Cell motility: can Rho GTPases and microtubules point the way?

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Journal of Cell Science 114, 3795-3803 (2001) © The Company of Biologists Ltd

Summary

Migrating cells display a characteristic polarization of the actin cytoskeleton. Actin filaments polymerise in the protruding front of the cell whereas actin filament bundles contract in the cell body, which results in retraction of the cell's rear. The dynamic organization of the actin cytoskeleton provides the force for cell motility and is regulated by small GTPases of the Rho family, in particular Rac1, RhoA and Cdc42. Although the microtubule cytoskeleton is also polarized in a migrating cell, and microtubules are essential for the directed migration of many cell types, their role in cell motility is not well understood at a molecular level. Here, we discuss the potential molecular mechanisms for interplay of microtubules, actin and Rho GTPase signalling in cell polarization and motility. Recent evidence suggests that microtubules locally modulate the activity of Rho GTPases and, conversely, Rho GTPases might be responsible for the initial polarization of the microtubule cytoskeleton. Thus, microtubules might be part of a positive feedback mechanism that maintains the stable polarization of a directionally migrating cell.

Keywords: Cell motility, Rho GTPases, Cytoskeleton, Microtubules, Actin

Introduction

Many metazoan cell types – for example, fibroblasts or epithelial cells – can become polarized as a response to an extracellular stimulus or spontaneously and migrate in a unidirectional fashion. This ability is essential for cells to function in their natural environment. For example, the development of the nervous system in vertebrates requires many complex patterns of cellular migration. Epithelial cells need to migrate in order to close wounds in the epithelial layer, whereas motile fibroblasts are crucial for tissue remodelling. Conversely, improper regulation of cell migration is the basis of many abnormal processes, resulting, for example, in the invasiveness of tumour cells.

Migrating vertebrate cells in tissue culture show a unique polarized morphology; a broad, flat lamella extending in the direction of migration that terminates in a ruffling lamellipodium (the leading edge) and a narrow, retracting tail at the rear of the cell (Abercrombie et al., 1970). The actin cytoskeleton provides the driving force for cell migration. Actin is regulated by small GTPases of the Rho family, and recent evidence indicates that microtubules might modulate the activity of Rho GTPases and thus influence the actin cytoskeleton. However, other recent experiments suggest that, in addition to organizing the actin cytoskeleton, Rho GTPases might also influence the organization and dynamics of microtubules. The potential mechanisms by which microtubules communicate with signalling molecules, particularly Rho GTPases, and the actin cytoskeleton to establish cell polarity and promote cell locomotion are the focus of this commentary. We do not discuss localized signalling events – for example, the activation of G-protein-coupled receptors in a chemotactic gradient or transient localized increases in intracellular calcium – that might modulate cell motility but occur as a response to extracellular stimuli (Lee et al., 1999; Parent et al., 1998).

Polarization of the actin cytoskeleton: asymmetries in contractility can drive cell locomotion

The morphological polarization of a migrating cell is reflected in the underlying polarization of its actin organization. Actin polymerisation is nucleated at the leading edge, which generates a highly crosslinked meshwork of actin filaments in the lamellipodium whose growing ends face the front of the cell (Henson et al., 1999; Small et al., 1978; Svitkina et al., 1997). The constant growth of these filaments, probably coupled with the action of a myosin motor, both pushes the leading edge forward and generates a retrograde flow of actin towards the cell centre (Henson et al., 1999; Lin et al., 1996; Wang, 1985; Waterman-Storer and Salmon, 1999; Cramer, 1997; Pollard et al., 2001). The organization of the actin cytoskeleton in the cell body is highly variable in different types of migrating cell (Small et al., 1998). Thus, no general model for how the cell body follows the leading edge has been established. However, in heart fibroblasts, actin is organized into long bundles of filaments that align along nearly the full length of the cell and in this case are thought to generate contraction to draw the cell body forward (Cramer et al., 1997). Cell motility requires the transmission of the forces generated by actin movements inside the cell to the matrix outside through regulated formation and dissolution of cell-substrate adhesions (Beningo et al., 2001). Thus, new substrate adhesion sites are formed at the edge of the lamellipodium, whereas old adhesion sites are broken down at the rear of the cell, which results in the retraction of the trailing cell body (Rottner et al., 1999; Laukaitis et al., 2001).

In the simplest case, a gradient of actin polymerisation and contractility seems to be sufficient to generate directed motility. This was demonstrated elegantly by Verkhovsky and colleagues in experiments using cytoplasmic fragments of fish skin keratocytes that are devoid of nuclei, most organelles and microtubules (Euteneuer and Schliwa, 1984; Verkhovsky et al., 1999). In symmetric, discoid fragments, actin polymerisation and retrograde flow occur equally from all edges towards the centre, and myosin II ribbons are distributed in a radially symmetric manner. When one edge of a discoid fragment is pushed by micromanipulation, this edge retracts, and myosin on the retracted side of the resulting half-moon-shaped fragment is condensed; this establishes an asymmetry in contractility. The other side of the fragment then automatically becomes protrusive, and this asymmetry self-perpetuates, causing continuous directional motility of the fragment (Verkhovsky et al., 1999). Similarly, local application of agents that inhibit myosin-dependent contractility release adhesion of one side of a symmetric fibroblast, causing that edge to retract, the opposite side to protrude, and the cell to become motile (Kaverina et al., 2000). Thus, asymmetries in contractility are sufficient to polarize both protrusion and adhesion.

