even individual molecules within the complex molecular mixture that we call cytoplasm. The main problem will still be how a sample — in this case a whole cell — can be frozen without introducing minute ice crystals, which would distort molecular arrangements. But even when this obstacle is overcome, we will still have to ask: fact or artefact, hazy truth or shining lie?

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OPINION

Evolutionary conservation of microtubule-capture mechanisms

Gregg G. Gundersen

The dynamic nature of microtubules allows them to search the three-dimensional space of the cell. But what are they looking for? During cellular morphogenesis, microtubules are captured at sites just under the plasma membrane, and this polarizes the microtubule array and associated organelles. Recent data indicate that the signalling pathways that are involved in regulating the different microtubule–cortical interactions are not only conserved between species, but also that they function in diverse processes.

Microtubules are one of the main elements of the cytoskeleton, and are essential for cell division, cell migration, vesicle transport and cell polarity. Since the 1980s, it has been clear that microtubules are highly dynamic structures (see the Perspective by Manfred Schliwa in this issue). In cells, they turn over every few minutes — or even seconds — due to an intrinsic property termed dynamic instability (BOX 1).

What has not been clear is why cells keep microtubules in such a highly dynamic state. GTP is hydrolysed during microtubule polymerization to allow for dynamic instability, so cells must derive some benefit from maintaining highly dynamic microtubules. In 1986, Marc Kirschner and Timothy Mitchison[1] proposed that dynamic instability allows microtubules to respond rapidly to external stimuli such as growth factors or cytokines. They proposed that dynamic microtubule ends could sample the three-dimensional space of the cell to interact with — and be stabilized by — sites just under the membrane (cortical sites) that had been activated by signals (FIG. 1).

This model is conceptually appealing because it does not require further information to explain how microtubules might respond in a localized fashion to external signals; the

Box 1 | Microtubule organization and dynamics

Organization. Microtubules are composed of the subunit protein tubulin, which is a heterodimer of the closely related α- and β-subunits. Because of the head-to-tail arrangement of tubulin within the microtubule lattice, microtubules have a structural polarity, and this influences the dynamic properties of the two ends of the microtubule. One end, termed the 'plus' end, grows about three times faster than the other end, termed the 'minus' end. The plus end also has a lower 'critical concentration' (the concentration of tubulin subunits that is necessary for elongation of the microtubule), so this is the preferred end for growth *in vivo*.

Nucleation of new microtubules is an unfavourable process and, in cells, microtubule nucleation is promoted by a microtubule-organizing centre (MTOC). The MTOC contains a nucleating complex composed of γ-tubulin and associated molecules that nucleates microtubules from their minus end so that new subunit addition occurs at the favoured plus end. In most cases, the minus ends remain attached to the MTOC, so the MTOC also contributes to the overall geometry of the microtubule array. For example, in most interphase mammalian cells grown in culture, there is a single MTOC, the centrosome, which nucleates microtubules all over its surface leading to the radial array of microtubules that is typically observed in these cells. During mitosis, duplicated and separated centrosomes each nucleate a radial array of microtubules, which interact with chromosomes to form the bipolar mitotic spindle. The MTOC in yeast is termed the 'spindle pole body' (SPB), and it carries out an analogous function to the mammalian centrosome. Unlike the mammalian centrosome, which resides in the cytoplasm adjacent to the nucleus, the SPB is embedded in the nuclear envelope.

Dynamics. Inoue's pioneering work with polarization microscopy in the 1960s showed that microtubules in mitotic spindles of marine invertebrates are dynamic structures that can form and reform in cells. However, it was not until the use of fluorescently tagged tubulin in the 1980s that the extent of the dynamics was shown. In interphase fibroblasts, the half-life of individual microtubules is 5–10 mins, whereas in mitotic spindles the half-life is only ~30 sec (REFS 60,61). So, an interphase array of several hundred microtubules will be completely remodelled in less than an hour, whereas remodelling of a mitotic spindle will take only minutes.

This remarkable turnover of microtubules is due to their 'dynamic instability' — an intrinsic property of microtubules that is characterized by periods of growth and shrinkage, which are punctuated by transitions between these states[62]. Growing microtubules will catastrophe to a shrinking state and either depolymerize completely or be 'rescued' and begin growing again. In most cells, the number of nucleating sites on the MTOC limits the number of microtubules, so a depolymerized microtubule will be replaced by a new microtubule that is formed at the exposed nucleating site.

Although dynamic instability provides a good model to explain the turnover of microtubules, recent studies have proposed that other cellular factors might contribute to microtubule turnover in cells. Catastrophe factors can enhance dynamic instability and so enhance microtubule turnover[63]. Also, in some cells, microtubules are released from their nucleating sites on the MTOC, and this would be expected to contribute to enhanced microtubule turnover[64].

dynamics of microtubules is sufficient to convey them to signal-activated targets. When this model was proposed, there were no known cases in which dynamic microtubules responded to external signals. Instead, Kirschner and Mitchison illustrated the importance of dynamic microtubules for finding targets with the capture of spindle microtubules by chromosomes during mitosis — and further evidence for chromosome capture of microtubules has accumulated[2].

Evidence is beginning to emerge that microtubule searching is also important for the generation of asymmetric cell shape. For example, when microtubule antagonists are applied at nanomolar concentrations, they decrease microtubule dynamics without causing microtubule breakdown or disorganization, and inhibit processes that depend

on polarized cell morphology, such as growth-cone motility[3] and fibroblast migration[4]. Evidence has also accumulated that dynamic microtubules are selectively stabilized during cell polarization and differentiation[5]. However, crucial features of the model — including the identities of the external signals and the cortical targets, and how they are regulated — have remained mysterious.

