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Rho GTPases at the crossroad of signaling networks in mammals

Impact of Rho-GTPases on microtubule organization and dynamics

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Abbreviations: MT, Microtubule; GEF, guanine nucleotide exchange factor; GAP, GTPase activating protein; GDI, Guanine Dissociation Inhibitor; PTM, Post-Translational modifications; MEF: Mouse embryonic fibroblasts; LPA, Lysophosphatidic acid; GPCR, G protein–coupled receptors; LARG, Leukemia-associated RhoGEF; DRF, Diaphanous-related formin; GBD, GTP-binding domain; DAD, Diaphanous autoregulatory domain; FH, Formin homology; FAK, Focal adhesion kinase; EB1, End-binding protein 1; APC, Adenomatous polyposis coli; BM, Basement membrane; KS, Kaposi's sarcoma; SDF, stromal cell–derived factor; MTOC, Microtubule organizing center; GA, Golgi apparatus; aPKC, Atypical protein kinase C; GSK-3β, glycogen synthase kinase-3beta; PAK, p21-activated kinase; MRCK, Myotonic related Cdc42-binding kinase; ERM, Ezrin, radixin, moesin; EGF, Epidermal growth factor; CLASPs, CLIP-associating proteins; TIRFM, Total internal reflection fluorescence microscopy; MAP, Microtubule-associated protein; PH, Pleckstrin homology

Microtubule (MT) organization and dynamics downstream of external cues is crucial for maintaining cellular architecture and the generation of cell asymmetries. In interphase cells RhoA, Rac, and Cdc42, conspicuous members of the family of small Rho GTPases, have major roles in modulating MT stability, and hence polarized cell behaviors. However, MTs are not mere targets of Rho GTPases, but also serve as signaling platforms coupling MT dynamics to Rho GTPase activation in a variety of cellular conditions. In this article, we review some of the key studies describing the reciprocal relationship between small Rho-GTPases and MTs during migration and polarization.

Introduction

It is now well established that members of the Ras superfamily of small GTPases along with their upstream regulators and downstream effectors function as fundamental signaling stations controlling a wide array of morphogenetic events.[1,2] The initial functional characterization of the prototypical members of this superfamily, namely RhoA, Rac, and Cdc42 established that the actin cytoskeleton was one of its main targets.[1] Later studies extended these observations revealing that small Rho GTPases in concert with guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs), guanine dissociation inhibitors (GDIs), and factors that control their lifespan (i.e., ubiquitin E3-ligase), serve as "spatiotemporal signaling modules[3] that also regulate MT organization, dynamics, plus-end capture and the cross talk with the actin cytoskeleton. Equally, MTs have emerged as key regulators of Rho GTPase functioning.[4-6]

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Microtubule organization and dynamics in short

MTs are non-covalent cytoskeletal polymers involved in virtually every aspect of cell biology, including mitosis, cell motility, adhesion, intracellular transport and polarity. The main component of MTs is the tubulin polymer, composed of α- and β- heterodimer subunits assembled into 13 linear protofilaments that form a 20 nm hollow tube; in addition, a wide variety of associated proteins decorate and/or interact with the MT lattice or tips.[6] MTs typically undergo cycles of rapid growth and disassembly, a phenomenon known as dynamic instability, which has been observed in vitro and in vivo.[7,8] This property allows MTs to probe the intracellular environment for interacting partners (e.g., search, capture, and stabilization at the cell cortex), to rapidly reorganize in response to environmental cues, and to spatially and temporally differentiate to generate cell polarization.[5,6] While most MTs are very dynamic, with a half-life ranging between 5–10 min (dynamic MTs), a minor population exhibits a more stable behavior, lasting for up to 20 h (stable MTs).[7] In non-polarized cells these two types of MTs arrays coexist intertwined in the same cytoplasm in which stable ones