Polarization of intracellular signalling: how to create a contractility gradient

These results show that under certain experimental conditions, simple gradients of actomyosin contractility and/or substrate adhesion can drive polarization and directional motility. However, how a cell generates these gradients under normal conditions is not clear. One possibility is that the activity of signalling proteins that control actin dynamics and adhesion formation is locally regulated within the cell. Prime candidates for this role are small GTPases of the Rho family, in particular RhoA, Rac1 and Cdc42Hs. These proteins act as molecular switches that can be activated by a variety of extracellular stimuli. Rho GTPases cycle between a GTP-bound active form, which can activate downstream effectors, and an inactive GDP-bound form. In fibroblasts, RhoA activity generates contractile actin bundles and large adhesions to the substrate, Rac1 activity induces actin polymerisation to drive lamellipodial protrusion and the formation of small adhesions, and Cdc42Hs generates polarity and induces formation of filopodia (Nobes and Hall, 1999; Hall, 1998; Van Aelst and D'Souza-Schorey, 1997). Rho proteins are tightly regulated by different classes of upstream factors that control the exchange of GDP for GTP and the rate of GTP hydrolysis (Kjøller and Hall, 1999; Symons and Settleman, 2000; Van Aelst and D'Souza-Schorey, 1997).

Because the leading edge of a migrating cell is protrusive and the central and rear regions contract, one can envision that differences in Rho-protein activity levels in the cell front and rear might be responsible for the polarized organization of actin in a migrating cell. A simplistic view would be that Rac1 is activated in the protruding edge whereas RhoA is activated in the cell body. This polarization is reflected not only in the organization of the actin cytoskeleton but also in the evolution of adhesion sites in a migrating cell: small focal complexes form at the leading edge in response to Rac1 activity and mature into larger focal contacts as the cell moves over them (Rottner et al., 1999). However, the mechanism controlling how contacts are released in the cell rear is still an open question.

The most compelling evidence that Rho proteins are localized to different regions of polarized cells originates from budding yeast. In yeast cells, localization of Cdc42p provides the main cue for the polarity of the actin cytoskeleton (Pruyne and Bretscher, 2000). In mammalian cells, however, the subcellular localization of Rho GTPases is less clear. Post-translational prenylation of Rho GTPases regulates their localization to different membrane compartments. Rac1 appears to be predominantly bound to the plasma membrane, whereas Cdc42Hs is associated with diverse intracellular membrane compartments. Interestingly, both Rac1 and Cdc42Hs redistribute from these compartments and a considerable cytosolic pool to localize primarily to lamellipodial membrane ruffles upon cell stimulation (Michaelson et al., 2001).

Where and when a Rho GTPase is *active*, however, is a different question from where it is *located*. Thus, localization of the activities of these proteins may be key to testing the hypothesis that they are responsible for generating the asymmetries of motile cells. Kraynov and co-workers have recently approached this question by developing a fluorescence resonance energy transfer (FRET) biosensor to detect GTP-bound Rac1 in migrating fibroblasts. This tool indicated the accumulation of GTP-bound Rac1 in ruffles and a gradient of activated Rac1 from the front to the rear of migrating cells at a wound edge (Kraynov et al., 2000). Similarly, autophosphorylated (and thus activated) Pak1, a downstream target of active Rac1 and Cdc42Hs, is predominantly found in protruding lamellipodia upon growth factor stimulation of fibroblasts (Sells et al., 2000). However, there has so far been no evidence, direct or indirect, for increased localization or activity of RhoA in the central region of a migrating cell, where it could promote contraction, although recent data demonstrate that RhoA is required for the retraction of the trailing cell body in motile monocytes (Worthylake et al., 2001).

One way in which Rac1 and RhoA activity might be localized to opposite ends of the cell is through their antagonistically regulated activity. In fibroblasts, activation of Rac1 by a specific exchange factor results in an inhibition of RhoA, whereas RhoA activation does not seem to affect the activity of Rac1 (Sander et al., 1999). Thus, in fibroblasts, a gradient in activity across the cell could be simply accomplished by a basal RhoA activity throughout the cell and a local upregulation of Rac1 at the leading edge. In a neuronal cell line, however, activation of RhoA inhibits growth-factor-induced activation of Rac1 (Yamaguchi et al., 2001). Thus, further characterization of the antagonistic regulation of Rho proteins is required.

Polarization of microtubule organization and dynamics in a migrating cell

How such a hypothetical Rho GTPase activity gradient might be generated in a migrating cell remains unclear, but emerging evidence indicates that microtubules might modulate Rho protein activity. Since the microtubule cytoskeleton itself is polarized in migrating cells (Fig. 1), we first describe this polarity and then discuss how microtubules could locally regulate Rho proteins.