Rho, Rac and Cdc42 are members of the Rho subfamily of Ras-related small GTPases, which have well-characterized roles in regulating the actin cytoskeleton, cell adhesion, gene expression and cell proliferation[6,7]. (There are several isoforms of both Rho and Rac in mammalian cells; unless indicated otherwise, all references to Rho and Rac refer to the well-characterized isoforms, RhoA and Rac1.) Recent studies in mammalian cells indicate
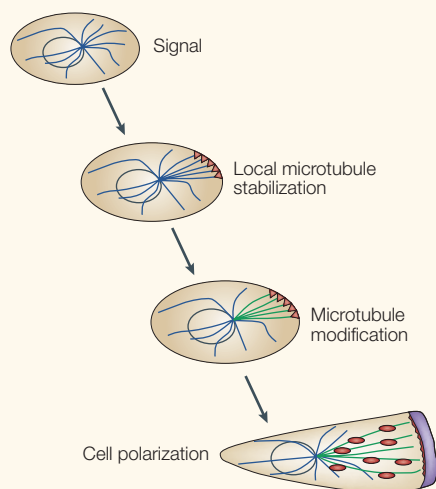
that the Rho and Cdc42 GTPases also regulate signalling pathways that have distinct effects on the microtubule cytoskeleton.

Examination of the Rho and Cdc42 signalling pathways that regulate microtubules in mammalian cells shows a striking similarity to pathways that were previously identified in budding yeast — both in the proteins involved and in the microtubule–cortical interactions that the GTPases regulate. This indicates that basic mechanisms for microtubule–cortical interactions might have been evolutionarily conserved between yeast and mammalian cells. The cellular processes that are regulated by these signalling pathways in yeast and mammalian cells are different (budding and directed migration, respectively), which indicates that there are only a limited number of basic mechanisms for regulating microtubule–cortical interactions, and that these are then adapted to carry out diverse cell functions. The characteristics of the proteins that are involved in the two pathways point to a general model for the regulated interaction of microtubules with the cell cortex.

Polarized responses of microtubules

In yeast, cytoplasmic microtubules that emanate from the spindle pole body — the yeast microtubule-organizing centre (MTOC) — become polarized along the mother–bud axis during cell division (FIG. 2a). Two cortical mechanisms contribute to the alignment of microtubules along this axis. One mechanism, known as 'microtubule capture and shrinkage', involves the end-on interaction of dynamic cytoplasmic microtubules with activated targets at the bud cortex[8–15]. This capture results in a transient stabilization followed by shrinkage of the attached microtubule[8]. The second mechanism, referred to here as 'microtubule capture and sliding', involves the lateral interaction of microtubules with activated targets in the bud cortex, but results in sliding of the microtubules[8,16–18]. Both types of cortical interaction contribute to alignment of the associated nucleus along the mother–bud axis, and proper segregation of nuclei to the mother and bud. But whereas capture and shrinkage occurs early in the cell cycle, aligning the spindle along the mother–bud axis and moving the nucleus towards the bud neck, capture

"What has not been clear is why cells keep microtubules in such a highly dynamic state"

Figure 1 | **Selective stabilization model for microtubule polarization.** In an unpolarized cell, an external signal is thought to activate stabilizing factors (red) in the cell cortex. Dynamic microtubules that encounter these sites become stabilized. Stabilized microtubules (green) are shown to acquire post-translationally modified tubulin. This, in turn, distinguishes them from their dynamic counterparts and allows for the preferential interaction of vesicles and organelles with the stabilized microtubules leading to a polarization of the cell. Adapted from Kirschner and Mitchison[1].

and sliding functions later, and is the predominant mechanism for pulling the nucleus through the bud neck[16].

Fibroblasts migrating to *in vitro* wounds have been a useful model for studying microtubule polarization, as microtubules become polarized in two ways in response to wounding: by reorientation of the MTOC to a position between the nucleus and the leading edge[19,20]; and by the selective stabilization of microtubules in the leading lamella[19–22] (FIG. 2b). Each of these contributes to the polarization of microtubules towards the leading edge, and might contribute to directed cell migration[20,23]. MTOC reorientation biases the bulk of the radial microtubule array towards the leading edge, whereas microtubule stabilization generates a localized subset of microtubules that accumulate post-translationally detyrosinated tubulin (BOX 2), and so are biochemically distinct from their dynamic counterparts.

MTOC reorientation is accompanied by reorientation of the Golgi apparatus, and this could contribute to the biased delivery of membrane precursors to the leading edge that has been observed in wound-edge fibroblasts[24]. Microtubule stabilization might also bias membrane transport to the leading edge, as microtubules with elevated detyrosinated tubulin bind the microtubule motor protein

kinesin better *in vitro* and participate in the recycling of endocytic vesicles to the cell surface[25,26]. As discussed below, the molecular pathways that regulate selective microtubule stabilization and MTOC reorientation in fibroblasts are similar to those that regulate microtubule polarization during budding in yeast, and are likely to involve similar microtubule–cortical interactions.

