



King's Research Portal

DOI:

[10.1080/21541248.2016.1232583](https://doi.org/10.1080/21541248.2016.1232583)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Haga, R. B., & Ridley, A. J. (2016). Rho GTPases: Regulation and roles in cancer cell biology: Regulation and roles in cancer cell biology. *Small GTPases*, 7(4), 207-221. <https://doi.org/10.1080/21541248.2016.1232583>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Rho GTPases: regulation and roles in cancer cell biology

Raquel B. Haga and Anne J. Ridley

Randall Division of Cell and Molecular Biophysics, King's College London, Guy's
Campus, London SE1 1UL, UK

Abstract

Rho GTPases are well known for their roles in regulating cell migration, and also contribute to a variety of other cellular responses. They are subdivided into two groups: typical and atypical. The typical Rho family members, including RhoA, Rac1 and Cdc42, cycle between an active GTP-bound and inactive GDP-bound conformation, and are regulated by GEFs, GAPs and GDIs, whereas atypical Rho family members have amino acid substitutions that alter their ability to interact with GTP/GDP and hence are regulated by different mechanisms. Both typical and atypical Rho GTPases contribute to cancer progression. In a few cancers, RhoA or Rac1 are mutated, but in most cancers expression levels and/or activity of Rho GTPases is altered. Rho GTPase signaling could therefore be therapeutically targeted in cancer treatment.

Introduction

The Rho family of GTPases is part of the Ras superfamily. Rho GTPases are highly conserved and found in nearly all eukaryotes. They contribute to several cellular processes including organisation of the actin and microtubule cytoskeletons, regulation of gene expression, vesicle trafficking, cell cycle progression, cell morphogenesis, cell polarity and cell migration ¹. Furthermore, Rho GTPases also play an important role in pathological processes including cancer progression, inflammation and wound repair ².

After cells are stimulated by extracellular factors such as soluble molecules, adhesive interactions or mechanical stresses, Rho GTPases can be activated and initiate signalling cascades through a wide range of effectors or targets including kinases and scaffold/adaptor-like proteins (Figure 1). The activation of Rho GTPases (GTP-bound form for the typical proteins) leads to changes in their conformation, increasing their ability to bind to effectors ^{3,4}.

The human Rho GTPase family consists of 20 proteins that are divided into 8 subfamilies, which are classified as typical or atypical depending on their mode of regulation (Figure 2) ⁵. Rac, Rho, Cdc42 and RhoF/RhoD subfamilies are considered typical because they act as molecular switches, cycling between an active GTP-bound form and an inactive GDP-bound form. The ratio of GTP-bound form/GDP-bound form is regulated by guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs) (Figure 3). The atypical Rho GTPases are predominantly GTP-bound, and so far there is no evidence that they are regulated by GEFs or GAPs. However, like several typical proteins, there is good evidence that atypical proteins are regulated by other mechanisms, including expression and post-translational modifications, and that their function involves their additional domains that are not found in typical Rho proteins ⁶. For

example, RhoBTB proteins have broad complex, tramtrack, bric à brac (BTB) domains that are able to interact with cullin3, a scaffold protein of ubiquitin ligase complexes involved in protein ubiquitination ⁷.

All Rho GTPases apart from the RhoBTB subfamily have been reported to alter cell morphology and/or the cytoskeleton when their expression is increased or reduced in cultured cells or model organisms. Here we describe how their activity is regulated, and their functions, particularly relating to cancer.

Regulation of typical Rho GTPases by GEFs, GAPs and GDIs

The three main types of proteins that regulate the activity of typical Rho GTPases in cells, GEFs, GAPs and GDIs (Figure 3), are often altered at the expression level in cancers, and some are mutated in a subset of cancers ⁸⁻¹¹.

GEFs are proteins that accelerate release of bound GDP that is replaced with GTP, activating the GTPase. RhoGEFs are divided into two unrelated families: Dbl-homology (DH) domain family and Dock Homology Region (DHR) domain family. Most RhoGEFs are part of the DH family and they consist of a catalytic Dbl-homology (DH) domain followed by a pleckstrin homology (PH) domain. The DH domain catalyzes the exchange of GDP for GTP and the PH domain is believed to play a role in localising Dbl proteins to plasma membranes, and/or to affect the catalytic activity of the DH domain ^{9, 12}. GEFs of the DHR family have two conserved domains called DHR-1 and DHR-2. The DHR-2 domain interacts with the nucleotide-free form of Rho GTPases and forms an intermediate in the catalytic reaction for exchange of GDP to GTP ^{8, 13}.

GTPase-activating factors (GAPs) are proteins that inactivate Rho GTPases by promoting the hydrolysis of GTP: they provide an essential catalytic group that accelerates the intrinsic GTPase activity of the GTPases. The human genome encodes around 80 RhoGAPs. However, less than half of them have been studied so far. RhoGAPs have a GAP domain of about 150 amino acids with a highly conserved arginine in a loop structure (arginine “finger”). This domain alone can bind to GTP-bound Rho proteins and catalyze their GTPase activity ^{14, 15}.

Guanine nucleotide dissociation inhibitors (GDIs) control the cycling of some Rho GTPases between cytosol and membranes; and regulate the activation and inactivation of Rho GTPases. There are three RhoGDIs in humans: RhoGDI1-3. RhoGDIs consist of a N-terminal domain that interacts with the switch 1 and switch 2 domains of Rho GTPases, restricting the flexibility that is important for the GDP/GTP cycling; and a C-terminal domain that includes the geranylgeranyl-binding pocket that is important to extract geranylgeranylated Rho GTPases from the membrane ^{11, 16}. RhoGDIs inhibit the activity of Rho GTPases by extracting them from their sites of action in membranes, holding them in an

inactive form in the cytosol. On the other hand, they can act as chaperones to take Rho GTPases between membranes, and so may contribute to their activation in some cases ^{11, 17}. Finally, RhoGDIs have also been shown to protect some Rho GTPases from proteasomal degradation ¹⁸, presumably by binding to them and preventing their interaction with ubiquitin ligases.

Not all Rho GTPases interact with RhoGDIs ^{11, 19}, and the interaction can also be regulated by post-translational modifications ²⁰. In addition, a Rac1 splice variant Rac1b has enhanced intrinsic guanine nucleotide exchange, impaired GTPase reaction and does not interact with RhoGDIs, leading to accumulation of the GTP-bound conformation of Rac1b in cells ^{21, 22}.

Regulation of Rho GTPases by post-translational modifications

In addition to cycling between GTP- and GDP-bound conformations, Rho GTPases are regulated by post-translation modifications, including lipid modifications, phosphorylation, ubiquitination and SUMOylation (Figure 3).

Most Rho GTPases undergo post-translational lipid modification at their C-terminal CAAX motif (where C represents cysteine, A is an aliphatic amino acid, and X is a terminal amino acid). This motif is post-translationally prenylated on the cysteine, either by a farnesyl or geranylgeranyl isoprenoid lipid, followed by proteolytic removal of the three C-terminal (AAX) amino acids and methylation of the prenylated cysteine. These modifications are important for the translocation of Rho GTPases to the plasma membrane and/or endomembranes and are required for their biological activity ²³. Another modification that occurs near the C-terminus of several Rho GTPases is palmitoylation. For example, RhoU and RhoV do not have a functional CAAX motif, but instead have a C-terminal CFV motif that can be palmitoylated and target these proteins to membranes. A Cdc42 splice variant is a brain-specific isoform that can be palmitoylated (Cdc42-palm) instead of or in addition to being prenylated (Cdc42-prenyl). This change in lipid modification has an effect on protein localisation and function ^{24, 25}.

Unlike all other Rho GTPases, the RhoBTB subfamily lack a CAAX motif and they are localized mainly in the cytoplasm ²⁶. So far there is no evidence that RhoBTB proteins are palmitoylated, but palmitoylation cannot be identified by amino acid sequence and requires biochemical analysis ²⁷.

As well as lipid modifications, Rho GTPases undergo several other types of covalent modifications that can regulate the proteins in a positive (activation) or negative (inactivation/degradation) way (Figure 3).