The most striking polarization of the microtubule cytoskeleton in many migrating cells is the orientation of the

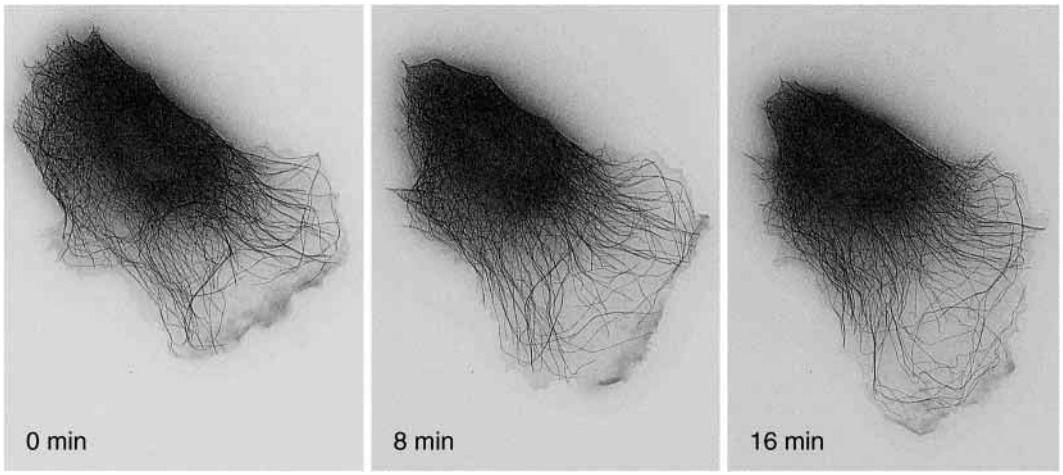

Fig. 1. Microtubule cytoskeleton in a migrating Swiss 3T3 fibroblast visualized by the microinjection of X-rhodamine-conjugated tubulin and fluorescence microscopy. The contrast of the images was inverted to show individual microtubules more clearly. Note how microtubules are aligned along the axis of migration and how growing microtubules fill in the protruding leading edge as the cell moves forward.

centrosome, the organizing centre of the radial interphase microtubule network, towards the direction of migration. However, not all cell types reposition the centrosome, and whether centrosome reorientation is the cause or a result of polarization has been a matter of debate (Euteneuer and Schliwa, 1992; Schliwa and Höner, 1993). In many cells migrating at the edge of a monolayer wound, the centrosome leads the way and is positioned in front of the nucleus, facing the leading edge (Gotlieb et al., 1981; Malech et al., 1977) (Fig. 2a). Partly as a result of centrosome position, microtubules themselves are polarized and tend to be aligned along the axis of cell migration; many of them, particularly stabilized, detyrosinated microtubules, are preferentially oriented with their plus ends facing the leading edge (Gundersen and Bulinski, 1988) (Fig. 1 and Fig. 2b).

In addition to this polarized organization of the microtubule cytoskeleton as a whole, microtubule polymerisation dynamics are polarized in a migrating cell. In living cells, microtubule ends undergo stochastic changes between polymerisation and depolymerisation, a property known as dynamic instability (Desai and Mitchison, 1997). In migrating cells, microtubules in the lamella behind the leading edge are moved backwards by actin retrograde flow, which indicates a coupling between the actin and microtubule networks (Waterman-Storer and Salmon, 1997; Yvon and Wadsworth, 2000). Since microtubule plus ends are often found close to the leading edge, they must undergo net growth as they are continuously swept backwards (Fig. 2c). Further, as the leading edge protrudes and the rear edge retracts, microtubules grow forward and fill in the advancing cellular space (Waterman-Storer and Salmon, 1997) (Fig. 1). Thus, although microtubules undergo dynamic instability throughout the cell, one would expect that microtubule growth is biased towards the leading edge. Indeed, microtubules in protruding lamellipodia appear to spend more time growing than microtubules in quiescent cell edges (Wadsworth, 1999; Waterman-Storer et al., 2000). In contrast, microtubules subjected to retrograde flow tend to buckle and break in the cell body, creating depolymerising microtubule ends (Fig. 2d).

These observations pose the question of how such regional differences in microtubule dynamics are generated. There has so far been no documentation of regional localization or regulation of stabilizing factors, such as microtubule-associated proteins, or catastrophe-promoting factors, in migrating cells. However, interesting candidates for regional microtubule regulation include a recently identified class of proteins that specifically bind to growing microtubule plus ends (Schroer, 2001; Schuyler and Pellman, 2001). One such protein, adenomatous polyposis coli protein (APC), forms granules that undergo plus-end-directed movement along microtubules and specifically accumulate on growing microtubule plus ends in actively protruding areas of cells (Mimori-Kiyosue et al., 2000; Näthke et al., 1996). In addition, APC stabilizes microtubules in vitro and in vivo (Zumbrunn et al., 2001). CLIP-170, the first protein described to bind to growing microtubule ends, does not seem to have any preference for certain areas of the cell (Perez et al., 1999), but recently described CLIP-170-associated proteins (CLASPs) preferentially bind to microtubule ends oriented towards the leading edge in serum-stimulated fibroblasts (Akhmanova et al., 2001). This polarized localization of CLASPs correlates with the orientation of microtubules in migrating cells, and CLASP2 appears to associate with the ends of acetylated microtubules (Akhmanova et al., 2001). Thus, plus-end-binding proteins such as APC or CLASPs might regionally regulate microtubule dynamics and promote microtubule growth into advancing lamellipodia (Fig. 2e).