Microtubule capture and shrinkage
The pathway that mediates capture and shrinkage in yeast includes proteins called Bni1, Kar9, Myo2, Bim1/Yeb1 and Kip3 (FIG. 3). Bni1 belongs to the Diaphanous-related formin (DRF) family of adaptor proteins that are involved in cell polarity through their ability to regulate the formation of actin fibres (known as 'cables' in yeast[27,28] or 'stress fibres' in mammalian cells[29–31]) and to regulate the microtubule cytoskeleton (discussed below). DRFs bind to, and are regulated by, Rho and Cdc42 GTPases. They contain conserved domains, termed formin-homology domains, that bind to other factors (such as profilin, Src and bud6) that might mediate effects on the actin and microtubule cytoskeletons[27–34].

In yeast, Bni1 is localized in the bud by cell-cycle cues and localizes Kar9 to the bud tip[9,10]. Bni1 does not bind Kar9 directly, but polarizes actin cables towards the bud. Kar9 binds Myo2, a type-V myosin motor, and this might carry Kar9 to the bud tip on the polarized actin cables[35]. Kar9, in turn, interacts with the microtubule-end-binding protein Bim1/Yeb1, and can mediate the binding of Kar9 to microtubules[11,12]. Bim1/Yeb1

is localized to microtubule growing ends in yeast[13], as is its mammalian homologue EB1 (REF. 36), and this indicates a model in which Bim1/Yeb1 at the ends of microtubules interacts with Kar9 positioned at the bud site by Bni1. In support of this, direct imaging has shown that deletion of Bim1/Yeb1 leads to reduced capture and shrinkage[8], and that Kar9 labelled with green fluorescent protein (GFP) is found at the ends of captured microtubules in the bud[14].

Microtubule stabilization in fibroblasts
The pathway that regulates selective microtubule stabilization in migrating fibroblasts involves Rho, mDia and, possibly, EB1 and the adenomatous polyposis coli (APC) protein (FIG. 3). This pathway has been deciphered with serum-starved wounded 3T3 fibroblasts, which require serum or a specific serum factor, lysophosphatidic acid (LPA), to generate stable microtubules after wounding[22,37]. Evidence for this pathway includes measurements showing that LPA stimulates the formation of Rho•GTP in fibroblasts[38], and that Rho is both necessary and sufficient for LPA-induced stabilization of microtubules in serum-starved fibroblasts[37]. Cdc42 and Rac are not involved (REF. 39; A. F. Palazzo and G.G.G., unpublished observations). mDia1, which is a mouse homologue of Bni1, is the only Rho effector that is necessary for microtubule stabilization in serum-starved fibroblasts, and active forms of mDia1 or mDia2 can stimulate stable microtubule formation in the absence of LPA or active Rho, which shows that mDia functions downstream of Rho[40].
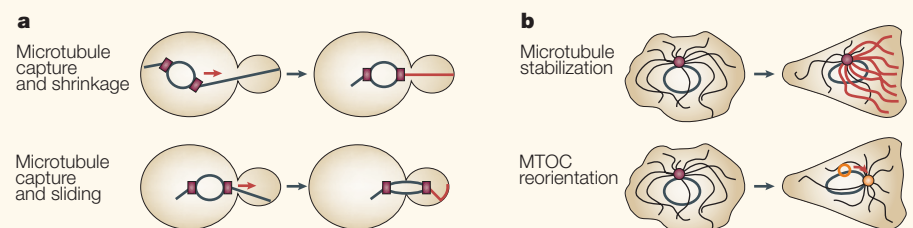

Figure 2 | **Microtubule polarization in budding yeast and migrating fibroblasts. a** | Microtubule capture and shrinkage, and microtubule capture and sliding in budding yeast. During cell division in budding yeast, two microtubule-capture mechanisms polarize microtubules along the mother–bud axis, and contribute to orientation of the nucleus and movements of the nucleus towards and into the bud. Microtubule capture and shrinkage (top) involves end-on capture of microtubules in the bud cortex followed by shrinkage of the captured microtubule. This aligns the spindle along the mother–bud axis and moves the nucleus to the bud neck. (Spindle pole bodies are indicated by boxes on the nucleus; spindle microtubules are not shown for clarity.) Microtubule capture and sliding (bottom) involves lateral capture of microtubules in the bud cortex, followed by sliding of the captured microtubule along the bud cortex. This pulls the nucleus through the bud neck. **b** | Microtubule stabilization and reorientation of the microtubule-organizing centre (MTOC) in migrating wound-edge cells. On receiving stimuli for migration, unpolarized cells (left) adopt a polarized morphology for directed cell migration (right). During the acquisition of the polarized morphology, microtubule arrays become polarized in two ways: by selectively stabilizing a subset of microtubules (top; shown in red) towards the direction of migration; and by reorienting the MTOC (bottom; shown in red) to a position between the leading edge and the nucleus.

Box 2 | Post-translational modification of tubulin

Tubulin is subjected to at least seven post-translational modifications — detyrosination, acetylation, tyrosine phosphorylation, serine phosphorylation, polyglutamylation, polyglycylation and removal of two carboxy-terminal residues. Most of these modifications occur on tubulin assembled into microtubules[65]. The best studied of these is detyrosination, in which the carboxy-terminal tyrosine residue of the α-tubulin is removed by a specific tubulin carboxypeptidase (reviewed in REF. 66). A second enzyme, tubulin tyrosine ligase, can re-add the tyrosine residue. Tubulin carboxypeptidase prefers polymeric tubulin as a substrate whereas the ligase works only on unassembled tubulin. In cells, the ligase maintains the pool of tubulin subunits in the tyrosinated form and the carboxypeptidase will detyrosinate microtubules that become stabilized; for example, by capture and capping (note that dynamic microtubules do not persist long enough to accumulate detyrosinated tubulin).