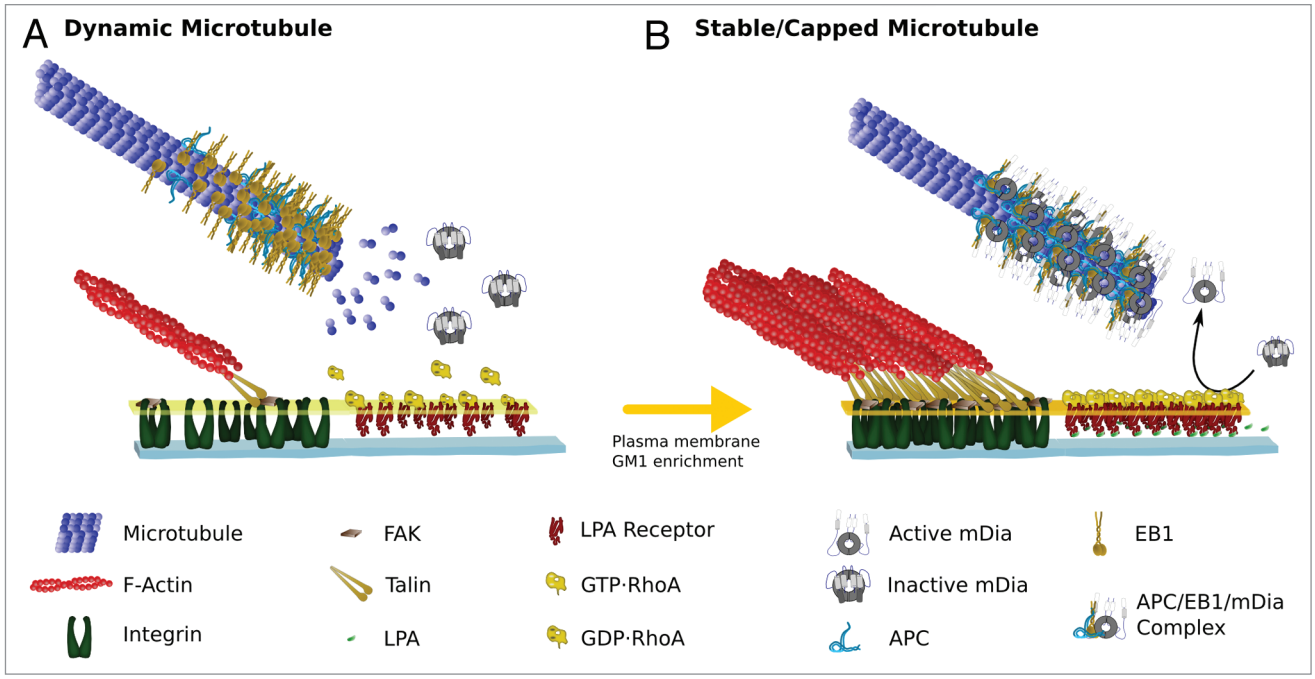

Figure 1. Drawing representing the MT-RhoA-mDia signaling pathway at the leading edge of migrating fibroblasts. Molecules are drawn schematically, roughly preserving relative molecular sizes and when possible, known crystallographic and/or functional spatial conformations. (**A**) Dynamic MTs: In the absence of LPA, most MTs in wound-facing fibroblasts are highly dynamic showing high rate of α/β heterodimer exchange on + tips. The MTs contain + tips proteins, like EB1 and APC having a comet-like appearance, being highly enriched at the most distal part of the MTs and tapering down within few micrometers. In the absence of LPA signaling, RhoA is inactive (GDP-RhoA), mDia (also inactive) doesn't bind MTs; there are no focal adhesions and hence FAK remains inactivated. (**B**) Stable and/or Capped Microtubule: Upon LPA stimulation, focal adhesions start assembling in the leading edge of wound-facing fibroblasts. Typically, focal adhesion formation involves integrin receptor activation and accumulation leading to further enrichment of several other proteins involved in the regulation of the actin network, providing mechanical and signaling means for F-actin binding. Of particular importance during FA formation is the activation of FAK, which, among other effects, promotes the accumulation of GM1 on the leading edge plasma membrane (big dark-yellow arrow). GM1-enriched membranes provide sites for the insertion of RhoA, and activation by LPA receptor signaling. GTP-RhoA activation releases mDia auto-inhibitory conformation leading to its binding to MT + tips; mDia also binds EB1 and APC forming a trimeric protein complex which functions as a MT plus cap. This cap prevents heterodimer exchange stabilizing MT at the leading edge.

(long-lived polymer) are formed and maintained next to the very dynamic population (short-lived polymer). In many cell types, including neurons, cell motility, and/or polarization have been mechanistically linked with the selective stabilization (temporal differentiation) and spatial segregation (spatial differentiation) of a subset of MTs.[5,6,9]

Stable MTs accumulate post-translational modifications (PTM) of α-tubulin, such as detyrosination and acetylation.[10-12] Detyrosination removes the gene-encoded C-terminal tyrosine resulting in tubulin monomers ending with glutamine; the resulting detyrosinated tubulin, also termed Glu-tubulin, can be further converted into Δ2-tubulin by the irreversible removal of the penultimate glutamate.[12] Early studies showed that acetylation of stable MT (no acetylation has been demonstrated on dynamic MT) occurs on lysine 40 of α-tubulin, which faces the MT lumen;[13-15] the biological significance of this finding has remained intriguing since most MT interactions occur on the outer surface of the polymer. Recent studies have identified novel acetylation sites on tubulin some of which are located on the outer MT lattice.[16] Neither detyrosination nor acetylation are the cause of MT stability, but appear to function as moieties that protect MTs from depolymerizing agents, such as members of the kinesin 13 superfamily, or as tags and/or markers allowing binding of MT-based molecular motors, such as Kif5, or preventing the one of certain types of plus-end microtubule-associates proteins or +TIPS.[12,17]