The role of microtubules in migrating cells: master regulators or obsolete?

What then is the evidence that microtubules are required to establish cell polarity during motility? It was observed some time ago that fibroblasts require an intact microtubule cytoskeleton to maintain their polarization (Bershadsky et al., 1991; Goldman, 1971; Tomasek and Hay, 1984; Vasiliev et al., 1970). In addition, in neurons, local application of microtubule-depolymerising drugs inhibits axonal growth (Bamburg et al., 1986), and low concentrations of colcemid result in a delocalisation of protrusive activity from the growth cone to the length of the neurite (Bray et al., 1978). The treatment of macrophages with colcemid causes a loss of cell polarity and the formation of more than one lamellipodium, resulting in an inhibition of directional migration (Glasgow and Daniele, 1994). Similarly, chemotaxing amoebae require microtubules for the stabilization of the pseudopod facing the

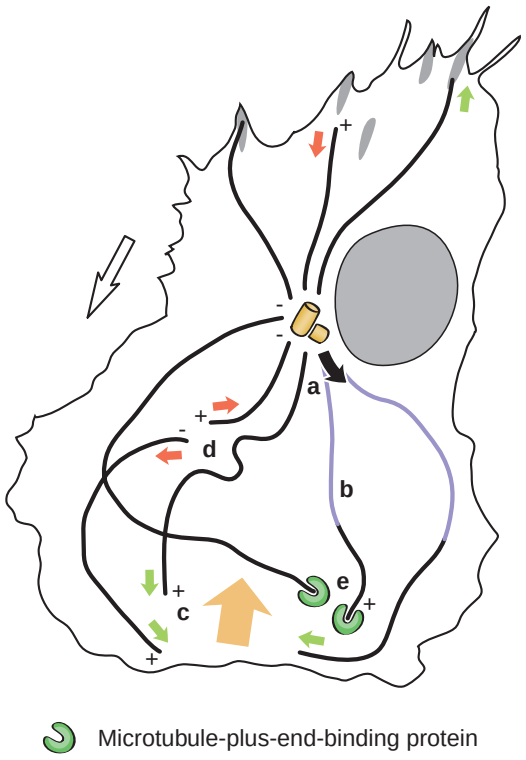

Fig. 2. Polarization of the microtubule cytoskeleton in a migrating cell. (a) In many cell types, the centrosome reorients towards the direction of migration (black arrow). (b) Stable, detyrosinated microtubules (purple) appear to be oriented preferentially in the direction of migration. (c) Microtubules exhibit net growth near the leading edge and, (d) as a result of actin-dependent retrograde flow (orange arrow) buckle and break in the cell body, creating depolymerising microtubule minus ends and dynamic plus ends. (e) Microtubule plus-end-binding proteins such as APC or CLASPs might stabilize growing microtubule ends in the leading edge. In this and all subsequent figures, the open arrow indicates the direction of cell migration. Thick black lines represent microtubules. Green and red arrows indicate growing or shrinking microtubules, respectively, and plus and minus signs indicate microtubule polarity.

source of chemoattractant (Ueda and Ogihara, 1994). Thus, microtubules appear to control the polarity of a migrating cell.

There are three major hypotheses for how microtubules contribute to cell polarity and migration. First, microtubules could serve as tracks for directed membrane and organelle transport towards the leading edge of the cell to provide building material for the protruding lamellipodium (Nabi, 1999). It was originally speculated that the primary reason for reorientation of the centrosome towards the direction of migration is in fact the requirement to orient the secretory apparatus. Indeed, secretion preferentially polarized towards the leading edge has been observed in migrating fibroblasts (Bergmann et al., 1983; Hopkins et al., 1994). The requirement for microtubule-based transport during cell locomotion was also demonstrated by microinjection of kinesin-specific antibodies, which inhibited cell motility in a way similar to microtubule depolymerisation (Rodionov et al., 1993). However, even low concentrations of nocodazole that inhibit microtubule assembly dynamics, but do not affect the overall organization of microtubules and, thus, should not inhibit microtubule-dependent transport, significantly reduce the speed of protrusion of fibroblasts into a wound (Liao et al., 1995). Curiously, the same antibody against kinesin that inhibits cell motility also suppresses microtubule dynamic instability (C.M.W.-S., unpublished). Thus, transport of membrane compartments is unlikely to be the only role of microtubules in cell motility.

