

I'm coming to GEF you

Regulation of RhoGEFs during cell migration

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Abbreviations: bFGF, basic fibroblast growth factor; ECM, extracellular matrix; DH, Dbl-homology; DHR, DOCK homology region; DOCK, dedicator of cytokinesis; EGF, epidermal growth factor; FA, focal adhesion; FN, fibronectin; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GDI, guanine nucleotide dissociation inhibitor; GPCR, G protein-coupled receptor; HGF, hepatocyte growth factor; LPA, lysophosphatidic acid; MII, myosin II; PA, phosphatidic acid; PDGF, platelet-derived growth factor; PH, pleckstrin-homology; PIP₂, phosphatidylinositol 4, 5-bisphosphate; PIP₃, phosphatidylinositol (3, 4, 5)-trisphosphate.

Cell migration is a highly regulated multistep process that requires the coordinated regulation of cell adhesion, protrusion, and contraction. These processes require numerous protein–protein interactions and the activation of specific signaling pathways. The Rho family of GTPases plays a key role in virtually every aspect of the cell migration cycle. The activation of Rho GTPases is mediated by a large and diverse family of proteins; the guanine nucleotide exchange factors (RhoGEFs). GEFs work immediately upstream of Rho proteins to provide a direct link between Rho activation and cell–surface receptors for various cytokines, growth factors, adhesion molecules, and G protein-coupled receptors. The regulated targeting and activation of RhoGEFs is essential to coordinate the migratory process. In this review, we summarize the recent advances in our understanding of the role of RhoGEFs in the regulation of cell migration.

At the center of this striking cytoskeleton reorganization is the Rho family of GTPases. Rho GTPases are versatile signaling molecules that regulate a diverse set of cellular functions. Rho GTPases function as molecular switches that cycle between an inactive GDP-bound and an active GTP-bound conformation. The activation of Rho proteins is mediated by guanine nucleotide exchange factors (RhoGEFs), which catalyze the exchange of GDP to GTP.³ Once in the active conformation, Rho GTPases interact with one of several downstream effectors that modulate a variety of intracellular processes.⁴ To turn the switch off, GTP has to be hydrolyzed to GDP, a reaction that is stimulated by GTPase-activating proteins (GAPs).⁵ In addition, inactive Rho GTPases are extracted from cell membranes by Rho-specific guanine nucleotide dissociation inhibitors (RhoGDIs) to prevent their inappropriate activation and to protect them from misfolding and degradation.⁶

Introduction

The cell migration cycle involves a series of highly coordinated steps that starts with polarization and membrane protrusion in the direction of migration.¹ These protrusions are then stabilized by forming adhesions that provide a link between the actin cytoskeleton and the extracellular matrix (ECM). These sites of adhesion are called focal adhesions (FA), and serve as traction points for the cell body to contract and move forward. Contraction also promotes the disassembly of the adhesions at the cell rear allowing it to detach. These processes involve hundreds of proteins forming a complex signaling network linked by multiple interactions.²

RhoGEFs

There are approximately 80 RhoGEFs in the human genome, encoded by two unrelated gene families:^{3,7} the Dbl family, which comprises 69 members in humans, and the DOCK family, with 11 members.^{3,7} The Dbl family is characterized by the presence of a Dbl homology (DH) catalytic domain, followed by an adjacent pleckstrin homology (PH) domain, C-terminal to the DH domain.³ Together, in most cases, they provide the minimal structural unit that is required to catalyze the exchange reaction *in vivo*.³ In most GEFs, the DH–PH domains are flanked by a diverse array of protein–protein and protein–lipid interaction domains.³ These domains help regulate the intrinsic catalytic activity of RhoGEFs, their intracellular localization, and their association with other proteins.

The DOCK (dedicator of cytokinesis) family of GEFs has been characterized more recently.⁷ DOCK proteins are structurally and mechanistically unrelated to the Dbl-family, and act on Rac and/or Cdc42, but not on RhoA. The DOCK GEFs are characterized by the presence of a conserved catalytic domain, the DOCK Homology Region 2 (DHR2), and a phospholipid-

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binding domain (DHR1) that can target the GEFs to the membrane.⁸ DHR2 domains share no primary sequence homology with DH domains.

Between the two RhoGEF families, there are approximately four times more RhoGEFs than Rho GTPases. This means that a single GTPase can be activated by multiple GEFs and may indicate some overlap or redundancy in their functions. Since several RhoGEFs can activate more than one GTPase, the effective number of RhoGEFs that can act on a single Rho GTPase is even higher. There are at least 25 RhoGEFs that can activate each of one of the major Rho proteins, RhoA, Rac1, and Cdc42.^{3,9} This number is probably an underestimation, since the specificities of many RhoGEFs have not been completely characterized yet. Since most of RhoGEFs are widely expressed, most cell types usually express several RhoGEFs for each of the GTPases at any given time. The diversity in their domain structure is what allows RhoGEFs with similar specificity to be regulated by different signaling pathways.

The last decade has seen an explosion in the number of publications characterizing the functions of different RhoGEFs, and there seems to be a perception that there is little left to learn. We have made significant progress in understanding how RhoGEFs are activated and targeted to particular locations in the cell; how they interact with other proteins, and how their expression is regulated. However, we are only starting to understand the mechanisms that control this complex network of regulatory proteins. Here, we discuss recent findings on the role of RhoGEFs on the regulation of cell migration. We focus our attention on the RhoGEFs for which a significant body of evidence has been accumulated linking them directly to cell motility, and for the sake of organization, have classified them in what we believe are their main functions associated with cell migration (see Table 1).

Polarization and Protrusion

In response to migration-inducing factors, cells polarize and form a protrusive area in the direction of migration and a

retracting rear that are defined by distinct cell signaling events.¹ Actin polymerization at the cell front drives the extension of the lamellipodium and filopodia. At the leading edge of the lamellipodium, the cell forms adhesions that connect the ECM to the actin cytoskeleton to anchor the protrusion and contract the cell body. Rho GTPases operate at each step to promote directional migration by regulating leading edge formation. Several studies have shown that RhoA, Rac1, and Cdc42 are activated at the front of migrating cells,¹⁰ and biochemical evidence suggest that extensive crosstalk between them may regulate one another.¹¹ However, their spatiotemporal regulation and coordination remains to be characterized, in particular, how the upstream (GEFs/GAPs) and downstream effectors are targeted and regulated in response to a particular stimulus.

One of the key events controlling cell polarization is the localized activation of Rac at the leading edge, which is critical for both establishing and maintaining polarized protrusion.¹ The Rac-GEFs β Pix and DOCK180 are implicated in Rac1 activation at the front of migrating cells.^{12,13} Both β Pix and DOCK180 are highly polarized and localized primarily in adhesions near the leading edge of protrusions. DOCK180 and β Pix are recruited to the plasma membrane through their interaction with the paxillin-p130Cas-CrkII and paxillin-GIT-PAK complexes, respectively.^{13,14} Non-receptor tyrosine kinases like Src or FAK phosphorylate paxillin create docking sites that recruit these complexes to adhesions.^{15,16} Thus, it appears that the dynamics of adhesion/protrusion may be controlled by different signaling mechanisms.