From Rho-GTPases to microtubules

Microtubule stabilization by Rho A-mDia in migrating fibroblasts

Perhaps one of the more striking examples of the role of Rho-GTPases in controlling MT organization and dynamics is cell migration.[18] Using wounded monolayers of serum-starved NIH-3T3 fibroblasts, Gregg Gundersen and colleagues[18-21] identified, several years ago, a RhoA signaling pathway that induces the formation of a stable Glu-MT subset involved in cell migration. These and related studies established that in different types of migrating cells the radially oriented array of MTs reorganizes and polarizes in response to an external cue by the spatio-temporal transformation of a group of dynamic MTs into a stable one that contains Glu- or acetylated α-tubulin. It also became clear that migration requires the formation of this stabilized subset of MTs.[20,21] These observations fit very well with the selective stabilization model proposed by Kirschner and Mitchison in the late 80s to explain the generation of cell

asymmetry; in this proposal external signals would locally activate cortical factors to stabilize dynamic MTs.[9] One such cue is lysophosphatidic acid (LPA), a cytokine known for its ability to stimulate RhoA activity and stress fiber formation.[22] Addition of LPA in wounded monolayers of starved fibroblasts results in a rapid (within 30–60 min) and robust activation of RhoA that precedes the appearance of the stable Glu-MT array that polarizes toward the leading edge;[23] inhibition of RhoA activity with both C3 botulinum toxin or dominant negative RhoA prevents the formation of stable Glu-MTs and cell migration.[23,24]

LPA receptors are coupled to heterotrimeric G proteins (Gi, Gq, Gα12/13 α-subunits) and elicit multiple cellular responses.[25-27] In scratch-wound directed cell migration of mouse embryonic fibroblasts (MEFs), the G protein–coupled receptors (GPCR) Gα12/13 are essential and sufficient for LPA-induced RhoA activation, Glu-MT formation, and cell progression.[28] Activated Gα13 tightly binds p115 RhoGEF and stimulates its capacity to catalyze nucleotide exchange on RhoA,[29] while activated Gα12 inhibits Gα13-induced stimulation. Thus, p115 RhoGEF can directly link heterotrimeric G protein α subunits to the regulation of RhoA activity upstream of MT stabilization[29]; on the other hand, MTs may have a role in targeting p115 RhoGEF to specific subcellular locations.[30] Leukemia-associated RhoGEF (LARG) has also been shown to promote Gα12/13 dependent RhoA activation in vivo.[31]

The question of how activated RhoA induces polarized MT stabilization and subsequent cell migration is complex and far from being solved. One straightforward possibility is the local activation of a MT stabilization pathway within the cell's leading edge, where RhoA activity has been detected.[32,33] Several lines of evidence suggest that LPA induces polarized stable MT formation in wound edge migrating fibroblasts through the mammalian homolog of Drosophila diaphanous (mDia), a formin that is a RhoA effector; in this model MTs that neither growth nor shrink, have a half-life of more than 1 h and contain Glu-tubulin are the result of capture and plus end stabilization or capping (**Fig. 1**).[4]

Formins are a family of multidomain proteins first described as potent actin regulators capable of modulating a wide variety of cellular processes such as cytokinesis, cell polarization and morphogenesis.[34,35] Diaphanous-related formins (DRF) are a subfamily bearing a GTP-binding domain (GBD) near the N-terminal region and a Diaphanous auto regulatory domain (DAD) near the C-terminal side of a formin homology domain (FH2) that facilitates intramolecular binding. The binding of Rho-GTP to GBD is thought to activate DRF by relieving an auto inhibitory interaction between an adjacent N-terminal inhibitory domain formed by armadillo repeats and DAD on the C-terminal region; RhoA uses its "switches" regions[1,2] for interacting with the two formin domains.[36] In addition to their known role in regulating actin-dependent processes DRF are also involved in regulating the MT cytoskeleton. In wound-edge migrating fibroblasts constitutively active mDia or activation of endogenous mDia with the mDia auto-inhibitory domain stimulates the formation of stable MTs.[37] Besides, mDia co-localizes with stable MTs when overexpressed and associates with MTs in vitro. These observations establish that mDia is sufficient to generate and orient stable MTs, and that DRF could be part of a conserved pathway that regulates the dynamics of MTs + ends.[37] A more recent study has provided evidence suggesting that actin capping protein induces release of mDia from actin filaments near the leading edge allowing binding to MTs and subsequent stabilization.[38]

Focal adhesions (FA) may serve as signaling platforms directing the RhoA-mDia pathway to stabilize MTs. It has been proposed that integrins and focal adhesion kinase (FAK) act as "adhesion checkpoints," coordinating both spatially and temporally the formation of stable Glu-MTs. FAK activation localizes ganglioside GM1 to specialized domains in the cell surface, perhaps in the form of lipid rafts, harboring regulatory proteins and enabling RhoA to stimulate mDia (**Fig. 1**).[39]

End-binding protein 1 (EB1) and adenomatous polyposis coli (APC), which target MT ends, directly bind to each other and to mDia. The three proteins are enriched on cortical MT + ends as detected by TIRF microscopy, where presumably active RhoA would accumulate.[20] Together these data suggest that upon LPA-GPCR activation near the cell cortex, an event facilitated by integrin/FAK signaling, RhoA activates mDia, which further binds EB1 and APC forming a cortical complex that captures and stabilizes dynamic MTs. The capture event would give enough time for tubulin modifying enzymes to detyrosinate and acetylate individual MTs, leading to further specialization (**Fig. 1**).