Although detyrosinated tubulin accumulates in stabilized microtubules, and antibodies to detyrosinated tubulin stain stable microtubules in cells, detyrosination itself is not involved in the microtubule stabilization. Instead, it is likely to regulate the interaction of motor proteins and organelles with stable microtubules[25,26].

Evolutionary conservation?

Given that Bni1 regulates a similar — albeit transient — microtubule stabilization in yeast, could the mammalian homologues of other proteins in the yeast capture and shrinkage pathway be involved in microtubule stabilization? Overexpressed mammalian EB1 induces selective stabilization of microtubules in serum-starved fibroblasts, and fragments of EB1 block the formation of stable microtubules that are induced by LPA or active Rho (Y. Wen, N. Cabrera-Poch and G.G.G., unpublished observations). So, EB1 might contribute to selective microtubule stabilization and could function in an analogous way to Bim1/Yeb1 in yeast microtubule capture and shrinkage.

There are no known homologues of Kar9 in mammals, *Caenorhabditis elegans* or *Drosophila melanogaster*. However, APC has been proposed as its functional homologue[41]. APC binds to EB1 (REF. 42), has a region with limited homology to Kar9 (REF. 41), and is found at the periphery of cells near microtubule ends[43]. Overexpressed APC in proliferating cells bundles microtubules and makes them resistant to the microtubule-depolymerizing drug nocodazole (although this might be due to its microtubule-binding domain rather than its EB1-binding domain[44]). In preliminary experiments, APC expression in serum-starved fibroblasts induces selective microtubule stability without bundling, and we are testing whether this is due to its EB1-binding domain. This supports the idea that APC might function in fibroblasts in an analogous way to Kar9 in yeast.

These results point to a striking similarity between the molecules that are involved in microtubule capture and shrinkage in yeast, and those that are involved in selective microtubule stabilization in mammalian cells. In addition, both pathways regulate events at the plus ends of the microtubules to prevent dynamic instability. In yeast, this has been shown directly by imaging the capture and subsequent shrinkage of microtubules at the bud cortex[8]. In migrating fibroblasts, selectively stabilized microtubules do not lose or gain subunits[45], and the mechanism that is responsible for this behaviour has recently been shown to be capping of the normally dynamic plus ends of the microtubule[46]. LPA, active Rho or active mDia[37,40] induce stable microtubules in fibroblasts that are capped in a similar way to those that are seen in epithelial cells, where capping was initially characterized[46]. Capped microtubules form within five minutes of treatment with LPA[37], so they are probably formed from dynamic microtubules that are captured at cortical sites. Indeed, direct recordings of fibroblasts activated by LPA show that a subset of dynamic microtubules, which encounter the leading edge, become non-dynamic and show long states of pausing, in which no subunit addition or loss is detected[37]. This is analogous to capture of yeast microtubules at the bud tip. As these long-paused microtubules are likely to be precursors of capped microtubules, selective stabilization of microtubules in fibroblasts can be considered to occur by a microtubule capture and capping mechanism.

Importantly, captured microtubules have different fates in yeast and fibroblasts. In budding yeast, captured microtubules are only transiently stabilized, and subsequently shrink. In fibroblasts, by contrast, captured microtubules become capped and do not shrink for hours. We do not yet know whether captured microtubules in fibroblasts always become capped or whether they can sometimes shrink; capped microtubules in fibroblasts will shrink under certain experimental conditions *in vitro*[46] and *in vivo*[47]. So, differences in the longevity of captured microtubules in yeast and fibroblasts might reflect differences in the degree of regulation rather than fundamental differences in the processes.

Further features of the yeast and mammalian pathways remain to be explored. For example, the Rho GTPase that regulates the activity of Bni1 in microtubule capture and shrinkage is unknown. Bni1 binds to Cdc42, Rho1, Rho3 and Rho4 in yeast[6,32,33]. We also need to determine the role of Bni1/mDia in capture. In yeast, Bni1 might mediate its effects on microtubule capture through actin[35], but in fibroblasts, mDia binds microtubules, which

> "Given that Bni1 regulates a similar — albeit transient — microtubule stabilization in yeast, could the mammalian homologues of other proteins in the yeast capture and shrinkage pathway be involved in microtubule stabilization?"

Yeast

Rho/Cdc42?
↓
Bni1
↓
Kar9
↓
Bim1/Yeb1
↓
Microtubule capture and shrinkage

Mammals

Rho
↓
mDia
↓
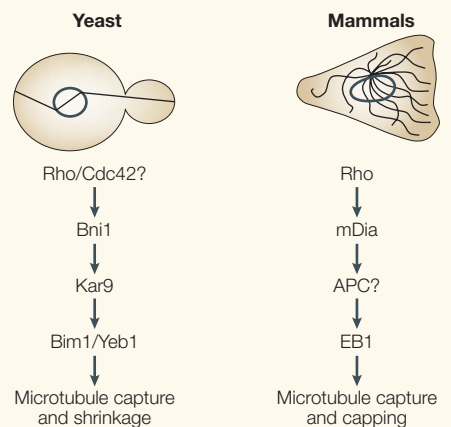APC?
↓
EB1
↓
Microtubule capture and capping

