations around each nucleus leads to the formation of a surface cell layer that develops apical/basal cortical polarity; this process is termed cellularization. Immediately after cellularization, ventral cells undergo an apical constriction that results in the invagination of the future mesoderm (gastrulation). Similar apical constrictions are observed in gastrulating cells in many embryos, as well as during placode invagination. Recent work has provided insight into how microtubules regulate cortical polarity during each of these processes.

How do microtubules regulate apical cortical constriction in gastrulating cells? The physical constriction of these cells requires activation of the conserved Rho1/Rho kinase/myosin light chain kinase/myosin II pathway at the apical cortex [Barrett et al. 1997, Hacker and Perrimon 1998]. Insight into how microtubules activate Rho1 at the apical cortex have come from pioneering biochemical experiments that identified a Rho-activating protein, RhoGEF2, as a binding partner of the microtubule plus end-binding protein EB1, which is required for apical constriction of gastrulating cells [Rogers et al. 2004]. Further experiments in the nonepithelial S2 cell line revealed that (1) RhoGEF2 requires EB1 for its association with growing microtubule plus ends; (2) the concertina Gα•GTP heterotrimeric G protein subunit triggers movement of RhoGEF2 from the microtubule plus end to the cortex; and (3) RhoGEF2 acts via Rho1 and Rho kinase to activate myosin II-dependent cortical contractions [Rogers et al. 2004]. This has led to a model in which microtubules deliver RhoGEF2 to the apical cortex, where it induces Rho1-dependent myosin II contractility during gastrulation. It is important to note that RhoGEF2 has not yet been observed on microtubule plus ends in gastrulating cells [only S2 cells], and it is possible that it acts by a different mechanism in each cell type. Other questions remain, including how the timing of apical constriction is regulated; one possibility is that the process is initiated by ligand-induced activation of the concertina Gα protein.

Microtubules are also used to induce cadherin cortical polarity in epithelial cells [Fig. 4A]. Cadherins are transmembrane proteins that mediate epithelial cell–cell adhesions through calcium-dependent homotypic binding [Takeichi 1991]. Several lines of evidence show that dynamic microtubules promote clustering of surface cadherin protein at the site of cell–cell contacts. First, the plus ends of highly dynamic, nonacetylated microtubules directly contact cadherin-based cell junctions [Stehbens et al. 2006]. Second, low concentrations of nocodazole abolish these dynamic microtubules without grossly affecting microtubule morphology, and result in the disruption of cell–cell junctions, suggesting that dynamic microtubules are required to maintain cell–cell adhesion [Waterman-Storer et al. 2000]. While microtubule motors are required to localize cadherin to the cell surface in myoblasts [Mary et al. 2002], localization of cadherin in epithelial cells may occur through a transport-independent mechanism. When dynamic microtubules are disrupted with a dominant-negative CLIP-170 protein, it results in the loss of cadherin clustering and cell adhesion without lowering the overall level of cell surface cadherin [Stehbens et al. 2006]. Moreover, photobleaching of cortical cadherin-YFP fluorescence led to rapid recovery in wild-type cells, but delayed recovery in nocodazole-treated cells, consistent with a defect in cadherin clustering [Waterman-Storer et al. 2000]. Thus, dynamic microtubule plus ends are required for proper cadherin clustering and cell–cell adhesion.

How do microtubules concentrate cadherin at sites of cell–cell contact? Recent work shows that microtubule-induced cadherin clustering requires the Rho/Rho ki-
nase/myosin light chain kinase/myosin II pathway, similar to the pathway of microtubule-induced apical cortical constriction in gastrulating cells [see above]. Microtubules are required for recruiting myosin II to sites of cadherin clustering, and pharmacological inhibition of Rho kinase or myosin II activity prevents cadherin clustering and proper cell–cell junctions [Braga et al. 1997; Takaishi et al. 1997; Vaezi et al. 2002; Shewan et al. 2005; Stehbens et al. 2006]. It is currently unknown how microtubules promote Rho activation, but it is appealing to consider a pathway in which microtubule plus ends deliver a Rho GEF to the cortex to stimulate Rho activity [Fig. 4B].