MT stabilization by RhoA/mDia in other biological systems

Evidence from different systems suggests that the signaling pathway involving RhoA, mDia, and + TIPS leading to MT stabilization is conserved across diverse cell types and cellular contexts. For example, in budding yeast the axis of cell division is determined by signaling molecules that control bud site selection; it has been proposed that this spot is established by interactions between cytoplasmic MTs and asymmetrically localized cortical proteins during cell cycle progression.[40] MTs captured at bud sites exhibit controlled shrinkage but do not persist for hours like those in mammalian cells; this pathway is regulated by Rho GTPases, such as RhoA and Cdc42 and the formin Bni1, the yeast ortholog of mDia. Budding yeast Kar9 serves as a functional homolog of APC and Bim1 as the one of EB1. The two yeast proteins have been directly implicated as direct Bni1 effectors in capturing and controlling MT dynamics in the nascent yeast bud.[40-42]

RhoA-dependent MT stabilization has also been observed in the epithelial to mesenchymal transition during gastrulation.[43] Basal membrane (BM) breakdown is the first recognizable step of this process and is controlled by loss of basally localized RhoA activity leading to loss of stable MTs. Reduction of RhoA activity in normal epithelium leads to BM breakdown while failure of RhoA downregulation during epithelial-mesenchymal transition to BM retention, a phenomenon partly due to RhoA-regulated MT stabilization.[44]

Kaposi sarcoma (KS)-associated herpes virus, which is implicated in the pathogenesis of KS and other lymphoproliferative disorders, infects a variety of target cells both in vivo and in

vitro.[45] Early during infection, the virus induces the activation of the RhoA/mDia2 pathway promoting the stabilization and subsequent acetylation of MTs. Using stable MTs the virus promotes the trafficking of viral capsids toward the cell nucleus and the establishment of infection. Capsids proteins colocalize along MT tracks supporting the need of an intact MT array for capsid trafficking. The inactivation of Rho by Clostridium difficile toxin B significantly reduces MT acetylation and delivery of viral DNA to the nucleus while constitutive active RhoA increases viral infection.[45] In T cells, the reorientation of the MT organizing center (MTOC) to the immune synapse (IS) during antigen presentation also involves Diaphanous 1 (DIA1, the human ortholog of mDia) and Formin-like-1 (FMNL1); besides, inverted formin 2 (INF2) mediates formation of an array of stable Glu-MTs that is necessary for MTOC reorientation to the IS.[46,47]

Finally, in developing cerebellar granule cells the RhoA/mDia pathway promotes axonal elongation in response to stromal cell–derived factor (SDF)-1α. The authors suggested that mDia-promoted axonal elongation could be related to the regulation of actin dynamics.[48] It will be of interest to test whether or not mDia also regulates axonal MT stability, a key event for initiating and maintaining neuronal polarity.[2,6]

Cdc42 and microtubule reorganization during cell migration

Several lines of evidence favor the view that Cdc42 also plays a major role in regulating MT organization and dynamics during cell migration. For example, it was initially established that Cdc42 regulates changes in centrosome orientation in T cells.[49] Then, using monolayers of primary astrocytes or fibroblasts in a scratch-induced migration assay, it was demonstrated that Cd42 is required for both polarized membrane protrusion at the leading edge and microtubule organizing center-Golgi apparatus (MTOC-GA) orientation toward the direction of migration (**Fig. 2**).[50,51]

In migrating astrocytes this phenomenon involves Par6, a scaffold protein that is a direct target of Cdc42 and PKCζ.[52] It was shown that both proteins directly interact regulating glycogen synthase kinase-3β (GSK-3β) to promote MTOC reorientation and control the site of protrusive activity.[53] In migrating fibroblasts Cdc42-GSK-3β pathway only regulates MTOC positioning; protrusive activity is controlled by the Cdc42-dependent activation of P21-activated protein kinase (PAK), which controls the localization of Rac activity through recruitment of β-Pix, a GEF for Cdc42 and Rac, to the leading edge.[54] Cdc42-dependent phosphorylation of GSK-3β occurs specifically at the leading edge of migrating cells, and induces the interaction of APC protein with MT + ends.[53] In fibroblasts, MTOC cell-centroid reorientation and/or maintenance requires dynein pulling on capped MT; the presence of APC, mDia, and EB1 at the end of MT contributes to their capping and stabilization.[20,55] The same signaling pathway has been implicated in axon formation, a phenomenon involving polarized protrusive activity, as well as remodeling of the growth cone actin and MT cytoskeleton.[56-60] Centrosome movement during astrocyte migration also requires dynein, Cdc42, and APC,[61,62] but does not involve MT plus end capture. Thus, it has recently been shown that a Cdc42 signaling pathway engaging the polarity protein disk large 1 (Dlg1) and the scaffolding protein GKAP, recruits dynein to leading edge MT.[61] In astrocytes, MTs bend in close proximity to the plasma membrane (approximately 3 μm) in a dynein-dependent manner; thus, dynein could provide a cortical anchor allowing minus end MT-based motor activity to pull the centrosome in the direction of migration.[62]