Ubiquitination is the covalent attachment of an ubiquitin to lysine residues in the target protein. This modification often leads to protein degradation. However, it can also change

the localisation of proteins or their activity²⁸. Ubiquitination of Rho GTPases can be triggered by bacterial toxins or growth factors²⁹, but other factors could be involved as well. Several Rho GTPases have been reported to undergo ubiquitination, including RhoA, Rac1, Rac1b, Cdc42, RhoB and RhoBTB2^{20, 30, 31}. Ubiquitination has been proposed to be a mechanism to control the local activity of Rho GTPases^{30, 32, 33} and it can affect either the GTP-bound form (active) or GDP-bound form (inactive), or both. For example, Rac1 is ubiquitinated only when in the active form and bound to the plasma membrane^{31, 34}. However, RhoA can be ubiquitinated in different conformations by different mechanisms. Nucleotide-free RhoA and GDP-RhoA are substrates of Smurf1 ubiquitin ligase³⁵ while only GDP-RhoA is a substrate of the BACURD-cullin3 complex³⁶. Recently, it has been proposed that GDP-RhoA and GTP-RhoA are substrates of SCF^{FBXL19} E3 ubiquitin ligase, facilitated by Erk2-mediated phosphorylation of RhoA³⁷. In all cases, the ubiquitination of RhoA leads to degradation by the proteasome.

SUMO is a ubiquitin-like protein that is similarly covalently linked to lysine residues. The only Rho GTPase that is known to be regulated by SUMOylation is Rac1, which becomes more active following addition of SUMO close to its C-terminus³⁸

Phosphorylation is another covalent modification that regulates Rho GTPases. Some protein kinases such as PKA, ROCK1, Src and Akt have been shown to phosphorylate RhoA, Rnd3 (RhoE), Cdc42 and Rac1, respectively²⁰. In most cases, phosphorylation seems to have a negative effect on the activity of Rho GTPases. RhoA can be phosphorylated on serine 188 (Ser188) by PKA and this allows RhoGDIs to bind with higher affinity to RhoA, thereby increasing its translocation from membranes to the cytosol. Although binding to RhoGDIs inhibits RhoA activity, the interaction can protect RhoA from ubiquitin-mediated degradation^{39, 40}. Phosphorylation of Rnd3 by ROCK1 and PKC on 7 Ser/Thr residues increases protein stability and translocates Rnd3 to the cytosol which reduces its activity in inducing stress fibre disruption^{19, 41}. Rac1 is phosphorylated on serine 71 (Ser71) by Akt and threonine 108 (Thr108) by ERK. Phosphorylation of Ser71 appears to decrease GTP-binding without affecting GTPase activity of Rac1 while phosphorylation of Thr108 inhibits interaction of Rac1 with PLC γ 1 and induces translocation of Rac1 to the nucleus^{42, 43}.

Transcriptional and post-transcriptional regulation of Rho GTPases

Expression of the best-characterized 'classical' Rho GTPases, RhoA, Rac1 and Cdc42, does not vary much between different tissues. However, some Rho GTPases are restricted to particular tissues and in some cases, specific stimuli increase their expression or genotoxic stress^{45, 151}. Atypical Rho GTPases have also been shown to be regulated in a transcriptional level. For example, expression of *Rnd3* is induced after Raf activation⁴⁶, and expression of *RhoU* is increased by Wnt-1 and Notch1^{47, 48}.

Rho GTPases undergo post-transcriptional regulation (Figure 3). microRNAs (miRNAs) are non-coding RNA molecules that can control the expression of mRNAs. These short sequences silence target genes by either inhibiting translation or degrading mRNA. Several Rho GTPases can be regulated by miRNAs. Most of the work on miRNAs has been done in cancer models, showing how the regulation of Rho GTPase expression by miRNAs can affect cancer progression ⁴⁹. For example, it has been shown that RhoA is target of miRNA-155 ^{50, 51} and miRNA-125a-3p ⁵²; RhoB is a target of miRNA-21 ⁵³; RhoBTB1 is a target of miRNA-31 ⁵⁴; and Cdc42 is a target of miRNA-29 ⁵⁵ and miRNA-137 ⁵⁶. Sometimes the same miRNA can target two different Rho GTPases. miRNA-185 has been reported to decrease the levels of RhoA and Cdc42, leading to inhibition of proliferation in human colorectal cancer cells ⁵⁷.

Rho GTPases and the actin cytoskeleton

A highly conserved function of Rho GTPases is the control of the actin cytoskeleton (Figure 1). Several cellular processes including cell migration, cell division, endocytosis and chemotaxis depend on the actin cytoskeleton ⁵⁸, and it plays a central role in cancer cell migration and invasion ⁵⁹.

Several Rho GTPases stimulate the first step of actin polymerization, known as nucleation, which involves the formation of a stable multimer of actin monomers that will function as a template to the elongation of the new filament. This is controlled by actin-nucleating proteins, including the actin-related protein 2/3 (Arp2/3) complex and formins. Each actin-nucleating protein acts in a distinctive mechanism. For example, Arp2/3 complex initiates a new actin filament that branches off an existing filament while formins promote nucleation of unbranched filaments at the barbed end of filaments ^{60, 61}.

The Arp2/3 complex is not an efficient nucleator and it requires the activity of nucleation promoting factor (NPF) proteins, including Wiskott-Aldrich Syndrome protein (WASP), neuronal WASP (N-WASP) and WASP-family verprolin-homologous protein (WAVE; also known as suppressor of cyclic AMP repressor (SCAR)) ^{60, 62}. These proteins have in common a WCA domain, which consist of a WASP-homology-2 (WH2 or W); and a central (also called cofilin-homology or connector) and acidic (CA) region. These regions are important to bring G-actin to the complex and to change the conformation of the Arp2/3 complex that leads to the initiation of actin polymerization. NPFs are regulated by several Rho GTPases, for example Cdc42 activates N-WASP, and Rac1 activates a protein complex called the WAVE-regulatory complex (WRC), that includes WAVE ⁶³⁻⁶⁵.

As well as stimulating Arp2/3 complex-mediated actin nucleation, Rho GTPases bind to and activate formins (Figure 1), which are large multidomain proteins defined by the presence of

a catalytic formin homology 2 (FH2) domain. There are eight formin families identified in humans including Dia, FMNL and FHOD, which are targets of Rho GTPases⁶⁶. Formins act both as nucleation and elongation factors. These proteins processively associate with barbed ends, allowing the addition of G-actin while inhibiting capping proteins from ending elongation^{63, 66, 67}.

Another family of proteins that control actin filament elongation is the Enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) proteins. All members of the family have an amino-terminal Ena/VASP homology 1 (EVH1) domain followed by a proline-rich central region and a carboxy-terminal Ena/VASP homology 2 (EVH2) domain. They act by binding at barbed ends of actin filaments and inhibiting filament capping by capping proteins. Ena/VASP proteins elongate filaments that have been initiated by W-based filament nucleators such as an Arp2/3/NPF complex, and interact with the WRC to enhance Rac-mediated actin polymerization⁶⁸. Ena/VASP proteins can also control actin filament branching by reducing the density of Arp2/3-dependent actin filament branches^{63, 68}.

Filaments grow until they are capped. Capping is important to control the length of the growing branches and localise where the filaments generate propulsive forces. The two main proteins involved in this process are capping protein (also known as CapZ in muscle) and gelsolin⁶⁹. While there are no direct interactions known between Rho GTPases and capping proteins, Rho GTPases regulate actin depolymerization, which is important for actin filament dynamics. The actin-depolymerizing factor (ADF)/cofilin family are involved in this step. These proteins are known to sever filaments and elevate the levels of monomeric actin. Both ADF and cofilin bind to ADP-bound subunits of the F-actin, promoting their disassembly. Cofilin can also bind to released ADP-actin monomers, controlling the recycling of disassociated actin subunits. ADF/cofilin activity is inhibited by LIMK-induced phosphorylation, and LIMK in turn is activated by the Rho effector ROCK and/or the Rac/Cdc42 effector PAK⁵⁸. Hence Rho signaling not only increases actin polymerization but reduces depolymerization. The extent of depolymerization of the actin filaments relies on several factors including the relative concentration of other actin-binding proteins^{70, 71}.

Rho GTPases and cell migration

Cancer cells can use several different modes to migrate, and indeed can transition from one mode to another during cancer progression and metastasis⁷². Single cells are able to migrate in two interchangeable modes of migration: amoeboid and mesenchymal, while cells migrating as groups can use different protrusive structures at the front^{73, 74}. Cell migration is a multistep process in which cells need to extend membrane protrusions in the cell front (lamellipodia, filopodia and/or membrane blebs), interact with the extracellular matrix or

neighbouring cells, contract the cell body and detach the cell rear from the surrounding environment (Figure 3). Rho GTPases are critical molecules in this process, sending signals from membrane receptors to the cytoskeleton and cell adhesions ⁷⁵.