β Pix

β Pix (also known as Cool-1 and ARHGEF7) has been initially characterized as a GEF for Cdc42 and/or Rac1, and is one of the most extensively studied RhoGEFs.¹⁷ Interestingly, β Pix not only interacts with Cdc42 and Rac1 through its catalytic domain, but also binds to Cdc42-GTP and to Rac1 in a

Table 1. RhoGEFs involved in cell migration

RhoGEFs	Alt. names	Specificity	Function	Localization	Refs*
p190RhoGEF	RGNEF	RhoA	adhesion	cytosol/FA	[108-112]
p63RhoGEF	GEFT	RhoA	contractility, polarization	leading edge	[99-100]
Syx	PLEKHG5/Tech	RhoA	polarization	cytosol/PM	[93-94, 96-97]
GEF-H1	Lfc, ARHGEF2	RhoA	contractility	microtubules	[122-126, 128-131]
p115RhoGEF	Lsc, ARHGEF1	RhoA	adhesion, polarization	cytosol/FA	[92, 114]
LARG	ARHGEF12	RhoA	contractility	cytosol/FA	[106, 114, 153]
PDZ-RhoGEF	ARHGEF11	RhoA	contractility, tail retraction	trailing edge	106-107]
Net1	ARHGEF8	RhoA	contractility, tail retraction	nucleus/FA	[105, 136-139]
P-REX1	-	Rac1/2	?	cytosol/PM	[84-86]
β PIX	ARHGEF7	Rac1/Cdc42	polarization/protrusion	cytosol/FA	[13, 14, 17-22, 25-27, 29-32]
Tiam1	-	Rac1	polarization/protrusion	cytosol/leading edge/FA	[63-65]
Asef1	ARHGEF4	Rac1/Cdc42	adhesion	cytosol/PM	[74-76]
Asef2	ARHGEF29	Rac1/Cdc42	adhesion	cytosol/PM	[71, 77-78]
DOCK2	-	Rac1	polarization	cytosol/PM	[79-83, 87-89]
DOCK3	MOCA	Rac1	polarization, \downarrow contractility	cytosol/PM	[147-148]
DOCK180	DOCK1	Rac1	polarization/protrusion	cytosol/PM	[46-47, 50-60, 63-65]
DOCK10	zizimin 3	Cdc42	contractility	?	[155]

* References listed refer specifically to the articles addressing the function of these GEFs during cell migration and their subcellular localization.

nucleotide-independent manner.^{18,19} β Pix was originally identified as a binding partner to p21-activated kinase (PAK) family of Cdc42/Rac1-activated kinases.¹⁷ Through the interaction with β Pix, PAK is targeted to focal complexes.^{17,20} PAK competes with Rac1 for binding to β Pix, and in the absence of PAK, there is more β Pix available for Rac1 binding, resulting in increased Rac1 activation and enhanced cell migration.¹⁹ Moreover, FAK can bind to and phosphorylate β Pix, and thereby, enhance β Pix binding to Rac1 and the translocation of Rac1 to adhesions.^{21,22}

In addition to binding to PAK, β Pix binds to a diverse array of adaptor and signaling proteins to modulate a complex protein–protein interaction network that regulates cell adhesion and motility.²³ Regulation of cell migration by β Pix involves the GIT proteins, an ARF–GAP family of scaffolding proteins that bind to β Pix, paxillin, and several other proteins.²⁴ The GIT family has two members, GIT1 and GIT2, and they both colocalize with β Pix at focal complexes.^{24–26} GIT may be required to target PAK and β Pix to adhesions through its association with paxillin (Fig. 1).²⁵ At adhesions, PAK may regulate actin remodeling and FA turnover, whereas β Pix could function as a GEF to activate Rac.^{25,27} However, the importance of the catalytic activity of β Pix is still a matter of debate, and it has been proposed it

may function exclusively as a scaffold to recruit PAK to adhesions.²⁸ Several studies have also demonstrated that β Pix, PAK, and GIT form a constitutively associated complex that shuttles between the endocytic compartment and the membrane.^{29,30} Recycling the GIT/PAK/ β Pix may be important to target it to the edge of lamellipodia where new adhesions are being assembled. Recently, Valdes and collaborators reported the interaction of the endosomal protein sorting nexin 27 (SNX27) with β Pix in complex with GIT family of proteins.³¹ SNX27 regulates the trafficking of the β Pix/GIT complex between the early endosome and FAs, and thereby, influences cell migration. Knockdown of SNX27 restricts the intracellular movement of β Pix to FAs leading to defects in cell migration.³¹ The precise function of the GIT complex in cell motility is still poorly understood, and there is conflicting evidence on whether it affects Rac-mediated protrusions positively¹³ or negatively.³²

β Pix belongs to a group of GEFs, which encode a PDZ-binding motif at their C terminus, a group that also includes Syx1 and Net1 among others.³³ PDZ domains are protein–protein interactions domains that act as scaffolds to concentrate signaling molecules at specialized regions in the cell. We have previously proposed that the interaction between Rho-GEFs and PDZ-

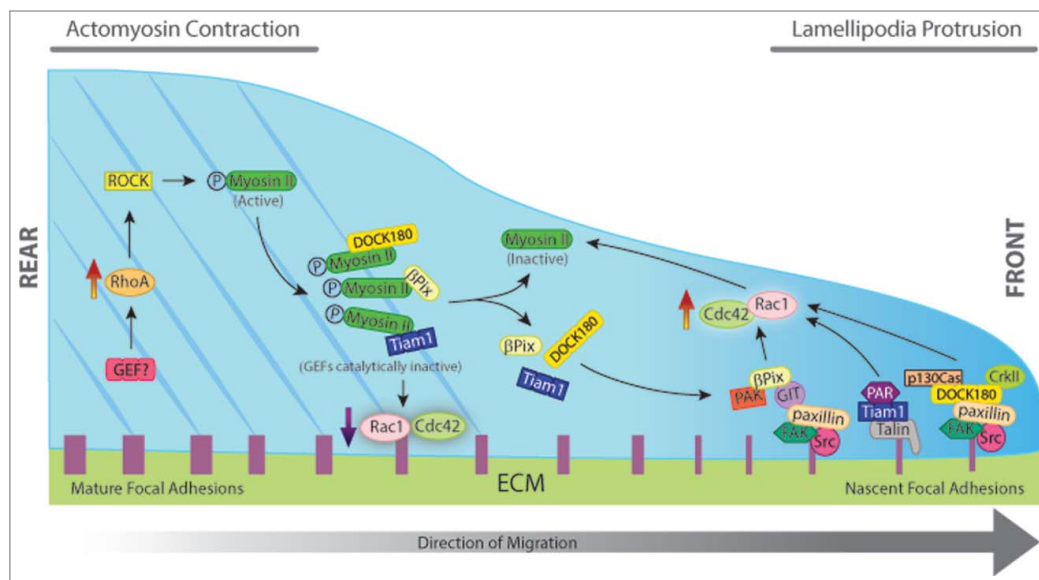


Figure 1. Schematic representation of the effects of RhoGEFs on focal adhesion dynamics and cell migration. Cell migration requires the coordinated spatiotemporal regulation of cell adhesion, protrusion, and contraction. Different GEFs such as β Pix, DOCK180, and Tiam1 are required at the cell front for lamellipodial protrusion and rapid nascent adhesion turnover. Following stimulation, β Pix, DOCK180, and Tiam1 are recruited to nascent adhesions through their interaction with kinases and adaptor proteins to form signaling complexes that control the local activation of Rac1 (GIT/ β Pix/PAK, p130Cas/DOCK180/CrkII, Talin/Tiam1/Par complex). Rac1 activation induces the assembly of dendritic actin networks by polymerizing actin filaments at the leading edge to push the membrane forward. At the cell rear, RhoA-mediated activation of ROCK induces phosphorylation of myosin light chain, which promotes the assembly of actin-Myosin II (MII) filaments. After MII becomes active, it interacts with β Pix, DOCK180, and Tiam1 colocalizing along the actin stress fibers. When in complex with MII, all three GEFs are catalytically inactive. Thus, increased activation of MII results in large actin bundles, large and stable adhesions, decreased signaling to Rac/Cdc42, and decreased protrusion. Inactivation of MII stimulates the release of the GEFs and activation of Rac1 and Cdc42. The resultant Rac1 activation further inactivates MII, thus forming a positive feedback loop, which contributes to persistence of directional migration.