Live imaging of fibroblasts has also revealed that at the onset of migration the nucleus moves rearward while the MTOC remains stationary.[63] Rearward nuclear movement is coupled to retrograde actin-myosin flow and is regulated by Cdc42 and its effector, Myotonic related Cdc42-binding kinase (MRCK).[63] Dynein is not involved in nuclear movement, but is essential to maintain the MTOC at the cell centroid. Together, these results reveal that there are at least two Cdc42 pathways involved in cell migration that regulate either nuclear movement or MTOC positioning and/or reorientation (**Fig. 2**).

Neutrophil chemotaxis is a form of directed migration that involves persistent polarization with a leading pseudopod, responsible for directional locomotion and the uropod, on the opposite site, mostly involved in cell-to-cell interaction and in a variety of leukocyte activities including activation, apoptosis, and immune interactions.[64] Several lines of evidence suggest that uropod formation involves microfilaments, ezrin-radixin-moesin (ERM) proteins, and integrin signaling, as well as rearrangement of the MTOC and activation of Cdc42. A recent study has also provided evidence suggesting that Cdc42 controls neutrophil polarity (pseudopod and/or uropod bipolar organization) by regulating WASP at the uropod.[65] WASP regulates the relocalization of CD11b integrin into a detergent resistant membrane domain, which in turn recruits EB1 to capture and stabilize MTs at the uropod.[65]

Rac-1/PAK and the regulation of MT dynamics during cell migration

In randomly migrating PtK1 cells (a marsupial kidney epithelial cell line), Rac1 and the effector PAK1 regulate the dynamics of a subset of growing MTs.[66] In these cells a group of MTs, that resembles "pioneer" MTs,[67] grow well into leading edge protrusions, showing markedly less catastrophes events and spending more time in a growing phase than the rest of the MT array. The study by Wittmann et al. (ref. 66) revealed that nearly all MTs in constitutively active Rac1-expressing cells exhibit a kinetic behavior similar to pioneer MTs, while most MTs in dominant-negative Rac1expressing cells behave as central MTs, in which catastrophe frequency and time spent in pause are high.

In various cancer cells and in PtK1 cells stathmin/Op18, a MT-destabilizing factor, becomes phosphorylated in response to treatment with epidermal growth factor (EGF) in a Rac1-PAK1-dependent manner.[67,68] PAK1 can directly phosphorylate Op18 at serine 16[67] inhibiting its catastrophe promoting activity in vitro, while inhibition of PAKs downstream of constitutive active Rac increases MT-destabilizing activity of Op18 in PtK1 cells, indicating that Op18 activity, and hence MT dynamics, can be regulated by PAKs in vivo.[68] Interestingly, this pathway operates in other cell types. Assembly and stabilization of growth cone dynamic MTs are key events during axon formation,[5] where