Figure 3 | **Similarities in pathways that regulate microtubule capture/capping in mammalian cells and microtubule capture/shrinkage in yeast.** In mammalian cells, Rho GTPase stimulates the formin mDia. mDia is thought to activate a protein (possibly adenomatous polyposis coli; APC), which then interacts with dynamic microtubules bearing EB1 to cap them. In yeast, the Rho or Cdc42 GTPases stimulate the formin Bni1. Bni1 localizes Kar9 and might activate it to interact with dynamic microtubules bearing Bim1/Yeb1 to capture them. Mammalian proteins that are homologous to those in yeast are known for each step, except for Kar9. APC is proposed to be the functional homologue of Kar9 on the basis of its binding to EB1 and other criteria.

indicates a more direct role[40]. And how do Bim1/Yeb1 and EB1 function in the capture events? EB1 is localized on the growing ends of microtubules, but it is not detected on the ends of capped microtubules in TC-7 cells[46] or wounded 3T3 cells (Y. Wen and G.G.G, unpublished observations). Perhaps EB1 acts transiently by initiating capture, but not by contributing to subsequent events? There are no reports so far as to whether Bim1/Yeb1 is on the ends of captured and shrinking microtubules in budding yeast. Nonetheless, during mating in yeast, microtubules are captured at the tip of the cell extension that forms to mediate mating ('shmoo tip'). Capture at the shmoo tip is analogous to that at the bud tip; however, at the shmoo tip the captured microtubules both shrink and grow[48]. Moreover, Bim1/Yeb1 is detectable on the ends of these captured microtubules only during periods of growth (P. Maddox and E. Salmon, unpublished observations).

Finally, the possibility that other factors participate in the capture pathways needs to be explored. Presumably, different proteins are necessary for captured microtubules to shrink (yeast budding and mating), grow (yeast mating) or become capped (fibroblasts). Genetic studies in yeast have implicated the kinesin motor Kip3 — which is localized along cytoplasmic and spindle microtubules — in capture and shrinkage[15]. Kip3 mutants have longer microtubules, which indicates that Kip3 might act as the 'shrinkage factor'. Kinesins can remain attached to the ends of depolymerizing microtubules in reconstitution experiments[49], making them candidates to modify the activity of captured microtubules. The kinesin inhibitor AMP–PNP blocks the ATP-induced shrinking of capped microtubules in extracted fibroblasts, which indicates that the mammalian cap might also contain a kinesin-like molecule[46].

Box 3 | Microtubule capture mechanisms in other systems

The mechanisms for microtubule capture have been best defined in budding yeast and in migrating cells in wounded monolayers. However, there is growing evidence that microtubule interactions with cortical determinants, which probably involve microtubule-capture mechanisms, are important in other systems[67–72].

Foremost of these are the systems in which the mitotic spindle is positioned asymmetrically in response to cortical cues, such as in early embryonic divisions of *Caenorhabditis elegans* or in dividing *Drosophila melanogaster* syncitial blastocysts and neuroblasts (TABLE 1). Microtubule–cortical interactions also seem to operate in the maintenance of mitotic-spindle position during the symmetrical divisions in cultured mammalian cells and in *Schizosaccharomyces pombe*, and in certain situations in cell polarity in non-dividing cells (TABLE 1).

The evidence for specific microtubule–cortical interactions in these dividing systems is based on observations that alterations in spindle position occur after expression of dominant-negative constructs or mutant versions of proteins that have been implicated in budding yeast or migrating-fibroblast-capture mechanisms. The functional data are supported, in most cases, by localization of the relevant proteins in the cortex at the appropriate position and time to affect spindle positioning. For example, Cdc42, Par-6, PKC-3 (an atypical PKC), dynein and dynactin, which are all implicated in MTOC reorientation in migrating cells, are also involved in the asymmetric division of *C. elegans* one-cell embryos (TABLE 1). Par-6, which was first described as a mutant that affects asymmetric divisions in *C. elegans* embryos, is localized in the anterior cortex of one-cell embryos, and forms a complex with PKC-3 and Par-3, which have been shown independently to regulate asymmetric cell divisions[73–75]. Cdc42 regulates the anterior localization of these proteins by interacting with Par-6 and is itself necessary for proper asymmetric division[76,77]. RNA interference (RNAi) of dynein and dynactin also disrupts spindle positioning in *C. elegans* one-cell embryos[78,79]. A Par-6/Par-3/atypical PKC complex is also required for asymmetric cell divisions of *Drosophila* neuroblasts and epithelial cells[80–82], and in maintaining mammalian epithelial integrity[83] (TABLE 1). APC2, a *Drosophila* homologue of APC, which has been implicated in the microtubule-stabilization pathway, has also been implicated in asymmetric cell division in *Drosophila* embryos[84,85].

In none of these systems, with the exception of *S. pombe*, has a detailed examination of the actual microtubule–cortical interaction been carried out. In *S. pombe*, microtubules interact with the cell tips, and this leads to microtubule catastrophe after a short delay at the cell tip, which perhaps reflects a transient microtubule capture[86–88]. This does not occur in cells that lack the CLIP-170/Bik1 homologue, Tip1 (REF. 86). Interestingly, dynein, which has a role in microtubule capture in yeast and MTOC reorientation in migrating mammalian cells, seems dispensable for nuclear positioning during division in *S. pombe*; instead, microtubules themselves seem to exert pushing forces on the nucleus (REFS 88,98; P. Tran and F. Chang, unpublished observations).