Second, growing microtubules could directly promote lamellipodial protrusion and thus be required for the selective stabilization of one particular leading edge to maintain a directed movement of the cell. The first support for this hypothesis was provided by the observation that, in fibroblasts, microtubules often grow into ruffling lamellipodia (Rinnerthaler et al., 1988) (Fig. 1). Furthermore, when microtubules are depolymerised in fibroblasts, membrane ruffling and protrusive activity that are normally confined to the leading edge are reduced, and residual ruffling is delocalised around the perimeter of the cell (Bershadsky et al., 1991; Waterman-Storer et al., 1999; Vasiliev et al., 1970). Conversely, microtubule regrowth after removal of the microtubule-depolymerising drug nocodazole induces the formation of ruffling lamellipodia (Waterman-Storer et al., 1999). Thus, growing microtubules might convey a signal that stimulates the protrusive activity of the cell.

This idea is countered by the third hypothesis: that microtubules do not regulate protrusion but instead locally regulate adhesion and contraction. In addition to the inhibition of lamellipodial protrusion, microtubule depolymerisation also causes increased contractility by the formation of focal adhesions and actin stress fibers (Bershadsky et al., 1996; Danowski, 1989). By imaging microtubules and focal adhesions simultaneously in living cells, Kaverina and colleagues have revealed that focal adhesions are targeted by microtubule plus ends undergoing dynamic instability and that repeated targeting leads to focal adhesion disassembly (Kaverina et al., 1998; Kaverina et al., 1999). In addition, when experimentally induced asymmetries in contractility are generated in cells lacking microtubules, the trailing cell body often remains stuck to the substratum (Ballestrem et al., 2000; Kaverina et al., 2000). Thus, microtubule targeting could release adhesions in the trailing part of the cell to allow detachment from the substratum. The molecular mechanism underlying this microtubule-dependent disassembly of focal adhesions is unknown but appears to involve kinesin, but not dynein (O. Krylyshkina, I. Kaverina and J. V. Small, personal communication) – which, remarkably, suggests that focal adhesion disassembly requires the transport of some factor towards the adhesion site (Fig. 3a).

The evidence for each of these hypotheses presents a conflicting picture – do microtubules promote protrusion or regulate adhesion – which is further confounded by the fact that some cells, such as keratocytes, neutrophils and lymphocytes, do not require microtubules for migration at all (Schliwa and Höner, 1993). It has been speculated that the microtubule dependence of cell motility correlates with cell size (Middleton et al., 1989; Schliwa and Höner, 1993) – that is, small cells can self-perpetuate asymmetries in contractility and adhesion to drive locomotion, whereas larger and more complex cells require an internal regulator to maintain their polarization. However, despite the almost identical size of amoebae and human neutrophils, some amoebae require

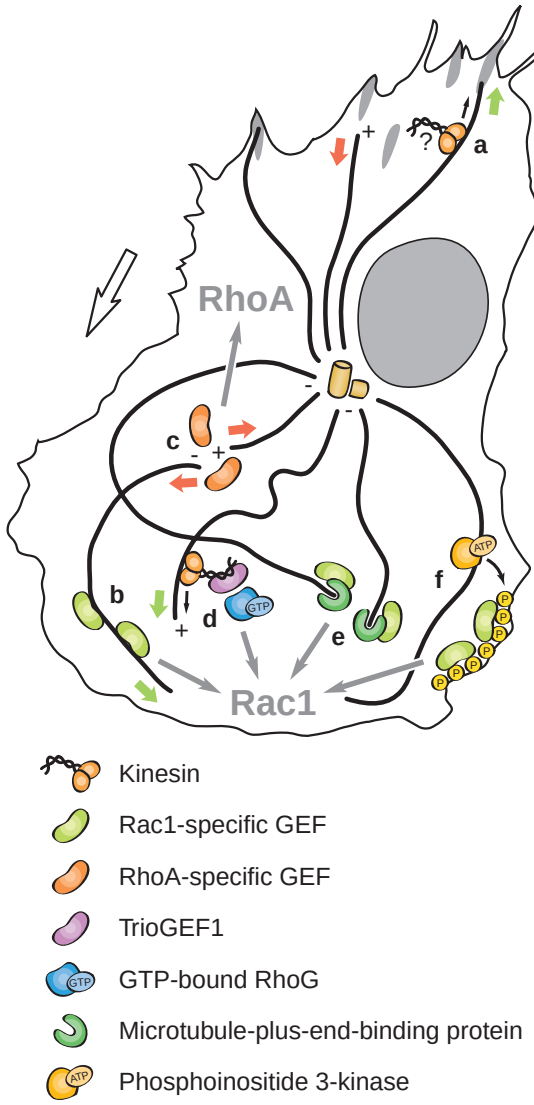

Fig. 3. Potential mechanisms for how asymmetries of the microtubule cytoskeleton might establish cell polarization. (a) Targeting of focal adhesions in the rear of the cell and kinesin-dependent transport of a focal-adhesion-dissociation factor to the adhesion sites might induce the retraction of the cell tail. (b-f) Microtubules might modulate the activity of Rho GTPases by a number of hypothetical mechanisms: (b) the activity of GEFs could be regulated simply by their association with the microtubule cytoskeleton; (c) in the case of RhoA, GEFs such as p190RhoGEF could be activated by their release from depolymerising microtubules in the cell body; (d) the local activation of a RhoG-specific GEF, TrioGEF1, and thus RhoG, a Rho protein upstream of Rac1 and Cdc42Hs, appears to be dependent on some sort of kinesin-mediated transport process; (e) association with the microtubule-plus-end-binding protein APC that is enriched in the lamellipodium could locally activate Rac1-specific GEFs such as Asef; (f) finally, microtubule-dependent regulation of phosphoinositide 3-kinase could activate Rac1 through PtdIns(3,4,5)P_3-binding GEFs such as Vav.