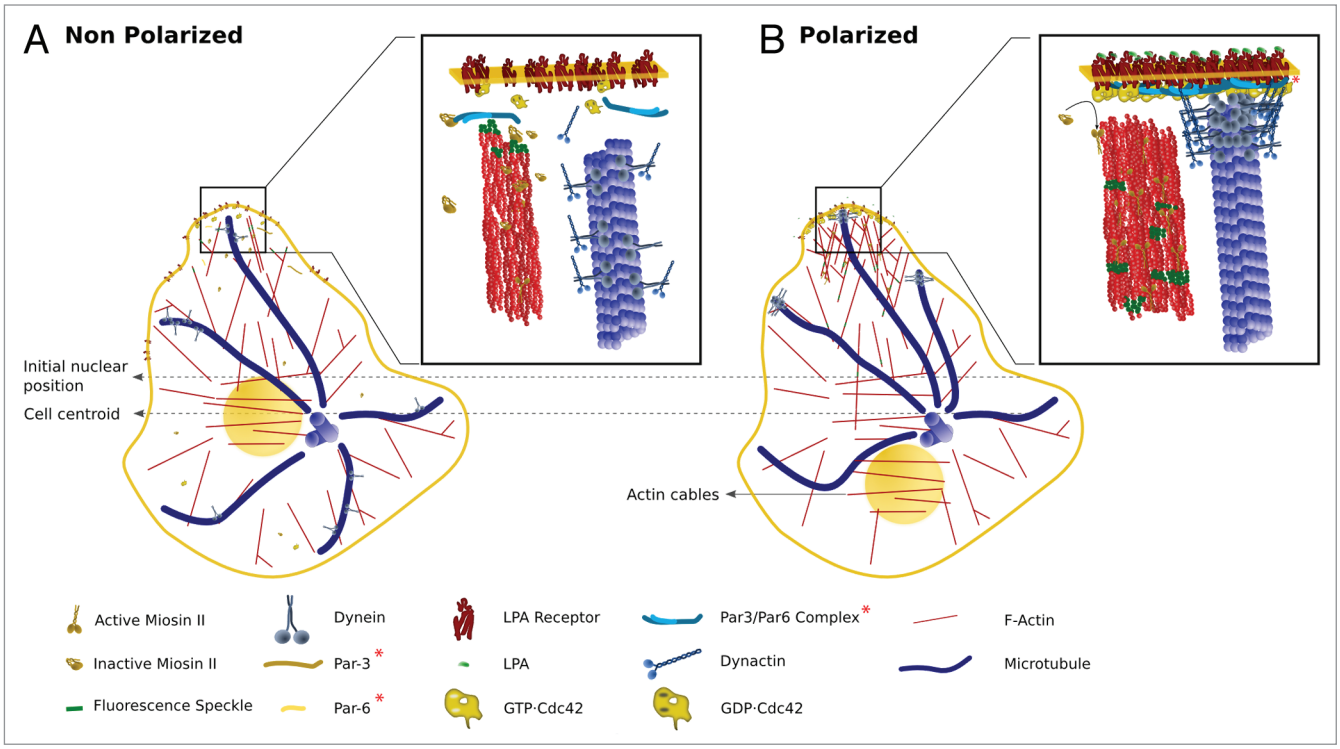

Figure 2. Drawings representing proteins involved in regulating nuclear movement and MTOC-GA orientation during cell migration. Molecules are drawn schematically, roughly preserving relative molecular sizes and, when possible, known crystallographic and/or functional spatial conformations. (**A**) Non-polarized MTOC-nuclear axis: In wound-facing fibroblasts and in the absence of LPA, MTs extending near the plasma membrane are dynamic and are neither stabilized nor anchored; these MTs also have dynein/dynactin complexes, most likely involved in carrying membranous organelles (organelles not shown). Under this condition RhoA is inactive and not bound to the plasma membrane. The signaling and scaffold proteins Par3 and Par6 are also inactive and membrane unbound. F-Actin, myosin-dependent rearward flow is very low with most myosin II in an inhibited (auto-inhibition) conformation. (**B**) Polarized MTOC-nuclear axis: Upon LPA stimulation, Cdc42 is activated (GTP·Cdc42) and accumulates at the leading edge. GTP·Cdc42 activates MRCK (not shown), which in turns releases the auto-inhibitory conformation of myosin II. Activated myosin II (phosphorylated) binds to the F-actin network promoting rearward actin movement, which is coupled with the rearward movement of transversal actin cables. These actin cables are coupled to the nucleus by transmembrane actin-associated nuclear (TAN) lines[98] (not shown) thereby moving the nucleus in the opposite direction of migration. GTP·Cdc42 also stimulates the assembly of the Par3/Par6/PKC (PKC not shown) polarity complex, which becomes activated and accumulates at the leading edge plasma membrane. The localization of Par3 is still a matter of debate.[71,99] One study reported that Par 3 is enriched at the leading edge of migrating keratinocytes,[71] while other that it associates with dynein at cell-cell contacts of migrating fibroblasts.[99] Regardless, it is likely that the polarity complex provides sites for binding to the dynein and/or dynactin complex. Upon binding to the cell cortex the dynein and/or dynactin generates an opposing force on MTs, which in turn oppose the rearward nuclear movement. Membrane-cortical factors, dynein and/or dynactin, and MTs generate a force that serves to maintain the MTOC at the cell centroid, while the nucleus is moved rearward by actin cables, polarizing the nucleus-MTOC-GA in the direction of migration.

Rac1 also plays a crucial role.[2] In primary cultured neurons DOCK7, a Rac GEF, selectively localizes to the tip of growing axons; DOCK7 and Rac activation lead to phosphorylation and inactivation of stathmin/Op18 in the nascent axon and this event is important for axon formation.[69]

It is likely that other factors downstream of Rac and/or PAK are involved in the regulation of either leading edge or growth cone MT dynamics, because PAK activity is necessary but not sufficient for Rac1-mediated promotion of MT growth.[66] It has been proposed that Rac1 and PAK-mediated inactivation of stathmin/Op18 initiates MT growth by locally lowering the catastrophe frequency, which could then be sustained with further MT stabilization by +TIPs, such as CLIP-170, EB1, or APC.[68]

Rac may also utilize different upstream regulators to control MT dynamics. For example, in wound-edge migrating primary mouse astrocytes, the Rac1 GEF Tiam1 is required for outgrowth of protrusions, wound closure and proper organization of the MT cytoskeleton.[70] Tiam1-deficient astrocytes contain reduced pools of Glu-tubulin, with stable MT restricted to an area close to the nucleus and failing to spread toward the leading edge, typical of migrating cells.[70] These results are in line with previous observations showing that Tiam1 can regulate migration and MT stability in single migrating keratinocytes[71] and that its downregulation results in reduced levels of acetylated tubulin.[72]