domain proteins can function as a general mechanism to control Rho-GEFs targeting and activation, helping to restrict and concentrate the exchange activity at appropriate subcellular destinations.³³ The intracellular distribution of β Pix has been shown to be modulated by PDZ domain-containing proteins, including Scribble, SNX27, and Tax-interacting protein 1 (TIP-1).^{31,34–36} Scribble is a conserved protein required for maintenance of epithelial cell polarization.³⁷ In astrocytes, Scribble binds to and regulates the targeting of β Pix to the leading edge during the establishment of cell polarity.^{34,35} Scribble controls some of the same processes regulated by Cdc42, including centrosome and Golgi re-orientation, protrusion formation, and cytoskeletal polarization. Depletion of either Scribble or β Pix expression inhibits Cdc42 recruitment to the leading edge, and the cells fail to form protrusion and

establish a polarized phenotype.³⁴ These results indicate that, at least in astrocytes, β Pix may be responsible for Cdc42 activation during polarization.³⁴ In fibroblasts, however, Cdc42 activates Pak1/2 at the leading edge of migrating cells, which promotes the recruitment of β Pix and the localized activation of Rac, suggesting that different pools of β Pix may independently activate Rac or Cdc42 at different cellular locations to control distinct processes during cell protrusion and polarization.³⁸

The PDZ protein TIP-1 modulates the interaction dynamics and the intracellular distribution of the Scribble/ β Pix complex by competing with Scribble in the selective binding to β Pix.³⁶ TIP-1 knockdown enhances the protein interaction between β Pix and Scribble and results in aberrant localization of β Pix. In addition, Rac activation during wound healing decreases significantly and is accompanied by an increase in active RhoA. These effects are also accompanied by impaired motility.³⁶

Myosin II (MII) has been recently reported to bind to the DH domain of β Pix, and is emerging as a major protein responsible for symmetry breaking and organization of the actin bundles that define the rear during the process of cell polarization.³⁹ Binding to MII inhibits β Pix activity toward Rac1 and Cdc42. Accordingly, inactivation of MII activity induces the release of β Pix from MII and activation of Rac1 and Cdc42 leading to lamellipodia and focal complexes formation.³⁹ In agreement with these results, when MII contractility is inhibited, β Pix accumulates in assembling nascent adhesions and promotes Rac1 activation. In contrast, during MII-mediated FA maturation, β Pix dissociates from FAs (Fig. 1).⁴⁰ Vicente-Manzanares and collaborators have also implicated β Pix in MII-dependent front-back polarity in migrating cells.⁴¹ They showed that both MIIA and MIIB coordinately define and assemble the rear and synergize to form actomyosin bundles. This MII-dependent bundling regulates adhesive signaling by restricting Rac activation.

In addition to β Pix, MII regulates a surprising number of Dbl GEF family members through direct binding with the DH-PH module, including FGD1, Kalirin, LARG, DOCK180, Tiam1, GEF-H1, Dbl, and Trio.³⁹ Although there are some quantitative differences among these interactions, this may represent a conserved molecular mechanism for GEF regulation by MII in cell protrusion and adhesion.

DOCK180

Another key regulator of Rac activation during cell migration is DOCK180.⁴² DOCK180 (DOCK1) is one of the best characterized members of the DOCK family of GEFs and was originally identified as a binding protein for the proto-oncogene product c-Crk.^{43,44} The targeting and activation of DOCK180 is regulated through its interaction with different signaling and adaptor proteins.⁴⁵ DOCK180 forms a complex with ELMO (engulfment and cell motility) through its SH3 domain.^{46,47} There are three ELMO proteins in mammals, and they all seem to be scaffold proteins, with no obvious catalytic activity.⁴² The formation of an ELMO/DOCK180 complex is essential for efficient Rac1 activation during cell polarization and migration.⁴⁷ The initial studies on DOCK180 suggested it functions as a bipartite GEF that is catalytically active only when bound to

ELMO.^{43,47} However, it is still not clear if binding to ELMO is required for DOCK180 to be catalytically active, and there is contradictory evidence on this matter.^{48,49} ELMO also functions as a RhoG effector, connecting RhoG with DOCK180 to regulate Rac activation.⁵⁰ Once activated, RhoG binds to ELMO and forms a ternary complex with ELMO and DOCK180. The interaction of RhoG with ELMO induces translocation of the ELMO/DOCK180 complex from the cytoplasm to the plasma membrane, which activates Rac and stimulates cell migration.⁵¹ Thus, activation of RhoG regulates cell migration through ELMO and DOCK180-dependent activation of Rac.⁵¹

DOCK180 and ELMO also function as a node between the Rho and the Arf families of small GTPases. The coordinated activation of Arf and Rac GTPases at the leading edge stimulates migration in epithelial cells.⁵² This crosstalk depends upon the assembly of a multi-protein complex that contains the Arf-GEF ARNO/cytohesin2, DOCK180, and ELMO, which are brought together through their interaction with the scaffolding proteins GRASP/Tamalin and IPCEF/CNK3.^{53,54} GRASP provides the physical link between Arf6 and Rac by binding both DOCK180 and ARNO.⁵³ This signaling pathway from ARNO to DOCK180 is independent of RhoG.⁵² The precise role of ARNO/Arf-mediated Rac activation remains to be elucidated, but it probably involves the regulated targeting of DOCK180 and Rac to a particular subcellular localization where they modulate actin protrusion and cell migration.

DOCK180 activity can also be regulated downstream of chemokine receptor-mediated pathways in breast cancer and endothelial cells.^{55,56} Binding of CXCL12 to CXCR4 induces the dissociation of heterotrimeric G-proteins into G α i and G β γ . The G α i2 subunit gets activated, and associates with ELMO, which recruits DOCK180 to the membrane, where it activates Rac1 and Rac2 and promotes actin polymerization required for cell migration and invasion.⁵⁵

The interaction between DOCK180 with CrkII is also required for cell migration.⁵⁷ Upon coexpression of DOCK180, ELMO, and CrkII, cells adopt a highly elongated morphology and migration is significantly increased.⁴⁶ Following integrin stimulation, DOCK180 is phosphorylated and forms a complex with CrkII,⁵⁷ which is then recruited to FAs by binding to the tyrosine phosphorylated adaptor proteins p130Cas and paxillin.^{12,14,57} Once at FAs, DOCK180 is activated and amplifies the downstream Rac signal (Fig. 1). Interestingly, the interaction of DOCK180 with CrkII is neither necessary for the formation and targeting of the RhoG/DOCK180/ELMO ternary complex, nor required for the downstream Rac activation associated to its formation.⁵¹ The association between DOCK180 and its binding partners can also be regulated by receptor tyrosine kinases. Both EGFR and PDGFR induce the phosphorylation of DOCK180 by Src family kinases, which promotes the association between DOCK180, CrkII, and p130Cas, resulting in Rac activation and increased migration of human glioma cells.^{58,59}