RhoA has been implicated in both lamellipodium-driven and bleb-driven migration in 3D environments (Figure 4). The bleb-driven mode of migration is characterized by actomyosin-based cortical contractility mediated by Rho/ROCK signalling. ROCK acts by increasing the phosphorylation of myosin light chain, which increases the interaction of myosin II with actin filaments ⁷⁶. The consistently high cortical tension results in blebbing, which contributes to cancer cell motility ^{76, 77}. Cells using lamellipodium-driven cell migration have an elongated morphology that depends on cell adhesion dynamics and traction forces between both poles of the cell. Lamellipodia drive protrusion of the plasma membrane at the front of the cell (Figure 4). Formation of integrin-based adhesions and actomyosin-mediated contractility is controlled by Rho/ROCK signalling ^{77, 78}.

Lamellipodia are transient structures and their formation is driven by actin polymerization. The Arp2/3 complex is associated with actin filaments throughout the cell front, and together with WAVE proteins, it stimulates the formation of a “dendritic” actin network that is important for lamellipodium extension ⁷⁹. Activation of Rac is necessary for lamellipodium formation (Figure 1, Figure 4). GTP-Rac binds directly to Sra1 protein, activating the WAVE regulatory complex (WRC) (hetero-pentameric complex formed by Sra1/Cyfit1, Nap1/Hem2/Kette, Abi2, HSPC300/Brick1 and WAVE1/SCAR) ^{88, 80}. WRC activity is controlled by inhibition of the verprolin-homology, central and acidic (VCA) region. It is believed that the interaction of GTP-Rac with Sra1, and perhaps with the meander region of WAVE1 (a meandering path across a concave surface of Sra1 that is formed by five helices ($\alpha 2$ - $\alpha 6$ and a series of intervening loops)), could lead to changes in the conformation of the complex and release of the VCA region, which is important for the activation of the Arp2/3 complex ^{81, 82}.

The presence of PI(3,4,5)P₃ increases GTP-bound Rac in several cell types. It is thought that PI(3,4,5)P₃ promotes GTP loading on Rac through direct interaction with Rac GEFs. Several Rac GEFs, including Tiam-1, β -PIX and DOCK180, are known to activate Rac to induce lamellipodia. Rac activation also stimulates PI3K ⁸³, which leads to the production of PI(3,4,5)P₃. This creates a positive feedback loop, accumulating active Rac at the cell front ^{75, 79, 84}. Another example of a mechanism to control actin polymerization in the cell front is through the PAK family of serine/threonine kinases. Activation of Rac and Cdc42 activates PAKs, which then phosphorylate and activate LIM kinase (LIMK). Activation of LIMK leads to phosphorylation and inactivation of cofilin. Cofilin is an important protein to promote filament treadmilling at the leading edge ⁷⁰.

Formins are also involved in lamellipodium extension ⁶⁰. For example, it was found that the formin mDia1, a RhoA effector (Figure 1), localizes at the leading edge of some cells,

including T-cells, and it can cooperate with the Arp2/3 complex to initiate lamellipodium formation^{85, 86}. RhoA is mainly active at the cell rear, but it has been shown to be active at the cell front as well (Figure 4). During membrane protrusion, RhoA is active at the leading edge, but it is inactivated during membrane retraction⁸⁷. Activation of mDia1 by RhoA in the cell front could act together with the Arp2/3 complex to stimulate actin polymerization^{85, 86}. Some cells use traction forces to pull on the extracellular matrix, which contributes to their migration. Active RhoA is associated with focal adhesion formation (sites of contact of the cell with the extracellular matrix) and cell contractility during cell migration. One important Rho effector that is involved in actin-myosin II filament assembly is the serine/threonine kinase ROCK. Activation of ROCK leads to phosphorylation and activation of LIMK, which then phosphorylates and inactivates cofilin. ROCK also phosphorylates myosin light chain (MLC) phosphatase, inactivating it. This leads to an increase in the levels of phosphorylated myosin light chain (pMLC), which then stimulates the cross-linking of actin filaments by myosin II and generates contractile forces. Contraction promotes membrane blebbing, movement of the cell body and contributes to detachment of the cell rear^{84, 88}. Another important effector of RhoA is mDia. This effector cooperates with ROCK for the assembly of actomyosin bundles such as stress fibres. mDia is found at the front and back of the cell, and its function is dependent on the cell type and conditions during cell migration^{84, 89}. Cell polarity is important for directional migration. Formation of protrusions and retraction of the cell rear are not enough to direct the cells to a specific place. The nucleus, Golgi apparatus and microtubule-organizing centre (MTOC) also need to be repositioned towards the leading edge⁹⁰. Cdc42 has been shown to regulate MTOC positioning through recruitment of Par6 and aPKC to the leading edge⁹¹. Nuclear movement can also be regulated by Cdc42, probably through actin flow controlled by actin-myosin regulatory kinase (MRCK)⁹²⁻⁹⁴.

Functions and regulation of atypical Rho GTPases

The atypical Rho GTPases have amino acid substitutions in their Rho domains that alter their GTP/GDP cycling. They consist of four subfamilies: RhoU/RhoV subfamily, RhoH subfamily, Rnd subfamily, and RhoBTB subfamily (Figure 1). Several of them, including Rnd3, RhoBTB2 and RhoH, have been implicated in cancer progression.

RhoU (also known as Wrch1) and RhoV (also known as Chp or Wrch2) proteins have significant sequence identity to Cdc42 (~52-55%), but they contain extra N- and C- terminal extensions that are critical for their activity^{95, 96}. For example, the N-terminal domain is able to regulate RhoU and RhoV function in a negative way, a characteristic not reported for other Rho GTPases^{96, 97}. Like Cdc42, RhoU and RhoV affect the actin cytoskeleton

(lamellipodium formation and filopodium formation, respectively), cell migration and formation of focal adhesions ⁶.

RhoU has a very rapid intrinsic guanine nucleotide exchange rate compared to Cdc42, and is believed to be mostly GTP-bound in cells ⁹⁶. RhoV is assumed to have similar properties, although this has not been tested biochemically. So far, RhoU and RhoV GAPs and GEFs have not been identified; although RhoU has been reported to bind to two GAPs, it is not yet known whether they act as GAPs for RhoU ⁹⁸. Expression of RhoU and RhoV is increased at the transcriptional level by Wnt-1; and RhoU has also been shown to be induced by STAT3 and Notch1 ^{47, 48, 99, 100}. The N- and C-terminal domains of RhoU and RhoV are quite different from each other. The N-terminal domain of RhoU but not RhoV contains proline-rich domains that bind to the SH3 domains of Grb2 and Nck ¹⁰¹, and its C-terminal domain is phosphorylated on tyrosine 254 by Src ^{96, 102}. Other known binding partners for RhoU and RhoV are the PAK family of kinases, which also interact with Cdc42 and Rac ^{47, 95, 103, 104}.

RhoH, also known as TTF (translocation three four), is expressed predominantly in hematopoietic tissues, similarly to Rac2 ¹⁰⁵. RhoH is GTPase-deficient, and is therefore assumed to be constantly in a GTP-bound form. Due to the lack of intrinsic GTPase activity, RhoH is unlikely to be regulated by GAPs and GEFs. However, RhoH was reported to interact with RhoGDIs, although the functional consequence of this has not been investigated. It has also been shown to be regulated at the transcriptional level and by phosphorylation ¹⁰⁶⁻¹⁰⁸. RhoH is important in the regulation of proliferation, survival, migration and engraftment of murine hematopoietic progenitor cells ¹⁰⁹; and it has been implicated in T-cell differentiation ¹¹⁰. RhoH does not seem to have any direct effect on actin reorganization ²⁶. However, RhoH can antagonize the activity of other Rho GTPases. For example, Rac1 and RhoA-mediated activation of NFκB is inhibited by RhoH through inhibition of IκB degradation ¹⁰⁸. Loss of RhoH in murine hematopoietic progenitor cells leads to an increase in Rac1-mediated migration and cortical F-actin assembly ¹¹¹, and RhoH deletion delays progression in a murine model for chronic lymphocytic leukemia ^{112, 113}.

Like RhoH, Rnd1, Rnd2 and Rnd3 (also known as RhoE) proteins are GTPase deficient, and are therefore constitutively in the GTP-bound form ¹¹⁴. One important function of Rnd1 and Rnd3 is that they can antagonize RhoA activity by interacting with p190RhoGAP. This interaction leads to loss of stress fibres and cell rounding ^{115, 116}. Other proteins interact with Rnd proteins, which could also contribute to Rnd-induced responses ¹¹⁴.