CLIP-associated proteins (CLASPs) are a group of +TIPs, which stabilize specific subsets of MT in response to signaling cues.[71] CLASPs relocalize to distal segments of MTs at the leading edge of motile fibroblasts, an event mediated by PI3-kinase and GSK-3β and regulated by Cdc42-Rac signaling.[71-74] Interestingly, MEFs lacking Tiam1 display alterations in migration and MT organization similar to those described in CLIP-associating

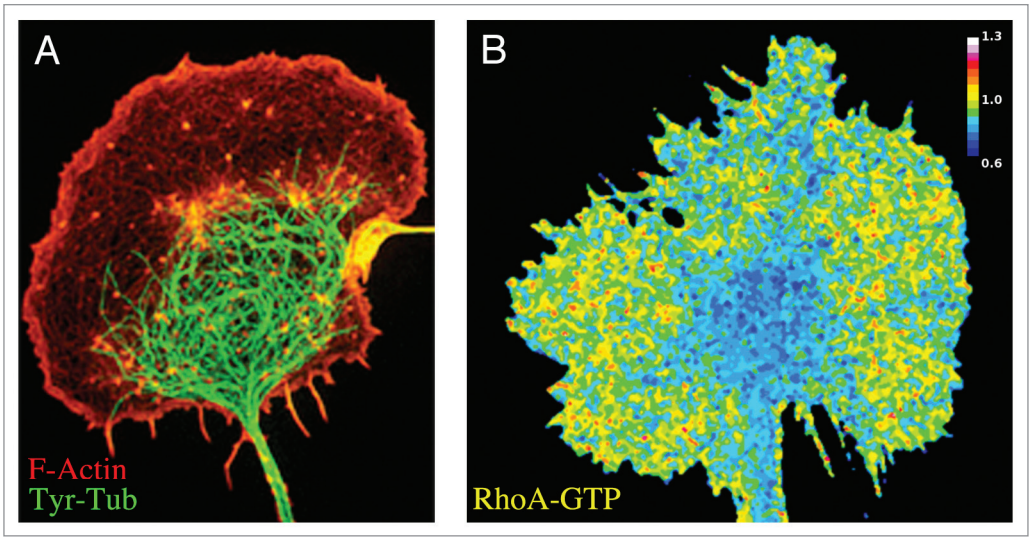

Figure 3. Distribution of MTs, F-actin, and RhoA-activity in growth cones of developing neurons. (**A**) Double immunofluorescence micrograph showing an axonal growth cone of a cultured dorsal root ganglion cell (DRG) stained with phalloidin (red; F-actin) and a monoclonal antibody against (mAb) against tyrosinated tubulin (green; Tyr-tubulin). Dynamic MTs containing Tyr-tubulin predominate in the central growth cone region (C domain); the peripheral lamellipodial veil contains very few MTs. (**B**) FRET map image showing Rho-A activity in a growth cone of a DRG neuron. FRET measurements were performed using a unimolecular RhoA biosensor[3,33] and a radiometric method. RhoA activity is low in the central MT-enriched growth cone domain and high in the actin-rich peripheral lamellipodial veil.

protein 2 (CLASP2)-deficient ones.[69] CLASP2, a member of this family, has been implicated in MT stabilization during migration presumably by capture and crosslinking of MTs to the cell cortex. In these cells, CLASP2 is required for the formation of a stable polarized MT array independent of CLIP-170 and APC-EB1 interactions.[75,76] In migrating PtK1 epithelial cells, although CLASPs associate with MT + ends in the cell body, they dynamically decorate the entire MT lattice in the leading lamella; interestingly, it was found that CLASP2 binding to the MT lattice is promoted by Rac.[77] Thus, by activating Rac1, Tiam1 could be an upstream regulator of CLASP2-mediated MT stabilization in migrating cells.

From microtubules to Rho GTPases

Pioneer work by C Waterman-Storer and T Salmon in the late 90s established that MT growth at leading-edge lamellipodia locally activates Rac1 to drive actin polymerization and lamellipodial protrusion required for cell migration.[78-80] It was also shown that MT depolymerization in fibroblasts resulted in an increase in the level of GTP-bound RhoA, a phenomenon paralleled by formation of contractile actin bundles and focal adhesions. These studies gave rise to the idea that MTs play a major role in regulating the crosstalk between Rho-GTPases and microfilaments to control polarized actin assembly dynamics during cell migration.[79,80] In addition, these observations implied that growing MTs might carry signals that promote Rac-mediated protrusive activity while shrinking ones factors that stimulate Rho activity and actin contractility. Subsequent studies identified several GEFs, such as GEF-H1, p190RhoGEF, Tiam1, and STEF (Tiam2) that link MTs with Rac and/or Rho and the actin cytoskeleton.[81-84]