Data consistent with a model of microtubule-induced Rho activation exists in several other cell types, but in no case is the entire pathway clearly elucidated. Rho-GEF2 or other Rho GEFs are required for Drosophila cellularization, pole cell cytokinesis, epithelial folding in Drosophila [Nikolaidou and Barrett 2004; Padash Barmschi et al. 2005]; and activated RhoA is specifically localized to cortical domains involved in pole cell and micromere cytokinesis in sea urchins and Xenopus [Bement et al. 2005; Ma et al. 2006], but whether microtubules deliver Rho GEFs to the cortex remains unknown. In contrast, there is evidence that microtubules induce cortical polarity during Drosophila epithelial planar cell polarity and dorsal closure. Planar cell polarity is established along the proximal/distal axis of an epithelial sheet in many organisms, and in Drosophila it results in the formation of an actin-rich protrusion from the distal side of the cell. Noncentrosomal microtubules are aligned along the proximodistal axis of these cells, with slightly more plus ends toward the distal side of the cell, and are required for clustering the transmembrane Frizzled receptor, E-cadherin, and several other proteins at the distal side of the cell [Shimada et al. 2006]. Microtubules are also essential for generating myosin-dependent epithelial cell protrusions during Drosophila dorsal closure (a process similar to epithelial wound healing) [Jankovics and Brunner 2006]. The mechanism of microtubule-induced cortical polarity remains unknown for both planar cell polarity and dorsal closure, and more specifically, it has not been tested whether microtubules act via delivery of a Rho GEF to the cortex in either cell type.

**Microtubules induce apical cortical polarity in Drosophila neuroblasts**

Asymmetric cell division requires that the dividing cell is polarized and the mitotic spindle is aligned with the polarity axis such that daughter cells are molecularly distinct. Neuroblasts are the stem cell-like progenitors of the Drosophila CNS that undergo asymmetric cell division to generate another proliferative neuroblast and a smaller differentiating daughter cell that generates neurons or glia. Mitotic neuroblasts have striking cortical polarity, with over a dozen proteins targeted to their apical cortex (future neuroblast) and at least six proteins targeted to the opposite basal cortex (future daughter cell). The mitotic spindle aligns with the cortical polarity axis, and the neuroblast undergoes asymmetric cell division, with the larger neuroblast inheriting apical proteins and the smaller GMC inheriting basal proteins. Establishment and maintenance of neuroblast cortical polarity requires microfilaments [Broadus and Doe 1997], but until recently there has been no evidence that microtubules are involved. Treatment of wild-type neuroblasts with microtubule inhibitors arrests the cells in mitosis but apical and basal cortical polarity can be established [Knoblich et al. 1995; Spana and Doe 1995; Broadus and Doe 1997]. The only early sign that microtubules play a role in neuroblast cortical polarity came from double drug studies, in which microfilament inhibitor treatment resulted in uniform cortical apical protein localization, but microfilament plus microtubule inhibitor treatment resulted in apical proteins delocalizing into the cytoplasm [Broadus and Doe 1997]. This suggested that microtubules might play a role in cortical targeting of apical proteins.

The breakthrough in understanding the role of microtubule-induced cortical polarity came from analysis of inscuteable (insc) mutants [Siegrist and Doe 2005]. The Insc protein links two evolutionarily conserved protein complexes at the apical cortex of the neuroblast. One is the Par complex, consisting of Par-3 (Bazooka in Drosophila), aPKC, and Par-6; the other is the Pins complex, consisting of Partner of Inscuteable (Pins), the heterotrimeric G protein subunits Gai/Ggo, and the tumor suppressor protein Discs large [Dlg]. In insc mutants, the Par complex is delocalized into the cytoplasm, but the Pins/Ga/Dlg complex still forms crescents [Siegrist and Doe 2005]. Importantly, the Pins/Ga/Dlg crescents are always over one spindle pole, even when the spindle is aligned perpendicular to the normal apical/basal axis [Fig. 5A].

To determine whether microtubules induce Pins/Ga/Dlg cortical polarity, or vice versa, insc mutant neuroblasts were treated with microtubule depolymerizing drugs. Treatment of mitotic insc mutant neuroblasts with either Colcemid (which abolishes all detectable microtubules) or nocodazole (which at the concentration used specifically abolishes astral microtubules) resulted in delocalization of Pins/Ga/Dlg proteins; a similar result was observed in fizzy insc double mutants, in which neuroblasts have a small barrel-shaped spindle that lacks astral microtubules [Siegrist and Doe 2005]. Thus, astral microtubules can induce Pins/Ga/Dlg cortical polarity in metaphase neuroblasts, suggesting that direct contact between microtubules and cortex is required to generate polarity [Fig. 5A].