microtubules for chemotaxis (Ueda and Ogihara, 1994) whereas human neutrophils apparently can do without them (Zigmond, 1977). Whether cells of the same type but different size have different requirements for microtubules during polarization and migration would be interesting to know.

Although microtubules might somehow promote lamellipodial protrusion, they are clearly not required for its basic mechanics, since both growth factor-stimulated fibroblasts and melanoma cells stimulated with phorbol 12-myristate-13-acetate (PMA) still produce lamellipodia after depolymerisation of microtubules (Gauthier-Rouvière et al., 1998; Ballestrem et al., 2000). Equally, in fibroblasts expressing a constitutively active mutant of Rac1, microtubule depolymerisation has no effect on lamellipodia formation (T.W. and C.M.W.-S., unpublished). Indeed, it has been proposed that protrusion of the leading edge could be merely a response to the contraction of the rear edge resulting in a recycling of actin to the front (Kaverina et al., 2000). However, there is no evidence that contraction of actin fibers in the rear of the cell increases the free monomer pool, and lamellipodial protrusion is dependent on actin polymerization as opposed to the movement of pre-existing actin filaments (Machesky and Hall, 1997; Turnacioglu et al., 1998). In addition, such a model cannot account for protrusion at the edge of a monolayer, since these cells do not have a retracting tail.

Microtubules as regulators of Rho protein activity

As described above, the depolymerisation of microtubules induces formation of contractile actin bundles and focal adhesions, whereas the induction of microtubule polymerisation leads to lamellipodia formation. The mechanism by which microtubules could cause such responses is unclear; however, one possibility is that they somehow regulate the activity of Rho GTPases.

Direct evidence for microtubule-dependent regulation of Rho GTPases has recently come from a biochemical assay that specifically detects GTP-bound, activated RhoA or Rac1. This demonstrated that the assembly state of microtubules can affect Rho protein activation. Depolymerisation of microtubules in fibroblasts resulted in an increase in the level of GTP-bound RhoA whereas polymerisation of microtubules after nocodazole washout resulted in activation of Rac1 (Ren et al., 1999; Waterman-Storer et al., 1999). Opposite effects of microtubule-destabilizing and -stabilizing drugs on contractility have also been observed in *Xenopus* oocytes (Canman and Bement, 1997; Mandato et al., 2000) and are accompanied by changes in the distribution pattern of Rac1 and Cdc42 activity (W. M. Bement, personal communication).

What then could be the molecular mechanism for a microtubule-dependent regulation of Rho proteins? The simplest explanation would be a direct interaction of RhoA and/or Rac1 with tubulin or microtubules. Although binding of Rac1 to renatured tubulin in blot-overlay assays has been described (Best et al., 1996), neither GFP-tagged Rac1 nor RhoA colocalize with microtubules in cells, and Rac1 does not bind to assembled microtubules in co-sedimentation assays or native tubulin dimers in affinity precipitation experiments (Michaelson et al., 2001) (T.W. and C.M.W.-S., unpublished).

The main mechanism cells use to regulate Rho protein activity appears to be the regulation of their corresponding guanine-nucleotide-exchange factors (GEFs). Interestingly, several GEFs have been proposed to interact with the microtubule cytoskeleton. A RhoA-specific exchange factor, p190RhoGEF, partly colocalizes with microtubules in tissue culture cells and binds to microtubules in vitro through its C-

terminal domain (van Horck et al., 2001). A similar colocalization with microtubules has been described for GEF-H1 and its mouse homologue, Lfc (Glaven et al., 1999; Ren et al., 1998). In these cases, the GEF appears to bind to the microtubule cytoskeleton throughout the cell, and there is no evidence for local accumulation (Fig. 3b). Microtubule binding has not yet been shown to affect the GEF activities of these proteins (van Horck et al., 2001). Thus, how binding of GEFs to all cellular microtubules could result in a local microtubule-polymerisation-mediated activation of Rac1 remains unclear. However, it has been hypothesized that microtubule depolymerisation could result in the release of a microtubule-bound RhoA activator from the microtubule lattice (Enomoto, 1996) (Fig. 3c). Whether p190RhoGEF can fulfil this role remains to be tested. Interestingly, p190RhoGEF might also interact with the microtubule-dependent motor kinesin through JIP scaffolding proteins, which have recently been identified as kinesin cargo (Meyer et al., 1999; Verhey et al., 2001). Thus, the intracellular distribution of Rho-GEFs could also be determined by microtubule-dependent motor proteins.