The Lfc-Tctex-1 connection and RhoA

GEF-H1, or its mouse homolog Lfc, is negatively regulated by MT binding[81] and is crucial for coupling MT dynamics to Rho A activation in different biological situations, including cell migration[82,83] and neuronal polarization.[84-86] Lfc mutants unable to bind MTs have increased GEF activity and stress fiber formation, while Lfc downregulation in non-neuronal cells attenuates LPA-mediated actin reorganization, raising the possibility that it may be one critical GEF mediating MT-microfilament crosstalk.[81]

Lfc participates in several aspects of neuronal development, including neurogenesis,[85] axon outgrowth,[84] and spine formation.[86] In primary cultured neurons, confocal and TIRF microscopy revealed that Lfc associates with MTs in the growth cone central region (C domain) where Rho activity is low (**Fig. 3**); it is also present at the growth cone P domain,[84] where few MTs are present and RhoA activity is elevated (**Fig. 3**). It has been proposed that a Lfc-RhoA-Rho-Kinase (RhoK) signaling pathway exerts an inhibitory control on axon formation by targeting Tiam1 and disrupting a self-activating module that includes STEF (Tiam2), the Par polarity complex, Cdc42, and Rac that drives growth cone MT/actin remodeling required for axon formation; thus, local inactivation of Lfc-RhoA could be a signal required to trigger axon formation.[6,84]

Recent studies have identified candidate molecules responsible for anchoring Lfc to the MT array and for regulating its activity. Tctex-1 is a dynein light chain that serves as an adaptor protein linking rhodopsin-bearing vesicles to the dynein motor complex for transport;[87] interestingly, Tctex-1 has dynein-independent functions, including promotion of Rac-mediated actin dynamics required for axon outgrowth.[88] Two recent studies[84,89] have demonstrated that Tctex directly interacts with Lfc, both in vitro and in vivo, inhibiting its GEF activity.

The Tiam1-MAP1B connection and Rac1

Reorganization of the growth cone cytoskeleton involving lamellipodial protrusion and expansion, shortening of actin ribs,

increased actin dynamics, Cdc42-Rac1 signaling, paralleled by MT growth, invasion, and capture within the P-domain are events essential for axon formation.[90,91] Tiam1-mediated activation of Rac1 is required for axon outgrowth and the rapid elongation of the newly formed axon;[91,92] besides, Par6-Par-3 mediated Cdc42-induced Rac activation involves Tiam1/Tiam2.[92,93] In cultured neurons, Tiam1 localizes to the growth cone of the nascent axon where it associates with dynamic MT and the subcortical cytoskeleton;[91] biochemical studies have confirmed these observation revealing that Tiam1 purifies with brain-derived MT.[91]

MAP1B is a microtubule-associated protein preferentially expressed in developing neurons that is enriched at the tip of growing axons and required for their development.[94,95] MAP1B-deficient neurons fail to properly elaborate an axon, displaying neurites with small growth cones containing fewer dynamic MT than their wild type counterparts.[94] It was also demonstrated that MAP1B deficiency decreases Rac1/Cdc42 while increasing that of RhoA, and that ectopic expression of Tiam1 rescues the MAP1B KO phenotype of cultured neurons.[96] A more recent study showed that Tiam1 interacts with MAP1B light chain 1 (LC1) through its pleckstrin homology (PH) domain; thus, MAP1B can be envisioned as a "flag" that targets Tiam1 to growth cone dynamic MTs to locally activate Rac1.[97] Together, with the Lfc-Tctex1 interaction, these observations illustrate the key role that MTs and MT-interacting components have in regulating Rho-GTPase signaling during axon formation, and its underlying events, namely neurite outgrowth (e.g., protrusion), growth cone advance (motility) and breaking of cell symmetry (polarization).

Conclusion and Future Directions

During the past decade great advances have been made in our understanding of the reciprocal relationship between MTs and member of the Ras family of small GTPases. Both protein systems are master regulators of key biological events including cell division, motility, and polarization. It is now evident that MTs, and not only actin filaments, are main cytoskeletal targets of RhoA, Rac, and Cdc42; more importantly, this regulation is central for controlling the dynamic organization of MTs, and hence most of its biological functions. Besides, due to its dynamicity and the presence of + TIPs, MTs can reach the cell cortex and interact with small Rho GTPases. This is of great functional importance since MTs also serve as platforms harboring factors, such as GEFs, capable of promoting the activity of at least RhoA and Rac. This reciprocal relationship generates positive and negative feedback loops, which have proved to be essential for events like cell migration and neuronal polarization.

One of the major challenges for future studies will be to correlate different MT behavior (growth, shrinkage, bundling, capture, etc.) with specific patterns of Rho-GTPase activation and/or signaling in time and space. The visualization of multiple Rho-GTPase activation patterns with next generation biosensors[3] and multiplexing techniques[33] in combination with live imaging of MT dynamics will certainly help characterizing the spatio-temporal circuits and programs involving MTs and small GTPases and how they relate with morphogenetic events.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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