How do astral microtubules induce Pins/Ga/Dlg cortical polarity? A key component is the kinesin 3 family member Khc73. Drosophila Khc73 is related to the human Gkin/Kif13b kinesin: Both Khc73 and Gakin have a conventional microtubule plus end-directed motor domain, a microtubule plus end-binding CAP-Gly domain, and a central conserved domain that can bind the C-terminal GK domain of human or Drosophila Dlg [Li et al. 1997; Hanada et al. 2000; Asaba et al. 2003; Siegrist and Doe 2005]. When Khc73 is depleted by RNA inter-
SH3 and GK domains are bound to other proteins (e.g., Khc73-association of its SH3-GK domains; (Dlg open) Dlg where its Mechanism of microtubule-induced cortical polarity. (Khc73) and sufficient to induce Pins complex cortical polarity. (B) absence of the Par complex, astral microtubules are necessary Pins complex (Dlg/Pins/Gαi, red) to the apical cortex. In the proteins (label, Par-3/Par-6/aPKC), which normally recruit the cortical polarity. Wild-type neuroblasts have apical Par complex — via transport of Dlg (Fig. 5B). It is not fully established how Khc73 induces cortical polarity in Drosophila neuroblasts. (A) Microtubules can induce neuroblast cortical polarity. Wild-type neuroblasts have apical Par complex proteins [label, Par-3/Par-6/aPKC], which normally recruit the Pins complex [Dlg/Pins/Gαi, red] to the apical cortex. In the absence of the Par complex, astral microtubules are necessary and sufficient to induce Pins complex cortical polarity. (B) Mechanism of microtubule-induced cortical polarity. [Khc73] Kinesin heavy chain 73; [Dlg closed] Dlg with intramolecular association of its SH3-GK domains; [Dlg open] Dlg where its SH3 and GK domains are bound to other proteins (e.g., Khc73-GK and Pins-SH3). See the text for details.

Figure 5. Microtubules induce apical cortical polarity in Drosophila neuroblasts. [A] Microtubules can induce neuroblast cortical polarity. Wild-type neuroblasts have apical Par complex proteins [label, Par-3/Par-6/aPKC], which normally recruit the Pins complex [Dlg/Pins/Gαi, red] to the apical cortex. In the absence of the Par complex, astral microtubules are necessary and sufficient to induce Pins complex cortical polarity. (B) Mechanism of microtubule-induced cortical polarity. [Khc73] Kinesin heavy chain 73; [Dlg closed] Dlg with intramolecular association of its SH3-GK domains; [Dlg open] Dlg where its SH3 and GK domains are bound to other proteins (e.g., Khc73-GK and Pins-SH3). See the text for details.

Microtubules/centrosomes induce cortical polarity in the C. elegans zygote

In C. elegans, the identity of the first two daughter cells is determined by the asymmetric protein localization during the first cell division. The newly fertilized C. elegans zygote is unpolarized (Goldstein and Hird 1996). The first sign of asymmetry is the loss of cortical contractility (smoothening) over the future posterior pole, while the anterior cortex remains active (ruffling). Within 2 min of posterior pole smoothening, cortical PAR complex proteins [PAR-3, PAR-6, and aPKC] are cleared from the posterior cortex, localizing to the anterior cortex coincident with the anterior movement of myosin II-containing foci. PAR-2, a ring domain protein, is then recruited from the cytoplasm to occupy the “vacant” posterior cortex leading to the production of two nonoverlapping, yet interdependent anterior and posterior cortical domains. These asymmetrically localized

cortical domains. These asymmetrically localized
cortical proteins, and others, are required for positioning the mitotic spindle and polarizing cytoplasmic cell fate determinants resulting in an asymmetric cell division (for review, see Schneider and Bowerman 2003). Altering the sperm entry site changes the position of the posterior cortical domain within the egg, and all later developmental events remain coordinated with the new polarity axis, showing that a sperm-associated structure induces functional cortical polarity (Goldstein and Hird 1996). Anucleate sperm can induce posterior cortical polarity, indicating that the polarizing cue is not the sperm nucleus (Sadler and Shakes 2000). What is the cue? Recent work suggests that microtubules are not involved, but rather the cue comes from the centrosome and/or a sperm provided membraneous organelle. It remains unclear whether either of these structures is sufficient to induce polarity or whether both are required and function in a single or separate pathways.