DOCK180 is a versatile molecule that can be assembled into different signaling modules to regulate independent pathways that respond to different stimuli.³³ The interaction of DOCK180 with the adaptor protein ANKRD28 represents a

good example of this scenario. ANKRD28 is a scaffolding protein comprised of 26 ankyrin repeat domains, which competes with ELMO for binding to DOCK180.⁶⁰ Through association with ANKRD28, DOCK180 regulates stability of FAs, via Rac, and thereby, regulates cell migration. ANKRD28 cooperates with DOCK180 to localize the FA proteins, CrkII, p130Cas, and paxillin to the peripheral region of the cells.⁶⁰ Depletion of ANKRD28 retards cell migration in mammalian cells, as does DOCK180.⁶⁰ Most of the effects observed when either ANKRD28 or ELMO expression is silenced appear to be similar. However, in cells overexpressing ANKRD28, but not ELMO, p130Cas gets hyperphosphorylated and cells have long tails that fail to retract, whereas ELMO overexpression induces the formation of lamellipodia along the circumference of the cell.⁶⁰ These results suggest that these two DOCK180 complexes may activate Rac at different locations of the cell to regulate lamellipodia formation and tail retraction.⁶⁰

DOCK180 has also been shown to couple PtdIns(3,4,5) P_3 signaling to Rac activation during cell migration.⁸ PtdIns(3,4,5) P_3 is a key player in the establishment of the initial asymmetry during polarization and interacts with the DHR-1 domain of DOCK180 in vitro and in vivo.⁶¹ PtdIns(3,4,5) P_3 mediates the translocation of the DOCK180-CrkII-ELMO signaling complex to the leading edge.⁶¹ Mutations in the DHR-1 domain of DOCK180 block Rac-dependent cell elongation and cell migration, even though Rac1 activation levels are not affected, suggesting both the activity and localization of Rac are required for proper DOCK180 signaling.⁸

Tiam1

The Rac-specific GEF Tiam1 has been mostly associated to the development of cadherin-mediated cell–cell adhesions.⁶² However, a series of recent studies have now shown that Tiam1 also plays a role in the development of front-rear polarity in free migrating cells.^{63–65} In epithelial cells, cadherins signal through Cdc42 and/or Rac to activate the Par polarity complex, which in turn, stimulates the formation of tight junctions (TJ). The Par polarity complex consists of Par3, Par6, and atypical PKC (aPKC), and regulates cell polarization in many different contexts, including apico-basal, neuronal, and front-rear polarity.⁶² Tiam1 has been shown to associate with the Par complex by binding to Par3 to regulate the assembly.^{66,67} There is evidence that during apical-basal polarity in epithelial cells, Tiam1 promotes the assembly of TJ through the activation of Rac and the Par complex but independently of Cdc42.⁶² Thus, by promoting the formation of cadherin-mediated adhesions and the development of an epithelial morphology, Tiam1 activity can inhibit cell migration, whereas the loss of Tiam1 can indirectly enable cell migration by promoting the disassembly of cell–cell junctions.⁶⁸ In contrast, in keratinocytes, which are highly polarized, the loss of Tiam1 (KO) correlates with reduced Rac activity and a complete loss of polarization.⁶³ Interestingly, random migration is only slightly affected, whereas directionality is completely lost and chemotaxis impaired.⁶³ In these cells, Tiam1 also binds to and activates the Par complex, and when Par3 is depleted directionality is also reduced. Blocking PKC ζ function also inhibits

directionality and chemotaxis. In cells that exhibit front-rear polarization, both Par3 and Tiam1, and to a lesser extent, PKC ζ , are enriched at the leading edge. These results suggest that Tiam1 controls chemotaxis and persistence through the Par polarity complex.⁶³

A recent article has shed some light into the mechanisms by which Tiam1 regulates polarized cell migration in glioma cells.⁶⁴ Using TIRF microscopy, Tiam1 is found at FAs where it partially colocalizes with the integrin-binding protein talin, preferentially at the front region of the cell. Tiam1 associates directly to talin and the interaction is required to target Tiam1 to FAs.⁶⁴ The activation of Rac downstream of integrins is reduced in Tiam1-depleted cells and almost completely abrogated when talin is depleted. Depletion of Tiam1 or talin delays spreading, inhibits polarization, and impairs FA turnover.⁶⁴ These processes require the association of talin with both Tiam1 and integrins. In addition, depletion of Par3, Par6, or PKC ζ expression inhibits Tiam1 recruitment to FA, which suggests the PAR complex works in concert with Tiam1 and talin to regulate FA formation and develop front-rear polarity (Fig. 1).⁶⁴ Interestingly, Tiam1 depletion only partially inhibits fibronectin (FN)-mediated Rac1 activation, suggesting there may be other Rac-GEFs involved. However, depletion of DOCK180 or α/β Pix does not affect Rac1 activation, whereas Vav2 depletion results in a slight decrease in Rac1 activation. In addition, β Pix overexpression cannot rescue Tiam1 deficiency. However, in a different glioma cell line, DOCK180 KD inhibits Rac1 activation to the same extent as Tiam1 depletion, suggesting different GEF requirements in different cell lines.⁶⁴

Asef

Asef1 and Asef2 are two highly homologous Rac1 and Cdc42-specific GEFs that interact with the tumor suppressor adenomatous polyposis coli (APC).^{69–71} Binding to APC stimulates the GEF activity of Asef1/2 by releasing intramolecular inhibition.^{70,72,73} Overexpression of Asef decreases cadherin-mediated cell–cell adhesion and promotes migration.^{69,74} Both Asef1 and Asef2 have also been shown to regulate Rac and Cdc42 activation downstream of growth factors. Different growth factors, including EGF, HGF, and bFGF, promote the translocation of Asef to the membrane where they stimulate cell migration in a PI3K-dependent manner.^{75,76}

Intriguingly, Asef2 function has been associated with both promoting and inhibiting cell migration depending on the ECM substrate.^{77,78} When HT1080 cells expressing GFP-Asef2 are plated on FN, Asef2 localizes to the leading edge and promotes cell migration by increasing the rapid turnover of adhesions through a mechanism that is dependent on PI3K and Akt.⁷⁷ In contrast, when the same cells expressing GFP-Asef2 are plated on type I collagen, migration is significantly inhibited.⁷⁸ The inhibition of migration requires Asef2 exchange activity, which activates Rac1, and induces MII phosphorylation. Phosphorylation of MII enhances cell contractility, and prevents FA disassembly at the leading edge, which results in impaired cell migration.⁷⁸ Asef2 effects on cell migration, both activation and inhibition, require the activation of Rac, but not of Cdc42.^{77,78} On FN

substrate, Asef2 also promotes the inactivation of RhoA, possibly regulated through Rac-mediated crosstalk.⁷⁷ How does the same GEF/GTPase module regulate a completely opposite cellular response? The mechanisms that control Asef2 in response to different substrates are currently unknown, but as we speculated for other GEFs, it is possible that by interacting with different scaffolding proteins Asef2 can be recruited to different signaling complexes that regulate distinct downstream responses.