Rnd1 and Rnd3 are mainly associated with membranes and RhoGDIs do not affect their localisation. Rnd2 seems to be localised predominantly in the cytoplasm ^{23, 114}. Since Rnd proteins have not been shown to interact with any GAPs or GEFs, these proteins should be regulated by other mechanisms such as phosphorylation, expression and localisation. In fact, it has been shown that Rnd proteins can be phosphorylated and phosphorylation (in

combination with farnesylation) allows these proteins to interact with 14-3-3 proteins. This interaction leads to translocation of Rnd proteins from the plasma membrane to the cytosol, inactivating these proteins¹⁹. Rnd3 is known to be phosphorylated on 5 serines and 1 threonine by ROCK1 and on serine 210 by PKC^{19, 41, 117}. Moreover, expression of Rnd3 is induced by activation of Raf, leading to changes in the actin cytoskeleton⁴⁶.

In mammals, RhoBTB1 and RhoBTB2 form the RhoBTB subfamily of Rho GTPases. Orthologues of RhoBTB1 and RhoBTB2 are present in vertebrates and insects, but not in plants and fungi^{118, 119}. Although RhoBTB3 shares some similarities with RhoBTB1 and RhoBTB2, this protein is not considered a Rho GTPase due to the low similarity of its Rho domain to other Rho proteins^{44, 118}. The Rho domain of RhoBTB1 and RhoBTB2 contains the amino acid motifs required for GTP-binding but is around 18 residues longer and rich in charged residues^{120, 121}. The GTP-binding domain of RhoBTBs is expected not to hydrolyse GTP, because they do not have key amino acids required for GTP hydrolysis, including a glycine equivalent to G12 and a glutamine equivalent to Q61 in Ras^{121, 122}. It has been reported that the GTP-binding domain of RhoBTB2 binds to GTP *in vitro*¹²⁰, but nothing has been published for RhoBTB1. RhoBTB1 and RhoBTB2 are also bigger than classical GTPases due to the presence of extra domains including 2 broad-complex, tramtrack, bric à brac (BTB) domains, which are conserved protein-protein domains. It has been shown so far that the BTB domains in RhoBTB proteins are important for the formation of Cullin-RING ubiquitin ligases (CRLs), the most prevalent class of E3 ubiquitin ligases^{7, 123}. Other BTB-containing proteins interact with cullin3, acting as adaptors that target proteins to the Cullin3-RING ubiquitin ligase complex (CRL3) for ubiquitination and hence degradation¹²⁴. Indeed, RhoBTB2 has been shown to be a substrate for the cullin3 ubiquitin ligase complex¹²³.

Relatively little is known about the functions of RhoBTB proteins. RhoBTB2 is involved in transporting vesicular stomatitis virus glycoprotein (VSVG) in a microtubule-dependent manner¹²⁵. It has been reported that RhoBTB2 has an active role in cell cycle progression and apoptosis through the transcription factor E2F1¹²⁶. Moreover, RNA profiling using HeLa cells has shown that RhoBTB2 affects cell cycle, apoptosis, cytoskeleton and membrane trafficking pathways¹²⁷. RhoBTB1 and RhoBTB2 were observed to localize to membrane vesicles, but the functional relevance of this is not known²⁶.

Rho GTPases in cancer

Rho GTPases are important signal transducers in signalling pathways that regulate cell migration, proliferation, survival and death. All these cellular processes are crucial for maintenance of normal tissues, but also contribute to cancer progression^{128, 129}. The development of human tumours is a multistep process that consists in dysregulation of cell

proliferation, resistance to growth suppressors, inhibition of cell death, uncontrolled replication leading to immortalization and activation of angiogenesis, invasion and metastasis¹³⁰.

Accumulation of mutations in genes that affect proliferation and survival is one of the key steps for primary tumour formation. Until recently, it was believed that Rho GTPases were rarely mutated in human tumours. However, it was found that proline 29 in Rac1 is mutated in a subset of melanomas, breast tumours and head and neck tumours¹³¹. RhoA is also mutated in some tumours including diffuse gastric cancer and angioimmunoblastic T cell lymphoma¹³²⁻¹³⁶. Although these mutations are likely to be important for tumour development, in most cases, Rho GTPases are found to be upregulated or to have their activity increased by changes in the expression of GAPs, GEFs and/or GDIs¹³⁷. Several Rho GTPases are often upregulated in human tumours including RhoA, RhoC, Rac1, Rac2, Rac3, Cdc42, RhoV and RhoF^{137, 138}.

The classical Rho GTPases, RhoA, Rac1 and Cdc42, have been shown to be important for the progression and metastasis of different human tumours including breast cancer, liver cancer, melanoma, testicular cancer and ovarian cancer¹³⁹⁻¹⁴¹.

RhoA appears to be involved in almost all stages of tumour progression. For example, in gastric cancer cell lines, RhoA is found to be hyperactive and its suppression leads to partial inhibition of the proliferation phenotype. This effect involves regulation of cell cycle through G1-S progression. Downregulation of the RhoA target mDia1 in gastric cancer cell lines leads to increase in the expression of cell cycle inhibitors p21^{Waf1/Cip1} and p27^{Kip1} while downregulation of ROCK increases the levels of another family of cell cycle inhibitors called INK4¹⁴². Several studies *in vitro* and *in vivo* suggest that RhoA has a role during tumour angiogenesis. One example is the knockout of $\text{G}\alpha_{13}$ receptors in endothelial cells in mice, which causes a decrease in tumour growth and normalization of tumour vasculature. $\text{G}\alpha_{13}$ induces expression of VEGF2 mediated by activation of RhoA which leads to transcription of NF κ B¹⁴³. Local invasion is an early step in the metastatic process by which cells need to detach from the primary tumour and migrate through the surrounding tissue. Although RhoA seems to play an active role in cancer progression, recent findings in angioimmunoblastic T cell lymphoma have shown that mutations in RhoA that lead to loss of GTPase activity are important to drive cancer progression¹⁴⁴.

RhoC has also been found to promote tumour progression while RhoB seems to act as a tumour suppressor^{145, 146}. RhoC promotes tumour progression in some tumours including melanoma, ovarian cancer and head and neck cancer¹⁴⁷⁻¹⁴⁹. RhoC is able to control invadopodium formation in tumour cells by locally regulating cofilin activity. Invadopodia are cell protrusions capable of penetrating and degrading the extracellular matrix, increasing the metastatic potential of tumour cells. RhoC activity is spatially regulated by activation of

p190RhoGEF outside the invadopodia and activation of p190RhoGAP inside the invadopodium core. This leads to restriction of cofilin activity only inside the invadopodium core¹⁵⁰. RhoB, on the other hand, is often downregulated in human tumours^{2, 151}. For example, the expression of RhoB in human lung tissues decreases going from normal tissue to invasive carcinoma. In addition, overexpression of RhoB in the lung cancer cell line A549 leads to inhibition of cell growth *in vitro* and *in vivo*¹⁵². Also, overexpression of RhoB in gastric cancer cell lines inhibits proliferation, migration and invasion; and increases chemosensitivity while overexpression of RhoA and RhoC leads to opposite effects¹⁵³.

Rac1 is another Rho GTPase that is found altered in several stages of tumour progression. Deregulation of Rac signalling can be caused by changes in the upstream signalling including tyrosine kinase receptors, PI3Ks, GEFs and GAPs. One example is the activation of Rac1 by Rac GEF P-Rex1 after stimulation of tyrosine kinase receptors and GPCRs in breast cancer cells¹⁵⁴. In colorectal tumours, the expression of a Rac1 splice variant, Rac1b, is increased in different stages of tumour progression¹⁵⁵. Expression of Rac1b has also been found in breast cancer and lung cancer^{156, 157}. Rac1b has been shown to be involved in MMP-3-mediated malignant transformation of mammary epithelial cells. MMP-3 induces the expression of Rac1b which increases the levels of cellular ROS, leading to expression of the transcription factor Snail, which induces epithelial-mesenchymal transition (EMT) as well as oxidative damage to DNA and genomic instability¹⁵⁸.

The role of Cdc42 in tumour progression may be tissue-specific. Cdc42 is found to be upregulated in many tumours including non-small cell lung cancer, colorectal adenocarcinoma, melanoma, breast cancer and testicular cancer. However, loss of Cdc42 in liver cancer leads to an increase in tumour development^{2, 159}. Some other Rho GTPases also appear to have a dual role in tumorigenesis. Rnd3 is downregulated in some tumours and acts as a tumour suppressor¹⁶⁰⁻¹⁶². However, in some cases, Rnd3 has been shown to be upregulated and mediate drug resistance in cancer cells^{163, 164}.