A more functional relationship between microtubules and a GEF has been demonstrated for TrioGEF1, an exchange factor specific for RhoG (Bateman and Van Vactor, 2001). RhoG is a Rho family protein that can activate both Rac1 and Cdc42Hs, and overexpression of TrioGEF1 or a constitutively active version of RhoG results in the formation of lamellipodia and filopodia. Interestingly, this activity of Trio GEF1 or RhoG is dependent on an intact microtubule cytoskeleton (Blangy et al., 2000; Gauthier-Rouvière et al., 1998). Neither TrioGEF1 nor RhoG is directly associated with microtubules. However, both proteins lose their localization to the cell periphery upon microtubule depolymerisation, which suggests a microtubule-dependent transport process (Fig. 3d). This could be mediated by a kinesin, since RhoG has been shown to bind to the kinesin-binding protein kinectin (Gauthier-Rouvière et al., 1998). In addition, both kinesin and kinectin appear to be required for RhoG-induced modifications of the actin cytoskeleton (Vignal et al., 2001).

Another prime candidate for microtubule-dependent regulation of Rac1 is Asef, a recently described Rac1-specific GEF (Kawasaki et al., 2000). The GEF activity of Asef is switched on by its binding to APC. This is very striking, since APC, as described above, moves along microtubules and collects at their growing plus ends in the protruding edges of the cell (Mimori-Kiyosue et al., 2000; Näthke et al., 1996). This could put Asef in a prime location for promoting local Rac1 activity and could explain how growing microtubules promote protrusion (Fig. 3e). However, whether the interaction between the Asef-APC complex and microtubules influences the GEF activity of Asef and whether it is required for microtubule-mediated Rac1 activation have not been tested.

An alternative mechanism for how microtubules might locally regulate Rho protein activity is by affecting the localization of the membrane lipid phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P_3). Much recent evidence suggests that PtdIns(3,4,5)P_3 is a key molecule specifying polarity in cells migrating in a chemotactic gradient (Firtel and Chung, 2000; Rickert et al., 2000). PtdIns(3,4,5)P_3-specific antibodies and EGFP-tagged pleckstrin homology (PH) domains that specifically bind PtdIns(3,4,5)P_3 accumulate at the leading edges of *Dictyostelium* amoebae, neutrophils and fibroblasts (Haugh et al., 2000; Meili et al., 1999; Servant et al., 2000). Although all Rho GEFs identified so far contain a PH domain, binding to PtdIns(3,4,5)P_3 and subsequent activation have only been demonstrated for the Rac1-specific GEF Vav, which could result in an asymmetric distribution of Rac1 activity (Han et al., 1998). Interestingly, there is some evidence that regulatory subunits of phosphoinositide 3-kinase (PI3K), the enzyme that generates PtdIns(3,4,5)P_3, interact with tubulin or microtubules (Inukai et al., 2000; Kapeller et al., 1993; Kapeller et al., 1995). Therefore, microtubules might regulate the activity and/or localization of PI3K upstream of Rac1 activation (Fig. 3f). Alternatively, PtdIns(3,4,5)P_3- and microtubule-mediated mechanisms of Rho-protein activation might represent entirely different and possibly redundant pathways in migrating cells.

Feeding back on microtubules: regulation of microtubules by Rho GTPases

In addition to microtubules modulating Rho protein activity, the reverse might also be true. Microtubules might become rearranged simply as a response to RhoA-mediated cell shape changes (Ishizaki et al., 2001). However, more direct effects of Rho GTPases on microtubules have been suggested. For example, in fibroblasts, the formation of stable, non-dynamic detyrosinated microtubules is induced by activation of RhoA and is mediated by its downstream effector mDia (Cook et al., 1998; Palazzo et al., 2001) (Fig. 4a). In contrast, in neuroblastoma cells, RhoA activation results in increased phosphorylation of the microtubule-associated protein tau, which should promote its dissociation from microtubules and result in their destabilization (Sayas et al., 1999) (Fig. 4b). Whether RhoA has similar effects on non-neuronal microtubule-associated proteins and whether this affects microtubule shortening in the cell body are unclear.

Recent data suggest that Rac1 and Cdc42Hs not only regulate the actin cytoskeleton but also influence microtubule dynamics. Growth-factor-induced activation of Rac1 and Cdc42Hs leads to Pak1-mediated phosphorylation of stathmin/Op18 (Daub et al., 2001). Phosphorylation, and thus inactivation of stathmin/Op18, is predicted to promote microtubule growth by decreasing the catastrophe frequency (Larsson et al., 1997; Andersen, 2000). This fits well with the idea that Rac1 is active in the leading edge of a migrating cell, where microtubule plus ends exhibit net growth (Fig. 4c). Furthermore, microtubule assembly also induces stathmin/Op18 phosphorylation in *Xenopus* egg extracts (Küntziger et al., 2001). Thus, one can envisage a positive feedback loop, in which microtubule growth activates Rac1 at the leading edge, and Rac1 in turn inactivates stathmin/Op18, thus propagating the growth of microtubules and at the same time promoting actin polymerisation and leading edge protrusion. Whether such a mechanism operates in vivo remains to be tested.