There are fewer known examples of microtubule–cortical interactions in non-dividing cells. Microtubule capture is probably important in other cell types in which the MTOC is reoriented in response to signals. For example, Cdc42 has been implicated in MTOC reorientation in T cells that respond to targets[89,90]. Microtubule capture might also be involved in MTOC reorientation in other migrating cells (such as macrophages[91] and endothelial cells[92]). Nuclear positioning observed in *Aspergillus nidulans* hyphae might also be regulated by microtubule capture, as dynein, dynactin and the dynein/dynactin-binding protein NudF (a Lis1 homologue) are essential for proper nuclear positioning, and mutants in dynein cause alterations in microtubule dynamics at the hyphal tip[93,94]. Dynein function also seems essential for maintaining spindle position in mammalian epithelial cells, as interfering with dynein or Lis1 leads to alterations in spindle positioning[95–97].

We will probably find more systems in which microtubule capture is important in cell polarity, and this will provide a deeper understanding of the different types of microtubule capture and how they act in distinct cellular contexts. Remaining questions include how the cortical signalling molecules regulate proteins that interact with and effect the changes in microtubule arrays, and whether individual cell-polarization events will require several microtubule-capture mechanisms, as has been found in budding yeast and wounded migrating cells.

Microtubule capture and sliding in yeast

The principal components in budding yeast microtubule capture and sliding are the microtubule motor protein dynein and its regulator, dynactin (FIG. 4). This pathway is less defined than the capture and shrinkage pathway, but clearly involves distinct proteins and a distinct interaction between microtubules and the cortex (FIG. 2).

Observations that support the idea of a separate pathway for microtubule capture and sliding in yeast include the fact that deletions in subunits of either dynein or dynactin are lethal in combination with deletions in members of the capture and shrinkage pathway, but deletions of more than one of the dynein or dynactin components do not increase the severity of the phenotype of single mutants[8,16–18]. Cortical microtubule sliding in the bud has been observed directly by fluorescent speckle analysis. Moreover, knockouts of Arp1 — a subunit of dynactin — reduce cortical-microtubule sliding, which links dynein and dynactin in this process[8].

MTOC reorientation in fibroblasts

Studies with wounded monolayers of serum-starved fibroblasts[39] and astrocytes[50] show that MTOC reorientation is controlled by a pathway that involves Cdc42, dynein and dynactin (FIG. 4). MTOC reorientation in

Table 1 | **Cortical determinants in other cell polarity systems**

System	Protein(s)	Localization	Evidence	References
Asymmetric cell division				
Caenorhabditis elegans embryos	Par-6/Par-3	Anterior cortex	Par-6 and Par-3 mutants fail to divide asymmetrically	73,74
	PKC-3 (atypical PKC)	Anterior cortex	RNAi of PKC-3 inhibits asymmetric division and Par-6/Par-3 localization	75
	Cdc42	?	RNAi of Cdc42 disrupts asymmetric division and Par-6/Par-3/PKC-3 localization	76,77
	Dynein/dynactin	Midbody (dynactin)	RNAi of dynein or dynactin leads to mispositioned spindles	78,79
Drosophila melanogaster neuroblasts and epithelia	Par-6/Bazooka (Par-3)/aPKC	Cortical crescent	Par-6, Bazooka and aPKC mutants lead to mislocalized spindles	80–82
	APC2/EB1	Adherens junction (APC2)	RNAi of APC2 or EB1 lead to mispositioning of spindles	84
Drosophila melanogaster syncitial blastocysts	APC2	Cortical caps and psuedo-cleavage furrows	Nuclei in APC2 mutants are not maintained in cortical location (drop-out phenotype)	85
Symmetric cell division				
Schizosaccharomyces pombe	Tip1 (CLIP-170 homologue)	Cell tips	Mutants lead to T-shaped cells	86
Non-polarized epithelial cells (NRKe)	Dynein	?	Inhibitory antibodies prevent alignment along long axis of cell	95
Polarized epithelial cells (MDCK)	Lis1 (dynein/dynactin-binding protein)	Cortical region	Lis1 overexpression results in non-parallel alignment of spindles and disruption of cortical Lis1	96
	Dynein/dynactin	Cortical region	?	97
Non-dividing Cells				
Polarized epithelia–MDCK	Par-6/Par-3/aPKC	Apical cortex	Dominant-negative aPKC disrupts Par-3 localization and epithelial integrity	83
T cells (complexed with target)	Cdc42	?	Dominant-negative and constitutively active mutants block MTOC reorientation	90
Aspergillus nidulans hyphae	Dynein/dynactin/ NudF (Lis1)	Hyphal tips	Mutants interfere with nuclear positioning in hyphae	93,94

MDCK, Madin–Darby canine kidney; MTOC, microtubule-organizing centre; aPKC, atypical PKC; RNAi, RNA interference.

serum-starved fibroblasts requires serum and, as with microtubule capture and capping, LPA is the main serum factor that triggers it[39]. However, for MTOC reorientation, LPA triggers a Cdc42-regulated pathway that involves dynein and dynactin as probable downstream components[39]. Although the downstream Cdc42 effector in fibroblasts has not been identified, in astrocytes it seems to be the adaptor protein Par-6 (REF. 50), a PDZ-domain-containing protein that specifically binds the GTP-bound forms of Cdc42 and Rac. Par-6 interacts with the atypical protein kinase C (PKC)-ζ, and inhibitors of PKCζ inhibit MTOC reorientation[50]. These studies indicate that there is a regulatory pathway for MTOC reorientation in migrating cells that is stimulated by LPA and involves Cdc42, Par-6/PKCζ, dynein and dynactin (FIG. 4). It is not yet clear whether Par-6/PKCζ directly or indirectly regulates dynein/dynactin.