RhoBTB1 and RhoBTB2 have been described as tumour suppressors. RhoBTB2 is also known as 'deleted in breast cancer 2' (DBC2) and its levels are reduced in some breast cancers¹⁶⁵. RhoBTB1 is downregulated in some cancers like head and neck cancer¹⁶⁶, and breast cancer and kidney cancer¹²². The *RhoBTB2* gene has been shown to be a target of E2F1, a transcription factor involved in cell cycle and apoptosis¹²⁶ and *RhoBTB1* is a target of microRNA-31 (miR-31) in human colon cancer⁵⁴.

These examples illustrate how complex the role of Rho GTPases in tumour progression is. Their contributions are dependent on cell type, extracellular stimuli and signalling pathways involved in the particular cancer type or cancer cell line.

Conclusions and future perspective

The classical Rho GTPases, RhoA, Rac1 and Cdc42, are well studied biochemically and for their roles in cancer progression. However, much less is known of the properties of most other family members, which do not appear to be mutated significantly in human cancers. The expression and activity of several family members has, however, been shown to be changed frequently in cancer. RhoC is perhaps the best studied Rho family member in cancer invasion and metastasis¹⁴⁶. Whether all Rho family members contribute to cancer progression, or have other unique physiological functions to distinguish them from the well-studied subset of Rho proteins, remains to be discovered.

References

1. Etienne-Manneville S and Hall A. Rho GTPases in cell biology. *Nature* 2002; 420:629-635.
2. Vega FM and Ridley AJ. Rho GTPases in cancer cell biology. *FEBS Lett* 2008; 582:2093-101.
3. Hall A Rho family GTPases. *Biochem Soc Trans.* 2012; 40:1378-82.
4. Schwartz M Rho signalling at a glance. *J Cell Sci.* 2004; 117:5457-8.
5. Heasman SJ and Ridley AJ. Mammalian Rho GTPases: new insights into their functions from in vivo studies. *Nat Rev Mol Cell Biol* 2008; 9:690-701.
6. Aspenstrom P, Ruusala A, and Pacholsky D. Taking Rho GTPases to the next level: the cellular functions of atypical Rho GTPases. *Exp Cell Res* 2007; 313:3673-9.
7. Berthold J, Schenkova K, Ramos S, Miura Y, Furukawa M, Aspenstrom P, and Rivero F. Characterization of RhoBTB-dependent Cul3 ubiquitin ligase complexes--evidence for an autoregulatory mechanism. *Exp Cell Res* 2008; 314:3453-65.
8. Gadea G and Blangy A. Dock-family exchange factors in cell migration and disease. *Eur J Cell Biol* 2014; 93:466-77.
9. Cook DR, Rossman KL, and Der CJ. Rho guanine nucleotide exchange factors: regulators of Rho GTPase activity in development and disease. *Oncogene* 2014; 33:4021-35.
10. Tcherkezian J and Lamarche-Vane N. Current knowledge of the large RhoGAP family of proteins. *Biol Cell* 2007; 99:67-86.
11. Garcia-Mata R, Boulter E, and Burridge K. The 'invisible hand': regulation of RHO GTPases by RHOGDIs. *Nat Rev Mol Cell Biol* 2011; 12:493-504.
12. Rossman KL, Der CJ, and Sondek J. GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. *Nat Rev Mol Cell Biol.* 2005; 6:167-80.
13. Laurin M and Cote JF. Insights into the biological functions of Dock family guanine nucleotide exchange factors. *Genes Dev* 2014; 28:533-47.

14. Jacobs T and Hall C, *Rho GAPs — Regulators of Rho GTPases and More*. RHO Family GTPases, ed. E. Manser. 2005, Dordrecht: Springer Netherlands. 93-112.
15. Moon SY and Zheng Y. Rho GTPase-activating proteins in cell regulation. *Trends Cell Biol* 2003; 13:13-22.
16. Cherfils J and Zeghouf M. Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiol Rev* 2013; 93:269-309.
17. DerMardirossian C and Bokoch GM. GDIs: central regulatory molecules in Rho GTPase activation. *Trends Cell Biol* 2005; 15:356-63.
18. Boulter E and Garcia-Mata R. RhoGDI: A rheostat for the Rho switch. *Small GTPases*. 2010; 1:65-68.
19. Riou P, et al. 14-3-3 proteins interact with a hybrid prenyl-phosphorylation motif to inhibit G proteins. *Cell*. 2013; 153:640-53.
20. Hodge RG and Ridley AJ Regulating Rho GTPases and their regulators. *Nat Rev Mol Cell Biol* 2016; 17:496-510.
21. Fiegen D, Haeusler LC, Blumenstein L, Herbrand U, Dvorsky R, Vetter IR, and Ahmadian MR. Alternative splicing of Rac1 generates Rac1b, a self-activating GTPase. *J Biol Chem* 2004; 279.
22. Singh A, Karnoub AE, Palmby TR, Lengyel E, Sondek J, and Der CJ. Rac1b, a tumor associated, constitutively active Rac1 splice variant, promotes cellular transformation. *Oncogene* 2004; 23:9369-80.
23. Roberts PJ, et al. Rho Family GTPase modification and dependence on CAAX motif-signaled posttranslational modification. *J Biol Chem*. 2008; 283:25150-63.
24. Kang R, et al. Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. *Nature* 2008; 456:904-9.
25. Wirth A, Chen-Wacker C, Wu YW, Gorinski N, Filippov MA, Pandey G, and Ponimaskin E. Dual lipidation of the brain-specific Cdc42 isoform regulates its functional properties. *Biochem J* 2013; 456:311-22.
26. Aspenstrom P, Fransson Å, and Saras J. Rho GTPases have diverse effects on the organization of the actin filament system. *Biochem J* 2004; 377:327-337.
27. Peng T, Thinon E, and Hang HC. Proteomic analysis of fatty-acylated proteins. *Curr Opin Chem Biol* 2016; 30:77-86.
28. Xu G and Jaffrey SR. The new landscape of protein ubiquitination. *Nat Biotechnol* 2011; 29:1098-100.
29. Visvikis O, Maddugoda MP, and Lemichez E. Direct modifications of Rho proteins: deconstructing GTPase regulation. *Biol Cell* 2010; 102:377-89.
30. de la Vega M, Burrows JF, and Johnston JA. Ubiquitination: Added complexity in Ras and Rho family GTPase function. *Small GTPases* 2011; 2:192-201.

31. Nethe M and Hordijk PL. The role of ubiquitylation and degradation in RhoGTPase signalling. *J Cell Sci* 2010; 123:4011-4018.
32. Mettouchi A and Lemichez E. Ubiquitylation of active Rac1 by the E3 ubiquitin-ligase HACE1. *Small GTPases* 2012; 3:102-6.
33. Tian M, Bai C, Lin Q, Lin H, Liu M, Ding F, and Wang HR. Binding of RhoA by the C2 domain of E3 ligase Smurf1 is essential for Smurf1-regulated RhoA ubiquitination and cell protrusive activity. *FEBS Lett* 2011; 585:2199-204.
34. Visvikis O, Lores P, Boyer L, Chardin P, Lemichez E, and Gacon G. Activated Rac1, but not the tumorigenic variant Rac1b, is ubiquitinated on Lys 147 through a JNK-regulated process. *FEBS J* 2008; 275:386-96.
35. Wang H-R, Zhang Y, Ozdamar B, Ogunjimi AA, Alexandrova E, Thomsen GH, and Wrana JL. Regulation of Cell Polarity and Protrusion Formation by Targeting RhoA for Degradation. *Science* 2003; 302:1775-1779.
36. Chen Y, et al. Cullin mediates degradation of RhoA through evolutionarily conserved BTB adaptors to control actin cytoskeleton structure and cell movement. *Mol Cell* 2009; 35:841-55.
37. Wei J, Mialki RK, Dong S, Khoo A, Mallampalli RK, Zhao Y, and Zhao J. A new mechanism of RhoA ubiquitination and degradation: roles of SCF(FBXL19) E3 ligase and Erk2. *Biochim Biophys Acta* 2013; 1833:2757-64.
38. Castillo-Lluva S, Tatham MH, Jones RC, Jaffray EG, Edmondson RD, Hay RT, and Malliri A. SUMOylation of the GTPase Rac1 is required for optimal cell migration. *Nat Cell Biol* 2010; 12:1078-85.
39. Rolli-Derkinderen M, Sauzeau V, Boyer L, Lemichez E, Baron C, Henrion D, Loirand G, and Pacaud P. Phosphorylation of serine 188 protects RhoA from ubiquitin/proteasome-mediated degradation in vascular smooth muscle cells. *Circ Res* 2005; 96:1152-60.
40. Ellerbroek SM, Wennerberg K, and Burridge K. Serine phosphorylation negatively regulates RhoA in vivo. *J Biol Chem* 2003; 278:19023-31.
41. Riento K, Totty N, Villalonga P, Garg R, Guasch R, and Ridley AJ. RhoE function is regulated by ROCK I-mediated phosphorylation. *EMBO J* 2005; 24:1170-1180.
42. Kwon T, Kwon DY, Chun J, Kim JH, and Kang SS. Akt Protein Kinase Inhibits Rac1-GTP Binding through Phosphorylation at Serine 71 of Rac1. *J Biol Chem* 2000; 275:423-428.
43. Tong J, Li L, Ballermann B, and Wang Z. Phosphorylation of Rac1 T108 by extracellular signal-regulated kinase in response to epidermal growth factor: a novel mechanism to regulate Rac1 function. *Mol Cell Biol* 2013; 33:4538-51.
44. Wennerberg K and Der CJ. Rho-family GTPases: it's not only Rac and Rho (and I like it). *J Cell Sci* 2004; 117:1301-1312.