Finally, mounting evidence indicates that Cdc42Hs activity might also have an effect on microtubule organization that parallels its role in regulation of polarity in yeast cells. This was first demonstrated in T cells, in which Cdc42Hs is required for the reorientation of the centrosome towards antigen-presenting cells (Stowers et al., 1995). More recently, using a wound-edge model system, two groups have now shown independently that the orientation of the centrosome towards

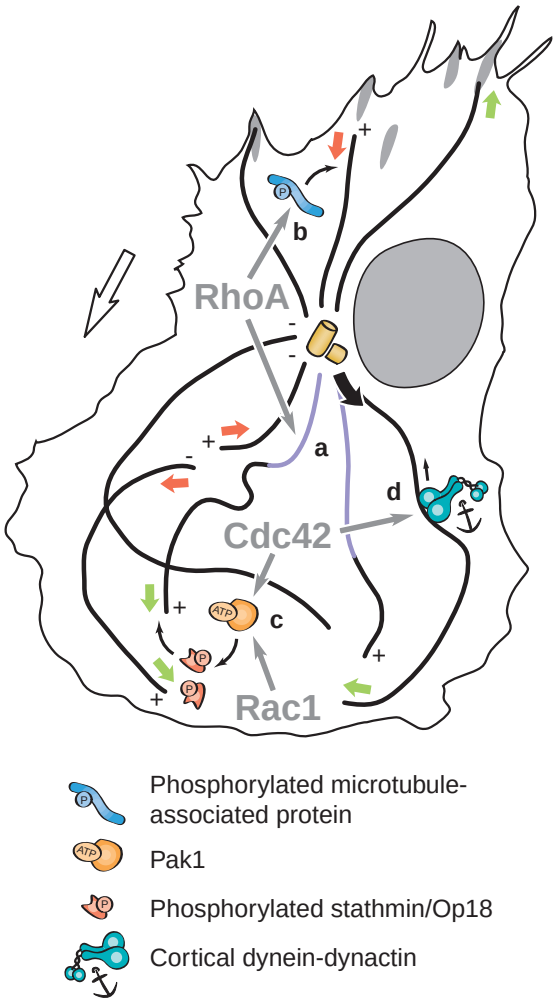

Fig. 4. Potential mechanisms for how Rho proteins could regulate aspects of the microtubule cytoskeleton. (a) Stable, detyrosinated microtubules (purple) are induced by RhoA. (b) RhoA might also cause the phosphorylation of microtubule-associated proteins and thus destabilize microtubules. (c) Rac1 and Cdc42Hs might decrease the microtubule catastrophe frequency and thus promote microtubule growth through Pak1-dependent phosphorylation of stathmin/Op18. (d) Cdc42Hs activity is required for the reorientation of the centrosome towards the direction of migration, which could occur through cortical cytoplasmic dynein activity.

the leading edge depends on Cdc42Hs function (Etienne-Manneville and Hall, 2001) (G. G. Gundersen, personal communication). Indeed, this is independent of changes in cell shape, because protrusion of the leading edge can be blocked by dominant negative Rac1 but the centrosome still reorients in a Cdc42Hs-dependent manner. The mechanism of centrosome reorientation has not been elucidated, but since microtubules are required (Gotlieb et al., 1983) one could imagine a microtubule-motor-driven mechanism analogous to the dynein-dynactin-dependent movements of the spindle poles during mitosis (Wittmann et al., 2001) (Fig. 4d) and, indeed, an involvement of the dynein-dynactin complex in Cdc42Hs-induced centrosome reorientation has been demonstrated (Etienne-Manneville and Hall, 2001) (G. G. Gundersen, personal communication). Since RhoA activates myosin through a Rho-kinase dependent phosphorylation of myosin light chain (Totsukawa et al., 2000; Katoh et al., 2001), this raises the intriguing possibility that Rho GTPases regulates both microtubule- as well as actin-based motor proteins.

Conclusion

Taken together, the observations discussed above paint a picture in which Rho GTPases not only control the actin cytoskeleton but also determine the polarity of the microtubule cytoskeleton. As in yeast, Cdc42Hs may turn out to be the master regulator of cell polarity in more complex eukaryotes. Once an initial asymmetry of the microtubule network is established, the feedback of microtubule effects on Rho protein activity could then promote the generation of asymmetries in actin contractility and substrate adhesion, which would ultimately result in polarization and directional movement of the cell.

We would like to thank N. Prigozhina for critical comments on the manuscript, and W. M. Bement, P. Fort, C. Gauthier-Rouvière, G. G. Gundersen, A. Hall, I. Kaverina and J. V. Small for sharing unpublished data. This work was supported by NIH grant GM61804-01 to C.M.W.-S. and an EMBO long term fellowship to T.W.

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