Polarization in Lymphocytes and Neutrophils

During inflammation, multiple chemokines are upregulated and attract lymphocytes and myeloid cells such as neutrophils, mast cells, and macrophages. Lymphocytes are highly motile and play central roles in surveying the body's lymphoid tissues for antigens. Lymphocytes differentiate in primary lymphoid organs, and migrate into secondary lymphoid tissues such as the lymph nodes and spleen via the blood. This process of chemotaxis is guided by chemokines such as CCL21, CCL19, CXCL13, and CXCL12, which signal through G protein-coupled receptors (GPCRs) to activate Rac. In lymphocytes, chemokine-induced Rac activation is mediated by the Rac-specific GEF DOCK2. DOCK2 expression, unlike other DOCK proteins such as DOCK180, which is expressed in various tissues, is restricted to hematopoietic cells.^{79,80} As a result, DOCK2-deficient (DOCK2 $-/-$) lymphocytes exhibit a severe defect in polarization and chemotactic responses.^{79,81-83}

Neutrophils are also highly motile and play key roles in the innate response to invading pathogens. Bacterial proteins are translated with an N-terminal formylated methionine, and the resulting formyl-peptides (fMLP) act as potent neutrophil chemoattractants. Several lines of evidence had initially pointed to the Rac-specific GEF P-Rex1 as an important regulator of neutrophil chemotaxis.^{84,85} However, neutrophil chemotaxis is only slightly reduced in P-Rex1-deficient (P-Rex1 $-/-$) neutrophils with only a mild defect in cell speed, and normal polarization and directionality.^{85,86} These results suggest the existence of another GEF responsible for Rac activation during neutrophil chemotaxis. As in lymphocytes, DOCK2 appears to be the main candidate. In the absence of DOCK2, Rac1 and Rac2 activation in response to fMLP is inhibited, and neutrophils cannot chemotact efficiently.⁸⁷ fMLP stimulation triggers a rapid translocation of DOCK2 to the plasma membrane in a PI3K-dependent manner, where it stimulates Rac activity and mediates the polarized accumulation of F-actin at the leading edge.^{87,88} DOCK2 binds to PIP3 (but not to PIP2), in a process that is enhanced by binding to its effector ELMO1. Rac and PIP3 are part of a positive feedback-loop, in which PIP3 is required for DOCK2 to translocate to the membrane, where it activates Rac and further stabilizes PIP3 at the leading edge.⁸⁷ Surprisingly, in cells lacking PI3K γ , the major generator of PIP3 in these cells, DOCK2 can still translocate to the plasma membrane in response to fMLP, albeit at later time points, suggesting the existence of a second mechanism of DOCK2 targeting.⁸⁸ This second mode of targeting requires phosphatidic acid (PA), which interacts with a

stretch of basic residues at the C terminus of DOCK2.⁸⁸ Thus, PA functions in a second late phase, following PI3K-mediated initial recruitment, to control polarized DOCK2 localization and stabilize the leading edge. This late recruitment phase depends on the PIP3-mediated early phase, since recruitment of DOCK2 to the membrane induced by addition of PA is attenuated by inhibition of PI3K.⁸⁸

Finally, DOCK2 has been shown to be essential for migration of dendritic cells.⁸⁹ Dendritic cells (DCs) are specialized antigen-presenting cells found as sentinels in peripheral tissues and lymphoid organs. DCs are classified into two populations, myeloid DCs (mDCs) and plasmacytoid DCs (pDCs), with distinct expression patterns of costimulatory molecules and Toll-like receptors. mDCs function as most potent antigen-presenting cells, whereas pDCs function in antiviral immunity by producing interferons type I.⁹⁰ Interestingly, DOCK2 deficiency impairs chemokine-mediated migration in pDCs but has no effect on mDCs. DOCK2 depletion in pDCs inhibits Rac activation, polarization, and chemotactic migration. Homing of pDCs to secondary lymphoid organs is also inhibited. How can the differences between pDCs and mDCs be explained? pDCs express only DOCK2, whereas mDCs express both DOCK2 and DOCK180 (DOCK1), so it is possible that in mDC, the absence of DOCK2, can be functionally compensated by DOCK180.⁸⁹

RhoA and Polarity

While polarity is mostly associated with Rac and Cdc42 function, other GTPases such as RhoA can also play a role in the development of polarity. RhoA has been originally thought to be restricted to contractility and tail retraction during migration.¹ However, recent studies using biosensors have demonstrated that active RhoA is also found at the leading edge in migrating cells.^{10,91}

Lsc, the mouse homolog of p115RhoGEF, plays a key role in the regulation of neutrophil migration.⁹² Following fMLP stimulation, Lsc relocates to both the leading edge and trailing edge. Neutrophils from Lsc $-/-$ mice generate and retract their protrusions rapidly at inappropriate locations in the cell and are unable to sustain a single-dominant leading edge or pseudopod, often developing multiple pseudopodia. In addition, Lsc $-/-$ neutrophils are significantly less adherent than their wt counterparts, and have reduced RhoA activity.⁹² As a result, they migrate twice as fast, but with reduced directionality. Taken together, these results suggest a role for Lsc in the development of polarization.

Syx1 (PLEKHG5) is one of the RhoA-GEFs that have been recently associated with RhoA-dependent cell polarity. When Syx1 expression is silenced in breast cancer cells, cells lose their elongated morphology and display a rounded flattened shape.⁹³ In a wound healing assay, Syx1 KD cells fail to form lamellipodia in the direction of the wound and are also defective reorienting the Golgi complex in the direction of migration. Chemotactic migration in a transwell assay is also inhibited.⁹³ Similarly,

silencing Syx1 in vivo in zebrafish results in inhibition of migration of endothelial cells in intersegmental vessels.⁹⁴ The loss in polarity is accompanied by an increase in the number and size of FA and a redistribution of actin to stress fibers and peripheral actin bundles.⁹³ The increase in stress fibers is unexpected when a RhoA-GEF is knocked down, and could reflect compensation by other RhoGEFs, as has been previously shown in other cases. For example, RhoA activity levels remain stable when p114RhoGEF is depleted, due to a compensatory activation of GEF-H1.⁹⁵ The fact that Syx1 KD does not change the overall RhoA activity, may also suggest a localized effect on RhoA activation. In addition to the effects on the actin cytoskeleton, Syx1 depletion also results in the dramatic reorganization of microtubules, which may also contribute to the loss of polarity.⁹³

Syx1 has been shown to localize to the membrane by interacting with PDZ domain proteins such as MUPP1/PatJ and synectin via its PDZ-binding motif.^{94,96-98} MUPP1/PatJ forms part of the Crumbs polarity complex (Crumbs-Pals-PatJ), which plays a role in the formation of cell-cell junctions.³⁷ Both the ability to bind PDZ proteins and to activate RhoA are required for Syx function in the establishment of polarity, suggesting the localized activation of RhoA is essential.⁹³ Interestingly, the Syx gene expresses two isoforms by alternative splicing; the full-length Syx1, and an almost identical isoform, Syx2, which is just two amino acids shorter and is unable to interact with PDZ domains.⁹⁶ Using a FRET RhoA-biosensor, Liu and colleagues showed that, in response to LPA, Syx1 stimulates RhoA activity specifically at the leading edge and promotes cell migration. Syx2 also promotes RhoA activation, but it is uniformly distributed in the cytoplasm and does not stimulate migration.⁹⁶ A similar series of experiments analyzed the localization of RhoA activity in the presence or absence of angiominin (Amot), a protein that forms a ternary complex with MUPP1 and Syx.⁹⁴ In randomly migrating endothelial cells, RhoA activity concentrates at the leading edge of extending lamellipodia. However, in Amot-deficient cells, the localized activation of RhoA is lost and the activity redistributes evenly throughout the membrane.⁹⁴ The molecular mechanisms that regulate polarization through Syx-RhoA are not completely characterized, but there is evidence that the formin Dial1 may be one of the main downstream effectors.⁹³

Another RhoA-GEF that has been recently associated to the development of polarity in migrating cells is p63RhoGEF, also referred to as GEFT. p63RhoGEF was found to be required for serum-induced chemotactic migration in MDA-MB-231 breast cancer cells.⁹⁹ p63RhoGEF signals downstream of GPCRs and binds directly to $G_{\alpha_{q11}}$, but not $G_{\alpha_{12/13}}$.¹⁰⁰⁻¹⁰² G_{α_q} activates p63RhoGEF allosterically by relieving intramolecular inhibition.¹⁰³ When cells are incubated in the presence of serum, RhoA is rapidly activated, and the activation of p63RhoGEF follows a similar pattern. When p63RhoGEF is knocked down, RhoA activation in response to serum is completely abrogated, and the cells form multiple lamellipodia around the cell periphery. In contrast, control cells display a polarized cell morphology with a single lamellipodium forming in the direction of migration. Chemotactic migration toward serum is also inhibited in the absence of p63RhoGEF, but not random migration, which

highlights the specificity of its function.⁹⁹ Supporting these results, overexpression of p63RhoGEF induces cell rounding and prevents lamellipodia formation. Taken together, these results support a role for p63RhoGEF in the development of polarity and in chemotactic migration. Although not directly addressed in these reports, the function of p63RhoGEF in cell migration is probably related to the ability of RhoA to regulate contractility through ROCK and MLC.