45. Fritz G, Kaina B, and Aktories K. The Ras-related Small GTP-binding Protein RhoB Is Immediate-early Inducible by DNA Damaging Treatments. *J Biol Chem* 1995; 270:25172-25177.
46. Hansen SH, Zegers MMP, Woodrow M, Rodriguez-Viciano P, Chardin P, Mostov KE, and McMahon M. Induced Expression of Rnd3 Is Associated with Transformation of Polarized Epithelial Cells by the Raf–MEK–Extracellular Signal-Regulated Kinase Pathway. *Mol Cell Biol* 2000; 20:9364-9375.
47. Tao W, Pennica D, Xu L, Kalejta RF, and Levine AJ. Wrch-1, a novel member of the Rho gene family that is regulated by Wnt-1. *Genes Dev* 2001; 15:1796-1807.
48. Bhavsar PJ, Infante E, Khwaja A, and Ridley AJ. Analysis of Rho GTPase expression in T-ALL identifies RhoU as a target for Notch involved in T-ALL cell migration. *Oncogene* 2013; 32:198-208.
49. Liu M, Bi F, Zhou X, and Zheng Y. Rho GTPase regulation by miRNAs and covalent modifications. *Trends Cell Biol* 2012; 22:365-73.
50. Bijkerk R, et al. MicroRNA-155 Functions as a Negative Regulator of RhoA Signaling in TGF- β -induced Endothelial to Mesenchymal Transition. *MicroRNA* 2012; 1:2-10.
51. Kong W, Yang H, He L, Zhao JJ, Coppola D, Dalton WS, and Cheng JQ. MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. *Mol Cell Biol* 2008; 28:6773-84.
52. Huang B, Luo W, Sun L, Zhang Q, Jiang L, Chang J, Qiu X, and Wang E. MiRNA-125a-3p is a negative regulator of the RhoA-actomyosin pathway in A549 cells. *Int J Oncol* 2013; 42:1734-42.
53. Liu M, Tang Q, Qiu M, Lang N, Li M, Zheng Y, and Bi F. miR-21 targets the tumor suppressor RhoB and regulates proliferation, invasion and apoptosis in colorectal cancer cells. *FEBS Lett* 2011; 585:2998-3005.
54. Xu RS, et al. The tumor suppressor gene RhoBTB1 is a novel target of miR-31 in human colon cancer. *Int J Oncol* 2013; 42:676-82.
55. Park SY, Lee JH, Ha M, Nam JW, and Kim VN. miR-29 miRNAs activate p53 by targeting p85 α and CDC42. *Nat Struct Mol Biol* 2009; 16:23-9.
56. Chen Q, Chen X, Zhang M, Fan Q, Luo S, and Cao X. miR-137 is frequently down-regulated in gastric cancer and is a negative regulator of Cdc42. *Dig Dis Sci* 2011; 56:2009-16.
57. Liu M, Lang N, Chen X, Tang Q, Liu S, Huang J, Zheng Y, and Bi F. miR-185 targets RhoA and Cdc42 expression and inhibits the proliferation potential of human colorectal cells. *Cancer Lett* 2011; 301:151-60.
58. Jaffe AB and Hall A. Rho GTPases: biochemistry and biology. *Annu Rev Cell Dev Biol* 2005; 21.

59. Fife CM, McCarroll JA, and Kavallaris M. Movers and shakers: cell cytoskeleton in cancer metastasis. *Br J Pharmacol* 2014; 171:5507-23.
60. Firat-Karalar EN and Welch MD. New mechanisms and functions of actin nucleation. *Curr Opin Cell Biol* 2011; 23:4-13.
61. Goley ED and Welch MD. The ARP2/3 complex: an actin nucleator comes of age. *Nat Rev Mol Cell Biol* 2006; 7:713-26.
62. Campellone KG and Welch MD. A nucleator arms race: cellular control of actin assembly. *Nat Rev Mol Cell Biol* 2010; 11:237-51.
63. Dominguez R. Actin filament nucleation and elongation factors-structure-function relationships. *Crit Rev Biochem Mol Biol* 2009; 44:351-66.
64. Ladwein M and Rottner K. On the Rho'd: the regulation of membrane protrusions by Rho-GTPases. *FEBS Lett* 2008; 582:2066-74.
65. Suetsugu S. Activation of nucleation promoting factors for directional actin filament elongation: allosteric regulation and multimerization on the membrane. *Semin Cell Dev Biol* 2013; 24:267-71.
66. Schonichen A and Geyer M. Fifteen formins for an actin filament: a molecular view on the regulation of human formins. *Biochim Biophys Acta* 2010; 1803:152-63.
67. Breitsprecher D and Goode BL. Formins at a glance. *J Cell Sci* 2013; 126:1-7.
68. Chen XJ, et al. Ena/VASP proteins cooperate with the WAVE complex to regulate the actin cytoskeleton. *Dev Cell* 2014; 30:569-84.
69. Pollard TD and Borisy GG. Cellular Motility Driven by Assembly and Disassembly of Actin Filaments. *Cell* 2003; 112:453-465.
70. Bamburg JR and Bernstein BW Roles of ADF/cofilin in actin polymerization and beyond. *F1000 Biol Rep.* 2010; 2:62.
71. Dos Remedios CG, Chhabra D, Kekic M, Dedova IV, Tsubakihara M, Berry DA, and Nosworthy NJ. Actin Binding Proteins: Regulation of Cytoskeletal Microfilaments. *Physiological Reviews* 2003; 83:433-473.
72. Friedl P and Alexander S. Cancer invasion and the microenvironment: plasticity and reciprocity. *Cell* 2011; 147:992-1009.
73. Sanz-Moreno V and Marshall CJ. The plasticity of cytoskeletal dynamics underlying neoplastic cell migration. *Curr Opin Cell Biol* 2010; 22:690-6.
74. Mayor R and Etienne-Manneville S. The front and rear of collective cell migration. *Nat Rev Mol Cell Biol* 2016; 17:97-109.
75. Ridley AJ Rho GTPase signalling in cell migration. *Curr Opin Cell Biol* 2015; 36:103-12.
76. Julian L and Olson MF. Rho-associated coiled-coil containing kinases (ROCK). *Small GTPases.* 2014; 5:e29846.