Genetic studies have shown that the two pathways that regulate microtubule capture and shrinkage, and microtubule capture and sliding in yeast act independently. Palazzo *et al.*[39] have shown that the pathways that regulate MTOC reorientation and selective microtubule stabilization in 3T3 fibroblasts also act independently — treatments that activate or inhibit one process do not activate or inhibit the other process. For example, stimulating microtubule stabilization with active Rho, or with an active form or autoinhibitory domain of mDia, does not induce MTOC reorientation[39]. Conversely, inhibiting MTOC reorientation with dominant-negative Cdc42, with antibodies to dynein, or by overexpression of the dynactin subunit dynamitin, does not block the formation of stabilized microtubules[39]. This rules out the possibility that microtubule stabilization is required for MTOC reorientation, or vice versa, and indicates that the two pathways are independently controlled in a similar manner to the two capture pathways in yeast.

A second conserved pathway?

The results discussed above point to a similarity in the molecular pathways that regulate microtubule capture and sliding in yeast and MTOC reorientation in mammalian cells. On the basis of the role of dynein and dynactin in

both processes, the microtubule–cortical interaction that mediates MTOC reorientation in mammalian cells is probably analogous to that involved in capture and sliding in yeast. Indeed, sliding (or pulling) of microtubules that are captured by active dynein anchored near the leading edge is a straightforward mechanism that can account for MTOC reorientation in migrating cells. However, direct imaging has not yet been done in mammalian cells, and this will be necessary to determine whether microtubule sliding occurs during MTOC reorientation.

Questions about microtubule capture and sliding remain to be addressed in both systems. The upstream regulators in the microtubule capture and sliding pathway in yeast have not been identified. On the basis of the work in mammalian cells, it is possible that Rho GTPases regulate dynein/dynactin in yeast. Although there is no equivalent to Par-6/ PKCζ in yeast, Pkc1 is a yeast PKC homologue that is regulated directly by Rho during budding[51]. The dynactin subunit Nip100 binds to active Rho1 and Rho2 in yeast two-hybrid screens, and this is another possibility for regulation[52].
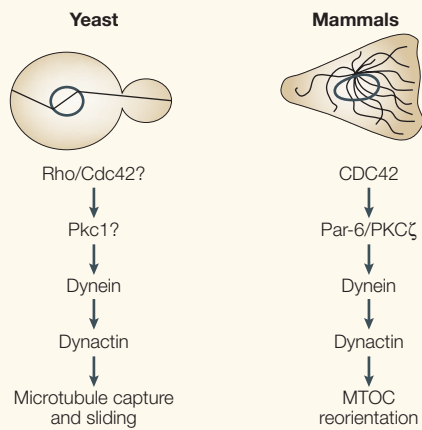
Figure 4 | Pathways that regulate MTOC reorientation in mammalian cells and microtubule capture and sliding in yeast. In mammalian cells, the Cdc42 GTPase stimulates Par-6/protein kinase C (PKC)ζ, which then regulates the activity of dynein or dynactin to induce MTOC reorientation. Dynein might interact with dynactin localized on the ends of microtubules. In yeast, dynein and dynactin are known to be involved in microtubule attachment and sliding. On the basis of comparisons with the mammalian pathway, it is proposed that the Rho or Cdc42 GTPases regulate this pathway — perhaps by interacting with the yeast homologue of PKC, Pkc1.

Another question is how the cortical capture that is regulated by this pathway occurs. Most models propose that cortically localized dynein pulls on microtubules. Support for this hypothesis comes from the discovery that a yeast protein called Num1 acts as a cortical anchor for dynein and functions genetically in the dynein pathway[17,18]. In mammalian cells, Par-6/PKCζ could have an analogous function, or it could interact with other cortical proteins that serve as the dynein anchor. For example, β-catenin has recently been shown[53] to bind dynein. Localization studies in both systems should help refine the mechanisms that are involved.

A general model: TMAPs and CMAPs

The pathways that regulate microtubule capture and shrinkage/capping, and microtubule capture and sliding use different molecules and result in different types of cortical interaction. Nonetheless, a general model for the interaction of microtubules with the cortex can be proposed.

There are four basic components of the model: a Rho family GTPase; an effector of the GTPase; a cortical microtubule attachment protein (CMAP), which acts as a microtubule receptor; and a tip-only microtubule-associated protein (TMAP), which acts as a CMAP ligand (FIG. 5). The Rho-family GTPase

acts as a regulatory switch and allows microtubule–cortical interactions to be initiated by external or internal signals and integrated with the actin cytoskeleton. In mammals, Rho GTPases can be switched on by soluble activators (such as LPA), and also by other molecules such as integrins. Indeed, we have found that integrin stimulation affects microtubule capture and capping (A. F. Palazzo, J. Yoon and G.G.G., unpublished observations). Integrins also seem to be involved in the MTOC-reorientation pathway in astrocytes[50]. In yeast, the factors that activate Rho-family GTPases are less clear, although cell-cycle and mating factors control the activation of Cdc42 (REFS 33,54). Cell-cycle regulation of microtubule capture and capping might also occur in mammalian cells; for example, during cytokinesis, where Rho GTPases are required.