Cell Adhesion and Focal Adhesion Formation

The polarized protrusions at the leading edge are stabilized by adhesion to the ECM through transmembrane adhesion receptors which link to the actin cytoskeleton. The formation of such adhesion sites generate traction forces at the leading edge that the cell uses to move forward. Adhesion assembly is regulated by Rho GTPases, which in turn, are regulated by adhesion-generated signals. Focal adhesion kinase (FAK) and c-Src (Src) are both non-receptor tyrosine kinases viewed as central regulators of FA turnover.¹⁰⁴ Activation of the FAK/Src complex promotes the recruitment and phosphorylation of multiple scaffolding and signaling proteins at FA that are involved in adhesion dynamics and motility.¹⁶ FAK has also been shown to bind to several RhoGEFs and plays a key role in targeting them to adhesion sites and regulating their activity. Therefore, FAK can function as a signaling hub that connects Rho GTPases to different processes associated to cell migration, including adhesion assembly and turnover, leading edge formation and tail retraction.

Most of the GEFs that bind to FAK are RhoA-specific, including Net1, LARG, PDZ-RhoGEF, and p190RhoGEF (RGNEF).¹⁰⁵⁻¹⁰⁸ FAK activation promotes the phosphorylation of p190RhoGEF, which in turn, stimulates RhoA activation.¹⁰⁸ When the expression of p190RhoGEF is silenced in fibroblasts, cells form fewer FAs, which correlate with lower levels of active RhoA. KD cells also migrate at reduced speed when plated on FN, suggesting p190RhoGEF is involved in the regulation of RhoA activity and FA formation downstream of integrins.¹⁰⁹ Similar results were obtained from fibroblasts isolated from a p190RhoGEF $-/-$ mouse, which also show reduced RhoA activity and FA number, as well as impaired migration.¹¹⁰ Interestingly, both FAK phosphorylation and tyrosine phosphorylation decrease in the absence of p190RhoGEF, suggesting the existence of a FAK-p190RhoGEF feedback loop involved in the regulation of cell adhesion and migration.¹⁰⁹ In addition, FAK expression increases in p190RhoGEF $-/-$ cells.¹¹⁰ Conversely, p190RhoGEF expression is upregulated in FAK $-/-$ cells, resulting in increased number of FA and higher RhoA-GTP levels.¹⁰⁹ These studies identify p190RhoGEF as one of the main regulators of FA formation during FN-stimulated cell motility. p190RhoGEF has also been shown to regulate RhoC activity at the lamellipodia of migrating cells.^{111,112} In EGF-stimulated cells, p190RhoGEF is rapidly translocated to the membrane, where it promotes the localized activation of RhoC, which functions to control the activity of cofilin and the formation of actin barbed ends. The activity of RhoC is spatially and temporally

regulated by the antagonistic actions of p190RhoGEF (activation) and p190RhoGAP (inactivation), which restrict the activity of RhoC to an area right behind the leading edge of the cell.^{111,112} Although depletion of RhoC does not affect the overall migration of cells in 2D, it inhibits protrusion formation and invasion in 3D.^{111,112}

The activation of RhoA downstream of FN-adhered cells is also regulated by p115RhoGEF/Lsc and LARG (Leukemia-associated RhoGEF), two RhoA-GEFs that belong to a subfamily of GEFs known as RGS-GEFs (which also includes PDZ-RhoGEF). The RGS-GEFs have been best characterized as exchange factors responsible for RhoA activation downstream of GPCRs.¹¹³ When fibroblasts are plated on FN, p115RhoGEF and LARG colocalize with paxillin-containing FAs and regulate FA and stress fiber formation.¹¹⁴ FAK plays a key role in targeting of RGS-GEFs to FA, and it has been shown to phosphorylate LARG and PDZ-RhoGEF (but not p115RhoGEF).¹⁰⁶ The FAK-related kinase Pyk2 also phosphorylates PDZ-RhoGEF following angiotensin II stimulation and promotes RhoA activation.¹¹⁵

In summary, p190RhoGEF, p115RhoGEF, LARG, and also Net1 (see below) seem to regulate similar processes in fibroblasts and downstream of integrins. However, it is still not known if their functions are redundant, if they act in parallel, or if they respond to different stimuli.

Microtubules and Polarization

The crosstalk between the actin cytoskeleton and microtubules contributes to the establishment of cell polarity and the directional movement of migrating cells.¹¹⁶ Microtubule polymerization at the leading edge stimulates Rac1 activation, which in turn, drives actin polymerization and lamellipodial protrusions.¹¹⁷ In contrast, microtubule disassembly results in activation of RhoA, which promotes actomyosin contractility.¹¹⁸⁻¹²¹ The activation of RhoA following microtubule depolymerization is mediated by GEF-H1, a microtubule-associated RhoA-specific GEF that is inactive when bound to microtubules.¹²²⁻¹²⁶ Once GEF-H1 is released, RhoA activation triggers a signaling cascade composed of ROCK/MLC that promotes contractility.^{124,126} Silencing GEF-H1 in HeLa cells inhibits FA turnover, perturbs protrusion dynamics, and decreases migration.¹²⁶

Recent studies using FRET-based biosensors revealed that RhoA is active at the leading edge during migration, and its activation pattern increases and decreases in synchrony with protrusion and retraction.¹⁰ This RhoA active zone at the leading edge of migrating cells is severely disrupted in GEF-H1-depleted cells.¹²⁶ The molecular mechanisms that regulate GEF-H1 targeting and activation are still being characterized, but one of the favored models is that localized microtubule depolymerization events control the temporal and spatial activation of RhoA.¹²⁷ Two recent studies have shown that GEF-H1 binding to microtubules may be regulated by Par1b, which plays a role in the regulation of cell polarity.^{128,129} Different studies have shown that Par1b binds to and phosphorylates GEF-H1 at different sites in

the C and N terminus, and that phosphorylation can affect its exchange activity and regulates its binding to microtubules.¹²⁹⁻¹³¹ Par1b and other kinases such as PAK1,4 and Aurora A/B, which also phosphorylate GEF-H1, may help regulate the localized binding and release of GEF-H1 from microtubules, as well as its catalytic activity in response to different stimuli.¹³²⁻¹³⁴ These results suggest GEF-H1 is a critical component of the locomotory machinery, coordinating multiple RhoA-dependent signaling pathways during the migration of cells.

Contractility and Tail Retraction

The migration cycle is completed with the disassembly and release of the rear adhesions and the retraction of the tail, which results in a net translocation of the cell body forward. The retraction of the tail requires RhoA-mediated activation of ROCK, which phosphorylates myosin phosphatase and MLC, increasing contractility, and promoting FA disassembly.