77. Pankova K, Rosel D, Novotny M, and Brabek J. The molecular mechanisms of transition between mesenchymal and amoeboid invasiveness in tumor cells. *Cell Mol Life Sci* 2010; 67:63-71.
78. Friedl P and Wolf K. Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer* 2003; 3:362-74.
79. Ridley AJ. Life at the leading edge. *Cell* 2011; 145:1012-22.
80. Krause M and Gautreau A. Steering cell migration: lamellipodium dynamics and the regulation of directional persistence. *Nat Rev Mol Cell Biol* 2014; 15:577-90.
81. Chen B, et al. The WAVE regulatory complex links diverse receptors to the actin cytoskeleton. *Cell* 2014; 156:195-207.
82. Chen Z, et al. Structure and control of the actin regulatory WAVE complex. *Nature* 2010; 468:533-8.
83. Fritsch R, de Krijger I, Fritsch K, George R, Reason B, Kumar MS, Diefenbacher M, Stamp G, and Downward J. RAS and RHO families of GTPases directly regulate distinct phosphoinositide 3-kinase isoforms. *Cell* 2013; 153:1050-63.
84. Raftopoulou M and Hall. A Cell migration: Rho GTPases lead the way. *Dev Biol* 2004; 265:23-32.
85. Heasman SJ, Carlin LM, Cox S, Ng T, and Ridley AJ. Coordinated RhoA signaling at the leading edge and uropod is required for T cell transendothelial migration. *J Cell Biol* 2010; 190:553-63.
86. Isogai T, van der Kammen R, Leyton-Puig D, Kedziora KM, Jalink K, and Innocenti M. Initiation of lamellipodia and ruffles involves cooperation between mDia1 and the Arp2/3 complex. *J Cell Sci* 2015; 128:3796-810.
87. Pertz O. Spatio-temporal Rho GTPase signaling – where are we now? *J Cell Sci* 2010; 123:1841-1850.
88. Ananthakrishnan R and Ehrlicher A. The Forces Behind Cell Movement. *Int J Biol Sci* 2007; 3:303-317.
89. Narumiya S, Tanji M, and Ishizaki T. Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. *Cancer Metastasis Rev* 2009; 28:65-76.
90. Vicente-Manzanares M, Webb DJ, and Horwitz AR. Cell migration at a glance. *J Cell Sci* 2005; 118:4917-9.
91. Etienne-Manneville S and Hall A. Integrin-Mediated Activation of Cdc42 Controls Cell Polarity in Migrating Astrocytes through PKC ζ . *Cell* 2001; 106:489-498.
92. Gomes ER, Jani S, and Gundersen GG. Nuclear movement regulated by Cdc42, MRCK, myosin, and actin flow establishes MTOC polarization in migrating cells. *Cell* 2005; 121:451-63.

93. Luxton GW and Gundersen GG. Orientation and function of the nuclear-centrosomal axis during cell migration. *Curr Opin Cell Biol* 2011; 23:579-88.
94. Sit S-T and Manser E Rho GTPases and their role in organizing the actin cytoskeleton. *J Cell Sci* 2011; 124:679-683.
95. Aronheim A, Broder YC, Cohen A, Fritsch A, Belisle B, and Abo A. Chp, a homologue of the GTPase Cdc42Hs, activates the JNK pathway and is implicated in reorganizing the actin cytoskeleton. *Curr Biol* 1998; 8:1125-1129.
96. Shutes A, Berzat AC, Cox AD, and Der CJ. Atypical mechanism of regulation of the Wrch-1 Rho family small GTPase. *Curr Biol* 2004; 14:2052-6.
97. Chenette EJ, Abo A, and Der CJ. Critical and distinct roles of amino- and carboxyl-terminal sequences in regulation of the biological activity of the Chp atypical Rho GTPase. *J Biol Chem* 2005; 280:13784-92.
98. Naji L, Pacholsky D, and Aspenstrom P. ARHGAP30 is a Wrch-1-interacting protein involved in actin dynamics and cell adhesion. *Biochem Biophys Res Commun* 2011; 409:96-102.
99. Faure S and Fort P. Atypical RhoV and RhoU GTPases control development of the neural crest. *Small GTPases* 2015; 6:174-177.
100. Schiavone D, Dewilde S, Vallania F, Turkson J, Di Cunto F, and Poli V. The RhoU/Wrch1 Rho GTPase gene is a common transcriptional target of both the gp130/STAT3 and Wnt-1 pathways. *Biochem J* 2009; 421:283-92.
101. Risse SL, Vaz B, Burton MF, Aspenstrom P, Piekorz RP, Brunsveld L, and Ahmadian MR. SH3-mediated targeting of Wrch1/RhoU by multiple adaptor proteins. *Biol Chem* 2013; 394:421-32.
102. Alan JK, Berzat AC, Dewar BJ, Graves LM, and Cox AD Regulation of the Rho family small GTPase Wrch-1/RhoU by C-terminal tyrosine phosphorylation requires Src. *Mol Cell Biol*. 2010; 30:4324-38.
103. Dart AE, Box GM, Court W, Gale ME, Brown JP, Pinder SE, Eccles SA, and Wells CM. PAK4 promotes kinase-independent stabilization of RhoU to modulate cell adhesion. *J Cell Biol* 2015; 211:863-79.
104. Saras J, Wollberg P, and Aspenstrom P. Wrch1 is a GTPase-deficient Cdc42-like protein with unusual binding characteristics and cellular effects. *Exp Cell Res* 2004; 299:356-69.
105. Troeger A and Williams DA. Hematopoietic-specific Rho GTPases Rac2 and RhoH and human blood disorders. *Exp Cell Res* 2013; 319:2375-83.
106. Delestre L, Berthon C, Quesnel B, Figeac M, Kerckaert JP, Galiegue-Zouitina S, and Shelley CS. Repression of the RHOH gene by JunD. *Biochem J* 2011; 437:75-88.

107. Gu Y, Chae HD, Siefring JE, Jasti AC, Hildeman DA, and Williams DA. RhoH GTPase recruits and activates Zap70 required for T cell receptor signaling and thymocyte development. *Nat Immunol* 2006; 7:1182-90.
108. Li X, Bu X, Lu B, Avraham H, Flavell RA, and Lim B. The Hematopoiesis-Specific GTP-Binding Protein RhoH Is GTPase Deficient and Modulates Activities of Other Rho GTPases by an Inhibitory Function. *Mol Cell Biol* 2002; 22:1158-1171.
109. Gu Y, Jasti AC, Jansen M, and Siefring JE. RhoH, a hematopoietic-specific Rho GTPase, regulates proliferation, survival, migration, and engraftment of hematopoietic progenitor cells. *Blood* 2005; 105:1467-75.
110. Dorn T, et al. RhoH is important for positive thymocyte selection and T-cell receptor signaling. *Blood* 2006; 109:2346-2355.
111. Chae HD, Lee KE, Williams DA, and Gu Y. Cross-talk between RhoH and Rac1 in regulation of actin cytoskeleton and chemotaxis of hematopoietic progenitor cells. *Blood* 2008; 111:2597-605.
112. Sanchez-Aguilera A, et al. Involvement of RhoH GTPase in the development of B-cell chronic lymphocytic leukemia. *Leukemia* 2010; 24:97-104.
113. Troeger A, Johnson AJ, Wood J, Blum WG, Andritsos LA, Byrd JC, and Williams DA. RhoH is critical for cell-microenvironment interactions in chronic lymphocytic leukemia in mice and humans. *Blood* 2012; 119:4708-18.
114. Riou P, Villalonga P, and Ridley AJ. Rnd proteins: multifunctional regulators of the cytoskeleton and cell cycle progression. *Bioessays* 2010; 32:986-92.
115. Oinuma I, Kawada K, Tsukagoshi K, and Negishi M. Rnd1 and Rnd3 targeting to lipid raft is required for p190 RhoGAP activation. *Mol Biol Cell* 2012; 23:1593-604.
116. Wennerberg K, Forget M-A, Ellerbroek SM, Arthur WT, Burridge K, Settleman J, Der CJ, and Hansen SH. Rnd Proteins Function as RhoA Antagonists by Activating p190 RhoGAP. *Curr Biol* 2003; 13:1106-1115.
117. Madigan JP, et al. Regulation of Rnd3 localization and function by protein kinase C alpha-mediated phosphorylation. *Biochem J* 2009; 424:153-61.
118. Boureux A, Vignal E, Faure S, and Fort P. Evolution of the Rho family of ras-like GTPases in eukaryotes. *Mol Biol Evol* 2007; 24:203-16.
119. Rivero F, Dislich H, Glöckner G, and Noegel AA. The Dictyostelium discoideum family of Rho-related proteins. *Nucleic Acids Res* 2001; 29:1068-1079.
120. Manjarrez JR, Sun L, Prince T, and Matts RL. Hsp90-dependent assembly of the DBC2/RhoBTB2-Cullin3 E3-ligase complex. *PLoS One* 2014; 9:e90054.
121. Ji W and Rivero F. Atypical Rho GTPases of the RhoBTB Subfamily: Roles in Vesicle Trafficking and Tumorigenesis. *Cells* 2016; 5.