The centrosome at the one-cell stage in C. elegans likely has dual functions: first, to nucleate microtubules required for spindle formation and equal DNA segregation during cell division, and second, to induce cortical polarity. A role for centrosome-induced cortical polarity independent of microtubules is based on several lines of evidence. First, ablation of the sperm-derived centrosome either genetically (spd-2 or spd-5) or by laser irradiation resulted in a delay or absence of posterior cortical smoothening and forming PAR-2 posterior cortical polarity (O’Connell et al. 2000; Hamill et al. 2002; Cowan and Hyman 2004). Second, spd-2(RNAi) centrosomes weakly nucleate microtubules but lack PAR-2 posterior cortical polarity, whereas γ-tubulin(RNAi) or α/β-tubulin(RNAi) centrosomes nucleate even fewer microtubules yet have normal PAR-2 posterior cortical polarity (Cowan and Hyman 2004). Finally, cortical polarity forms normally in embryos treated with microtubule depolymerizing drugs (Strome and Wood 1983; Sonnville and Gonczy 2004). Thus, formation of PAR-2 posterior cortical polarity and posterior cortical smoothening is not correlated with the presence or abundance of microtubules, but requires a functional centrosome.

What is the centrosomal component that induces PAR-2 cortical polarity? The first break in symmetry is a localized weakening in the actomyosin contractile network, either due to reduced myosinII activity or fewer microfilaments. This weakening in the network, which lies just below the cell cortex, occurs at a cortical site close to the centrosome and results in a massive rearrangement of the cortical actin network leading to asymmetry in cortical contractility and cytoplasmic flows (Munro et al. 2004). Recent results from several labs suggest that network weakening requires CYK-4, a RhoGAP highly enriched on sperm-associated membranes; weakening also requires the maternally provided RHO and ECT-2 [RhoGEF] proteins (Jenkins et al. 2006; Motegi and Sugimoto 2006; Motegi et al. 2006; Schonegg and Hyman 2006). cyk-4(RNAi), rho(RNAi), and ect-2(RNAi) embryos all fail to establish cortical polarity but for different reasons. Cortical contractility remains uniformly high in cyk-4(RNAi) embryos coincident with uniform high levels of cortical phosphorylated myosin light chain. Conversely, cortical contractility is dramatically reduced in rho(RNAi) and ect-2(RNAi) embryos with no phosphorylated myosin light chain detected at the cortex. These results suggest that CYK-4 may induce weakening in the contractile network by locally inactivating Rho leading to reduced cortical myosin II activity and a localized cortical smoothening that is then propagated toward the anterior. Whether canonical Rho signaling transducers such as Rho kinase are required for myosin II phosphorylation remains unknown. In addition, it is unclear what the relationship is between the centrosome and the CYK-4-containing membrane. Both remain spatially close to one another within the egg, with CYK-4 localizing asymmetrically to the cortex. Is the centrosome required for assembly or function of CYK-4+ membranes, or does the centrosome provide an independent cue that regulates cortical polarity? The latter model is supported by data from a wide range of systems showing centrosomal regulation of cortical polarity (Piel et al. 2001; de Anda et al. 2005; Gromley et al. 2005; Stinchcombe et al. 2006). In the future, it will be interesting to determine what happens to CYK-4-containing membranes when centrosomes are ablated and vise versa. Perhaps one role for the centrosome is to temporally regulate initiation of cortical polarity, thereby preventing premature polarity establishment prior to completion of meiosis.

While microtubules may not be required for establishing cortical polarity during the one-cell stage, microtubules are required for the asymmetric localization of PAR-2 over the meiotic spindle in the egg, and may be important for the formation of the polar body cortical domain (Wallenfang and Seydoux 2000; Cowan and Hyman 2004). Thus, microtubules induce anterior PAR-2 cortical polarity during polar body formation (Fig. 6, left). The mechanism by which meiotic spindle microtubules...