The regulation of tail retraction and the role of the Rho GTPases in this process are still poorly understood. There are only a few RhoGEFs, mostly RhoA-specific, that have been associated with the regulation of contractility at the cell rear. The RGS-GEF PDZ-RhoGEF, in conjunction with FAK, has been shown to play a role in regulating adhesion dynamics at the trailing edge in migrating fibroblasts. Knockdown of PDZ-RhoGEF, or FAK, inhibits tail retraction downstream of LPA, in a process that also requires ROCKII, but not Dia1.¹⁰⁷ FAK binds to, and phosphorylates PDZ-RhoGEF, and they both colocalize at FAs, which suggest that activation of FAK and PDZ-RhoGEF are closely coupled.^{106,107} The effect of PDZ-RhoGEF depletion can be attributed to impaired FA turnover, and/or a decrease in Rho-generated contractility in these cells.¹⁰⁷ These results suggest that FAK and PDZ-RhoGEF stimulate RhoA-dependent contractility at the trailing edge in response to LPA. There is evidence that another RGS-GEF, p115RhoGEF/Lsc, also plays a role in tail retraction, as Lsc $-/-$ marginal-zone B (MZB) cells are unable to release properly from integrin-mediated adhesions. As a result, they develop long trailing tails and migration to sphingosine 1-phosphate is severely impaired.¹³⁵

In an interesting story that highlights another layer of the intricate relationship between Rac and Rho, Frost and colleagues have shown that Net1 plays a role in the regulation of adhesion formation and contractility.^{105,136} Net1 is one of only two RhoGEFs that localize to the nucleus at steady-state and must be relocated to the cytosol in order to activate RhoA.¹³⁷ A recent report found that active Rac1 promotes the translocation of Net1 out of the nucleus and the subsequent activation of RhoA in the cytosol, and that Net1 relocalization is necessary for efficient adhesion and spreading.¹³⁶ In the absence of Net1, cells are more elongated and spindly and have decreased levels of pMLC, suggesting a role for Net1 in contractility and tail retraction.^{105,136} Net1 is also required for LPA-mediated RhoA activation and regulates cell migration in breast and gastric cancer cells.^{105,138,139} Net1 localizes in puncta that co-localize with active FAK at FA along the leading and trailing edges.¹⁰⁵ Targeting to FAs is mediated

through direct association with FAK, and when Net1 is silenced, cells have spreading defects with a reduced number and size of FAs. Net1 binding to FAK is enhanced by RhoA activation and inhibited when FAK, Src, or contractility is inhibited.¹⁰⁵ This suggests a feedback loop mechanism in which Net1 is recruited to FA by FAK, where it activates RhoA and stimulates contractility, which in turn, results in additional Net1 recruitment and enhanced FAK activation. Net1 is not the only RhoA-GEF that responds to LPA, as several others have been described, including p115-RhoGEF, PDZ-RhoGEF, and LARG.^{140,141} Thus, it is likely that LPA controls different aspects of cell migration by simultaneously activating multiple RhoA-GEFs.

Migration in 3D

One of the limitations in the migration field is that many of the key concepts about cell migration were established by studying cells on rigid two-dimensional surfaces (2D). Over the past decade, there has been a shift to study cell motility in three-dimensional (3D) environments, which represent more physiological *in vivo* conditions. These studies have revealed substantial differences between 2D and 3D migration. Cells migrating on 2D use actin polymerization to extend the leading edge of the plasma membrane during lamellipodia-based migration. This mesenchymal-type movement can also be seen in 3D environments and is characterized by an elongated morphology, resulting from Rac-dependent protrusions at the leading edge. In a 3D environment, cells can utilize other mechanisms of migration that are usually not observed in 2D. Studies in normal and tumor cells have shown that cells can switch between different modes of migration depending on the properties of the extracellular environment.^{142,143} Tumor cells can move in 3D in either mesenchymal or amoeboid fashion in a process controlled mainly by Rac and RhoA, respectively.^{142,144} Amoeboid cells are characterized by a rounded, less adhesive phenotype, which displays high levels of actomyosin contractility downstream of RhoA-ROCK, and can squeeze through the matrix by deforming the cell body, in a proteolysis-independent manner.^{142,144,145} Importantly, these two types of movement are interconvertible or plastic, and cells can undergo amoeboid-to-mesenchymal and mesenchymal-to-amoeboid transitions.

The regulation of Rac1 activity is central to regulate the switch between amoeboid and mesenchymal movement. Activation of Rac promotes mesenchymal movement and suppresses the amoeboid phenotype.^{146,147} In A375M2 cells, Rac1 is activated by DOCK3.^{147,148} Silencing DOCK3 expression (but not DOCK180) inhibits the mesenchymal phenotype.¹⁴⁷ DOCK3 association with NEDD9, a member of the p130Cas family, is required to activate Rac, which signals through WAVE2 to drive mesenchymal movement.^{146,147} WAVE2 is a member of the WASP family of proteins, which regulates actin assembly through the activation of the Arp2/3 complex (Fig. 2).

Besides promoting mesenchymal movement through actin assembly, Rac suppresses amoeboid movement by inhibiting actomyosin contractility. Silencing Rac expression leads to

increased phosphorylation of MLC and faster amoeboid movement.¹⁴⁷ This requires DOCK3, NEDD9, and the Rac effector WAVE2, but not PAK. WAVE2 acts downstream of Rac to suppress MLC phosphorylation, and thus, inhibit actomyosin contractility. NEDD9 also contributes to inhibiting contractility by inactivating ROCKII in a Src and integrin β 3-dependent manner.¹⁴⁹ Conversely, in rounded cells, Rac activity is kept low through the activation of a Rac-specific GAP, ARHGAP22, in a process that depends on actin contractility.¹⁴⁷ Activation of ARHGAP22 is RhoA and ROCK-dependent, but it does not seem to involve direct phosphorylation of the GAP by ROCK (Fig. 2).¹⁴⁷

Very little is known about the identity of the GEFs that regulate RhoA during amoeboid migration, but a recent report suggests that Net1 may be important.¹⁰⁵ In the absence of Net1, amoeboid invasion is inhibited in MDA-MB-231 cells, and the majority of the cells switch to a mesenchymal morphology. The increase in mesenchymal phenotype is accompanied by the upregulation of MT1-MMP, which is characteristic of this type of migration.¹⁰⁵ GEF-H1 has also been associated to the regulation of RhoA during amoeboid migration. In the absence of GEF-H1, cell migration and invasion in 3D are inhibited.^{126,150} Supporting these results, Eitaki and colleagues have shown that, when they treat human gastric adenocarcinoma cells with the anti-cancer drug vincristine, a microtubule depolymerization agent, GEF-H1 is activated and promotes amoeboid migration in a RhoA-ROCK-dependent manner.¹⁵¹ GEF-H1 has been recently shown to respond to cellular tension and ECM stiffness,^{150,152} suggesting that different matrix environments may control its activity and determine the type of migration utilized. Finally, LARG has been associated to amoeboid migration in 3D matrices.¹⁵³ In this study, the RhoA effector Dia1 was found to associate and activate LARG downstream of LPA, but not other RGS-GEFs. Both LARG and Dia1 are required for LPA-mediated RhoA activation, and silencing LARG or Dia1 expression decreases ROCK activity, pMLC, and invasion of MDA-MB-435 cells through matrigel containing LPA. LARG or Dia1 knockdown has no effect on 2D migration, which is slightly increased compared with control cells.¹⁵³ These results describe a novel role for Dia1 functioning upstream of RhoA through its interaction with LARG, in addition to its role as a RhoA downstream effector, and establish a positive feedback loop that may play a role in the regulation of tumor cell invasion (Fig. 2).