Rho GTPases do not seem to regulate the activity of the CMAPs directly; instead, they act through their GTPase effectors. This might reflect the complexity of microtubule attachment to cortical sites, and the effectors that have been identified so far — Bni1/mDia and Par-6/PKCζ — all associate with the cortex and bind other proteins through interaction domains. These effectors also interact with proteins that regulate the actin cytoskeleton, which points to an integrative function and the importance of interacting with cortical actin.

The least-understood aspect of the model is the nature of the CMAPs, and how they become associated with the cortex. Kar9 and APC are candidate CMAPs for the microtubule capture and shrinkage/capping pathways, although it is not yet clear whether they act as the structural tether to microtubules.

"… the repertoire of cortical interactions is much richer than previously predicted in the original Kirschner and Mitchison model: interactions involve not only microtubule capture, but also distinct consequences for the captured microtubules — shrinkage, growth, capping and sliding."
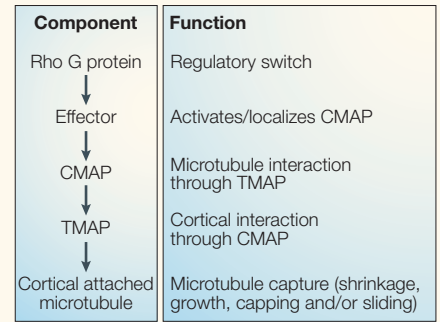

Figure 5 | Model for regulated microtubule–cortical interactions. This model is based on the two pathways for regulating microtubule–cortical interactions in mammalian cells and yeast. The first component is a member of the Rho family of GTPases, which functions as a switch in response to external, cell-cycle or cell-adhesion signals. The activated Rho GTPase then interacts with an effector, which might alter the activity of the effector, its location or its interaction with other proteins. The activated effector then stimulates the activity of the cortical microtubule attachment protein (CMAP). CMAPs initiate the interaction with microtubules through their ability to bind tip-only microtubule-associated proteins (TMAPs). This interaction might be modified further to generate attached microtubules that shrink, grow, remain stabilized or slide along the cortex.

Kar9 and APC bind to the TMAP EB1, and this could mediate the initial interaction between a growing microtubule and the cortex. However, EB1 is not detectable on captured and shrinking microtubules in yeast, or on captured and capped microtubules in mammalian cells. Perhaps Kar9 (and APC) promote the interaction of microtubule ends with still-to-be-identified CMAPs that mediate the actual tethering.

Dynactin is localized on the ends of growing microtubules[55], so it is a TMAP-like EB1. Dynactin that is localized at microtubule tips could stimulate cortically localized dynein and/or provide interaction sites for dynein to initiate capture and sliding of microtubules. Such a model extends an earlier idea — that the localization of dynactin at microtubule tips provides docking sites from which vesicles that contain dynein initiate their movement towards the minus end (retrograde movement)[55]. For example, endosomes are moved towards the centre of the cell by dynein. In the case of microtubule–cortical sliding, dynactin on the growing ends of microtubules would interact with dynein that is immobilized in the cortex rather than on mobile vesicles, and this would cause the microtubule (and attached MTOC) to move towards the active dynein. One test of this

idea will be to see whether dynactin is localized on the ends of captured and sliding microtubules.

CLIP-170 (cytoplasmic linker protein p170) is another candidate TMAP. CLIP-170 was originally identified as a protein that binds to microtubules in a nucleotide-dependent fashion and was shown to be specifically localized to the growing ends of microtubules[56,57]. Recently, Galjart and colleagues[58] identified CLASPs (CLIP-associating proteins) as a new family of CLIP-170-binding proteins. CLASPs might act as CMAPs, as they are localized near microtubule ends and are redistributed to the leading edge of wounded fibroblasts in response to serum[58]. Although the factors that regulate CLASPs are not yet known, CLASPs themselves seem to be involved in serum-induced microtubule stabilization. CLIP-170 also interacts with dynein and modifies its activity, which indicates that it might participate in the microtubule capture and sliding pathway. In yeast, the CLIP-170 homologue Bik1 seems to function in dynein microtubule capture and sliding[59]. Whether CLIP-170 and CLASPs regulate a new microtubule–cortical interaction or modify known cortical interactions will require further study.

In summary, Kirschner and Mitchison's selective stabilization model has provided a useful theoretical framework for exploring the importance of dynamic microtubules and their possible interaction with cortical sites that are regulated by signalling pathways. By identifying the molecules that mediate and regulate the cortical interactions, more recent studies have highlighted the conservation of the regulatory pathways from yeast to mammals. As the conserved pathways regulate different cellular activities in yeast and mammalian cells, it could be that there are only a few basic microtubule–cortical interactions, and that these are then modified to carry out specific cellular functions. Indeed, there are a number of other systems in which molecules that regulate microtubule capture in yeast and fibroblasts affect cellular asymmetry (BOX 3; TABLE 1). In any case, the repertoire of cortical interactions is much richer than previously predicted in the original Kirschner and Mitchison model: interactions involve not only microtubule capture, but also distinct consequences for the captured microtubules — shrinkage, growth, capping and sliding.

The regulatory pathways that control microtubule–cortical interactions have also turned out to be more intricate than could have been predicted, and this might allow for fine tuning and integration with other cellular responses. Given the repertoire of cortical interactions and the regulatory mechanisms that control them, it is likely that the contribution of microtubules to cell polarity is more varied than previously thought.

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