![Figure 6. Microtubules/centrosomes induce cortical polarity in the C. elegans zygote. (Left) Microtubules of the meiotic spindle (black line) induce Par2 cortical polarity at the anterior side of the oocyte. (Right) The mechanisms inducing Par2 cortical polarity at the site of sperm entry could include local inhibition of Rho by the CYK-4 RhoGAP protein localized to the sperm-derived membranes (gray) and/or by an unknown factor present on the sperm-derived centrosome (black). See the text for details.](image-url)
promote cortical PAR-2 localization is completely unknown. Based on work in other systems, where the position of the meiotic spindle is correlated with a spot of active CDC42 surrounded by a ring of active Rho at the cortex (Ma et al. 2006), it would be interesting to see if a similar cortical polarity was induced prior to PAR-2 cortical localization, whether microtubules are involved in generating this cortical domain, and whether this cortical domain is required for polar body extrusion.

Despite the new data showing a role for centrosomes and membranes, it may be premature to rule out a role for microtubules. First, microtubules are sufficient to induce PAR-2 cortical polarity during meiosis, and may have a related function after sperm entry. Second, the best evidence against a role for microtubules is that spd-2(RNAi) embryos nucleate microtubules but fail to induce cortical polarity (Cowan and Hyman 2004), but it is possible that these microtubules are functionally abnormal. If microtubules have a role in stimulating posterior PAR-2 localization, they could act independently or in concert with the centrosome/membrane pathways. For example, even a single microtubule could promote plus end-directed transport of CYK-4(RhoGAP)+ sperm membranes or the RhoGEF ECT-2 toward the posterior cortex. It is unknown whether the evolutionarily conserved +TIPs—including APC, EB1, and CLIP-170—are required for any aspects of cortical polarity in the one-cell C. elegans embryo; however, the APC ortholog, apr-1, is required at the four-cell stage for the EMS blastomere to generate two distinct cell types, E and M5. While Wnt signaling is required for this cell fate decision, it remains unknown whether APC functions on the microtubule plus end to polarize the cortex. The identification of additional proteins required for cortical polarity will be necessary to distinguish the relative importance of each sperm-derived structure in the induction of zygotic cortical polarity.

Conclusions and future directions

From these different contexts, one common theme for how microtubules regulate cell polarity is immediately apparent. Microtubules can impart polarity by signaling to the cortex via regulating the localized activation of small G-protein signaling (Table 1). From these systems, it remains unclear how many of the proteins will be conserved in microtubule/cortical polarity pathways and what are and will be the cell type specific differences. It is likely that common components of microtubule-based polarity pathways will include plus end-directed kinesin motor proteins, astral microtubule plus end-stabilizing proteins such as CLIP-170 and EB1, and proteins that function as molecular switches. Once the switch protein (i.e., a GEF or GAP) is delivered to the cortex, conserved (Rho-type GTPase, APC, formin) and unique (PAR-2, Pins/Dlg, PAK) proteins may be activated to respond, interpret, and induce appropriate readouts that are cell type specific. Another shared feature among many cell types is that microtubules and cortical polarity form a positive feedback loop—microtubules induce cortical polarity and then cortical polarity proteins anchor microtubules—that allows microtubules to induce and/or stabilize the axis of cortical polarity in cells lacking a stable extrinsic polarity cue.

Table 1. Microtubule-induced cortical polarity pathway components

<table>
<thead>
<tr>
<th>Cell type</th>
<th>S. pombe</th>
<th>Fibroblast</th>
<th>Drosophila neuroblast</th>
<th>Epithelia (gastrulation)</th>
<th>Epithelia (cadherin)</th>
<th>C. elegans oocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical polarity output</td>
<td>Polarity cell growth</td>
<td>Directed cell migration</td>
<td>Asymmetric cell division</td>
<td>Apical constriction</td>
<td>Cell–cell adhesion</td>
<td>Polar-body extrusion</td>
</tr>
<tr>
<td>MTs required for polarity effect</td>
<td>No</td>
<td>No</td>
<td>Yesa</td>
<td>?</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>MTs required for polarity position</td>
<td>Yes</td>
<td>Yes</td>
<td>Yesa</td>
<td>?</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>MT motors</td>
<td>tea2p (kinesin-like)</td>
<td>Khc73</td>
<td>?</td>
<td>EB-1</td>
<td>clip-170</td>
<td>?</td>
</tr>
<tr>
<td>MT cortical polarity inducers</td>
<td>tea1p, tea4p, rhl1p [rho1GFP]</td>
<td>iqgap1 Trio Asef Gef-1</td>
<td>Khc73</td>
<td>RhoGEF2?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

*aIn absence of Par complex only.
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Microtubule-induced cortical cell polarity

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