Cdc42 has been typically associated with mesenchymal-type migration in 2D, where it regulates cell polarization in the direction of migration.¹⁵⁴ However, Gadea and colleagues have recently shown that Cdc42 also plays a role in amoeboid migration.¹⁵⁵ Using a siRNA library, they identified DOCK10 as a GEF involved in the regulation of amoeboid migration in A375M2 cells, a melanoma cell line which exhibits predominantly a rounded phenotype.¹⁵⁵ DOCK10 is a Cdc42-specific GEF that is expressed in several tissues.^{155,156} When DOCK10 expression is silenced, a significant number of cells switch to an elongated, polarized morphology that moves in a mesenchymal fashion. These phenotypic changes correlated with a decrease in

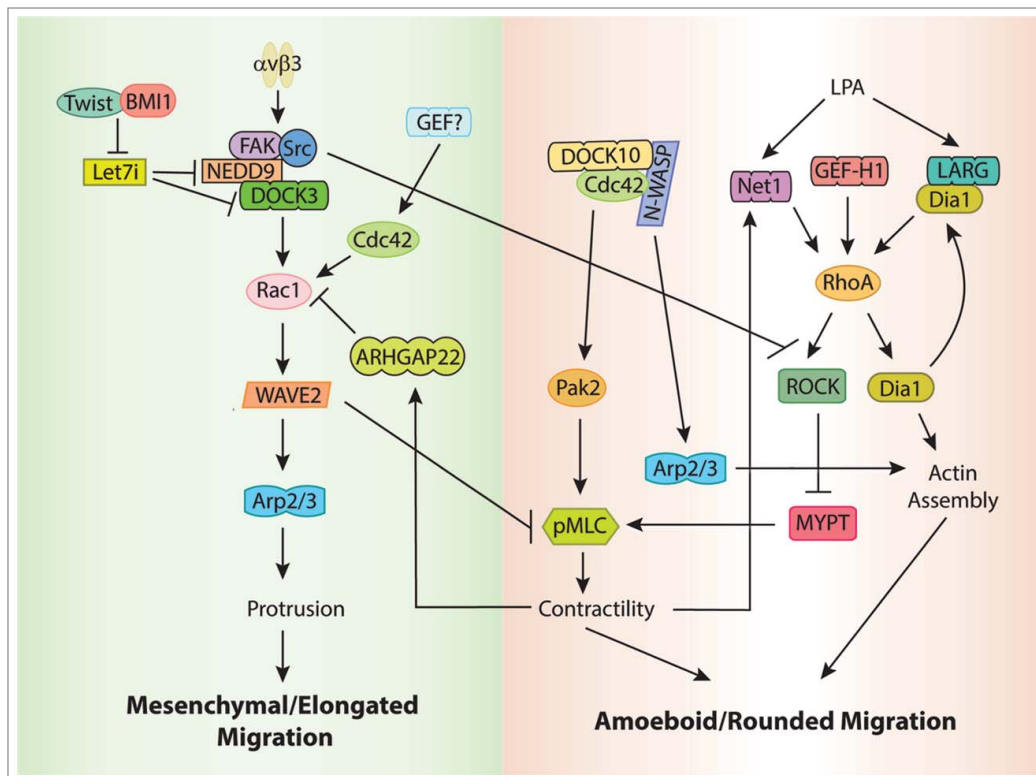


Figure 2. RhoGEF signaling pathways involved in 3D migration. During mesenchymal movement, Rac1 is activated by DOCK3. DOCK3 association with NEDD9 is required to activate Rac1, which signals through WAVE2 to drive mesenchymal movement and regulates actin assembly through the activation of the Arp2/3 complex. Twist1 and BMI1 negatively regulate the expression of the miRNA let-7i, which results in NEDD9 and DOCK3 upregulation and Rac1 activation. Activation of Rac1 suppresses amoeboid movement by inhibiting actomyosin contractility in a WAVE-dependent manner. Integrin-mediated Src activation also inhibits contractility and amoeboid migration by phosphorylating and inhibiting ROCK. In contrast, during amoeboid movement contractility activates a Rac1-GAP, ARHGAP22, which inhibits Rac and suppresses mesenchymal movement. Amoeboid migration is characterized by rounded cells, and high levels of actomyosin contractility downstream of RhoA-ROCK. The RhoA-GEFs Net1, GEF-H1, and LARG have all been associated with amoeboid migration. A positive feedback loops involving Net1 and DOCK10, in a pathway that involves both PAK2 and WASP.

probably regulating actin assembly. As expected, this study shows that interfering with Cdc42 also affects mesenchymal-type movement, and suggests that Cdc42 is required for both amoeboid and mesenchymal morphology.¹⁵⁵ However, the activation of Cdc42 by DOCK10 functions specifically during amoeboid movement and the Cdc42-GEFs required for mesenchymal morphology and migration have not been identified yet (Fig. 2).

Who Controls Cdc42?

Cdc42 has been recognized as the master of polarity, so it is really surprising that the identity of the Rho-GEFs that control its localized activation during polarization has remained elusive.¹⁵⁴ There are some isolated examples of Cdc42-specific GEFs that regulate polarity migration, which include βPix, Tuba, FGD1, FGD4, Ect2, and the Cdc42-specific DOCK proteins (DOCK6–11).^{7,34,158–161} However, most of these reports describe a specific situation, cell type, or a particular type

of migration that has not been yet validated as a conserved mechanism.

RhoA activity and contractility, and higher levels of active Rac1, an antagonistic effect that has been extensively documented.¹¹ The signaling pathway downstream DOCK10 and Cdc42 that regulates contractility involves PAK2 and MLC. Silencing the Cdc42 effectors PAK2 and N-WASP (but not others) phenocopies the effects of DOCK10 KD, with formation of elongated cells.¹⁵⁵ Conversely, overexpression of both PAK2 and N-WASP stimulates mesenchymal to amoeboid transition. However, only PAK2 induce pMLC phosphorylation. PAK2 has been previously shown to phosphorylate MLC in a Cdc42-dependent manner to induce contractility.¹⁵⁷ The role of N-WASP during amoeboid migration is still not clear, but N-WASP was found in a complex with DOCK10 supporting the idea that a signaling complex that includes the RhoGEF and the effector may function to confer selectivity to a particular GTPase pathway.^{33,155} These results suggest that both PAK2 and N-WASP function downstream of Cdc42 and regulate parallel pathways, the former involved in phosphorylation of MLC and contractility, and the latter

of migration that has not been yet validated as a conserved mechanism.

Conclusions and Future Directions

As we make progress in the understanding of these processes, we seem to have gotten to a point in which there are more new questions than answers. It appears that several GEFs can control the same processes and we still don't understand the subtleties of the system. Are all the GEFs associated with contractility required to control tail retraction? Do they act in different pathways converging into the same process? Do they synergize or act redundantly? Does the loss of one induce the compensation by others? What are the differences between 3D and 2D migration? Maybe our readouts are not sensitive enough to distinguish the specificity of each GEF. Maybe there are cell-specific, substrate-specific, or context-specific effects that we don't understand.

Maybe the conditions used *in vitro* eliminate some of the differences that occur in a more complex *in vivo* environment. We are only starting to shed light on some of these questions. Some of these questions can be addressed by analyzing several GEFs simultaneously and trying to dissect both their temporal and spatial regulation in relation to each other. Another aspect that needs to be addressed is the fact that many of these proteins function in “signaling units” that comprise GEFs, GTPases, effectors, adaptor proteins, and sometimes even GAPs. These signaling units can recruit different components dependent on the stimulus. For example, the same GEF may bind a different scaffold to associate with a different GTPase and effector (see β Pix/Scribble/Cdc42, β Pix/GIT/PAK/Rac above). That means that in order to understand the role of a particular GEF, we need to identify the components of its particular signal unit that are associated with a specific function. Our future efforts should be focused on trying

to address some of these exciting challenges and will enhance our understanding of this complex network of interactions that control cell migration.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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