122. Berthold J, Schenkova K, and Rivero F. Rho GTPases of the RhoBTB subfamily and tumorigenesis. *Acta Pharmacol Sin* 2008; 29:285-95.
123. Wilkins A, Ping Q, and Carpenter CL. RhoBTB2 is a substrate of the mammalian Cul3 ubiquitin ligase complex. *Genes Dev* 2004; 18:856-861.
124. Genschik P, Sumara I, and Lechner E. The emerging family of CULLIN3-RING ubiquitin ligases (CRL3s): cellular functions and disease implications. *EMBO J* 2013; 32:2307-20.
125. Chang FK, Sato N, Kobayashi-Simorowski N, Yoshihara T, Meth JL, and Hamaguchi M. DBC2 is essential for transporting vesicular stomatitis virus glycoprotein. *J Mol Biol* 2006; 364:302-8.
126. Freeman SN, Ma Y, and Cress WD. RhoBTB2 (DBC2) is a mitotic E2F1 target gene with a novel role in apoptosis. *J Biol Chem* 2008; 283:2353-62.
127. Siripurapu V, Meth J, Kobayashi N, and Hamaguchi M. DBC2 significantly influences cell-cycle, apoptosis, cytoskeleton and membrane-trafficking pathways. *J Mol Biol* 2005; 346:83-9.
128. Sahai E and Marshall CJ. RHO-GTPases and cancer. *Nat Rev Cancer* 2002; 2:133-42.
129. Toksoz D and Merdek KD. The Rho small GTPase: functions in health and disease *Histol Histopathol* 2002; 17:915-927.
130. Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144:646-74.
131. Alan JK and Lundquist EA. Mutationally activated Rho GTPases in cancer. *Small GTPases* 2013; 4:159-63.
132. Kakiuchi M, et al. Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. *Nat Genet* 2014; 46:583-7.
133. Sakata-Yanagimoto M, et al. Somatic RHOA mutation in angioimmunoblastic T cell lymphoma. *Nat Genet* 2014; 46:171-5.
134. Wang K, et al. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat Genet.* 2014; 46:573-82.
135. Yoo HY, et al. A recurrent inactivating mutation in RHOA GTPase in angioimmunoblastic T cell lymphoma. *Nat Genet* 2014; 46:371-5.
136. Zhou J, Hayakawa Y, Wang TC, and Bass AJ. RhoA mutations identified in diffuse gastric cancer. *Cancer Cell* 2014; 26:9-11.
137. Porter AP, Papaioannou A, and Malliri A. Deregulation of Rho GTPases in cancer. *Small GTPases* 2016:1-16.

138. Pajic M, Herrmann D, Vennin C, Conway JR, Chin VT, Johnsson AK, Welch HC, and Timpson P. The dynamics of Rho GTPase signaling and implications for targeting cancer and the tumor microenvironment. *Small GTPases* 2015; 6:123-33.
139. Gomez del PT, Benitah SA, Valeron PF, Espina C, and Lacal JC. Rho GTPase expression in tumorigenesis: evidence for a significant link. *Bioessays* 2005; 27.
140. Kamai T, Yamanishi T, Shirataki H, Takagi K, Asami H, Ito Y, and Yoshida K. Overexpression of RhoA, Rac1, and Cdc42 GTPases is associated with progression in testicular cancer. *Clin Cancer Res* 2004; 10.
141. Wang D, Dou K, Xiang H, Song Z, Zhao Q, Chen Y, and Li Y. Involvement of RhoA in progression of human hepatocellular carcinoma. *J Gastroenterol Hepatol* 2007; 22:1916-20.
142. Zhang S, et al. RhoA regulates G1-S progression of gastric cancer cells by modulation of multiple INK4 family tumor suppressors. *Mol Cancer Res* 2009; 7:570-80.
143. Sivaraj KK, Takefuji M, Schmidt I, Adams RH, Offermanns S, and Wettschureck N. G13 controls angiogenesis through regulation of VEGFR-2 expression. *Dev Cell* 2013; 25:427-34.
144. Chiba S, Enami T, Ogawa S, and Sakata-Yanagimoto M. G17V RHOA: Genetic evidence of GTP-unbound RHOA playing a role in tumorigenesis in T cells. *Small GTPases* 2015; 6:100-3.
145. Ridley AJ. RhoA, RhoB and RhoC have different roles in cancer cell migration. *J Microsc* 2013; 251:242-9.
146. Bravo-Cordero JJ, Hodgson L, and Condeelis JS. Spatial regulation of tumor cell protrusions by RhoC. *Cell Adh Migr* 2014; 8:263-7.
147. Horiuchi A, Imai T, Wang C, Ohira S, Feng Y, Nikaido T, and Konishi I. Up-Regulation of Small GTPases, RhoA and RhoC, Is Associated with Tumor Progression in Ovarian Carcinoma. *Lab Invest* 2003; 83:861-870.
148. Islam M, Lin G, Brenner JC, Pan Q, Merajver SD, Hou Y, Kumar P, and Teknos TN. RhoC expression and head and neck cancer metastasis. *Mol Cancer Res* 2009; 7:1771-80.
149. Ruth MC, Xu Y, Maxwell IH, Ahn NG, Norris DA, and Shellman YG RhoC promotes human melanoma invasion in a PI3K/Akt-dependent pathway. *J Invest Dermatol.* 2006; 126:862-8.
150. Bravo-Cordero JJ, Oser M, Chen X, Eddy R, Hodgson L, and Condeelis J. A novel spatiotemporal RhoC activation pathway locally regulates cofilin activity at invadopodia. *Curr Biol* 2011; 21:635-44.
151. Huang M and Prendergast GC. RhoB in cancer suppression. *Histol Histopathol* 2006; 21:213-218.

152. Mazieres J, Antonia T, Daste G, Muro-Cacho C, Berchery D, Tillement V, Pradines A, Sebti S, and Favre G. Loss of RhoB Expression in Human Lung Cancer Progression. *Clin Cancer Res* 2004; 10:2742-2750.
153. Zhou J, et al. A distinct role of RhoB in gastric cancer suppression. *Int J Cancer* 2011; 128:1057-68.
154. Wertheimer E, Gutierrez-Uzquiza A, Rosembliit C, Lopez-Haber C, Sosa MS, and Kazanietz MG. Rac signaling in breast cancer: a tale of GEFs and GAPs. *Cell Signal* 2012; 24:353-62.
155. Jordan P, Brazao R, Boavida MG, Gespach C, and Chastre E. Cloning of a novel human Rac1b splice variant with increased expression in colorectal tumors. *Oncogene* 1999; 18.
156. Schnelzer A, Prechtel D, Knaus U, Dehne K, Gerhard M, Graeff H, Harbeck N, Schmitt M, and Lengyel E. Rac1 in human breast cancer: overexpression, mutation analysis, and characterization of a new isoform, Rac1b. *Oncogene* 2000; 19:3013-3020.
157. Zhou C, et al. The Rac1 splice form Rac1b promotes K-ras-induced lung tumorigenesis. *Oncogene* 2013; 32:903-9.
158. Radisky DC, et al. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature* 2005; 436, 123-7.
159. Stengel K and Zheng Y. Cdc42 in oncogenic transformation, invasion, and tumorigenesis. *Cell Signal* 2011; 23:1415-23.
160. Grise F, et al. Rnd3/RhoE Is down-regulated in hepatocellular carcinoma and controls cellular invasion. *Hepatology* 2012; 55:1766-75.
161. Karlsson R, Pedersen ED, Wang Z, and Brakebusch C Rho GTPase function in tumorigenesis. *Biochim Biophys Acta*. 2009; 1796:91-8.
162. Muller PA, Vousden KH, and Norman JC. p53 and its mutants in tumor cell migration and invasion. *J Cell Biol* 2011; 192:209-18.
163. Li K, Lu Y, Liang J, Luo G, Ren G, Wang X, and Fan D. RhoE enhances multidrug resistance of gastric cancer cells by suppressing Bax. *Biochem Biophys Res Commun* 2009; 379:212-6.
164. Zhang C, et al. Overexpression of RhoE has a prognostic value in non-small cell lung cancer. *Ann Surg Oncol* 2007; 14:2628-35.
165. Hamaguchi M, et al. DBC2, a candidate for a tumor suppressor gene involved in breast cancer. *Proc Natl Acad Sci USA* 2002; 99:13647-52.
166. Beder LB, et al. Identification of a candidate tumor suppressor gene RHOBTB1 located at a novel allelic loss region 10q21 in head and neck cancer. *J Cancer Res Clin Oncol* 2006; 132:19-27.

Figure legends

Figure 1. Signalling pathways regulated by RhoA and Rac1

Examples of signalling by RhoA and Rac1 to the actin cytoskeleton are shown. Different types of cell surface receptors, such as integrins, G-protein-coupled receptors (GPCRs), and tyrosine kinase receptors, activate guanine nucleotide exchange factors (GEFs) for RhoA and Rac1. RhoA and Rac1 then interact with and activate a variety of effectors that are involved in actin polymerization, focal adhesion and stress fibre formation, including protein kinases (e.g. ROCKs, PAKs) and proteins that stimulate actin polymerization (e.g. mDia formins, the WAVE regulatory complex that stimulates the Arp2/3 complex).

Figure 2. The human Rho GTPase family

The Rho GTPase family consists of 20 genes in humans, which are present in all mammals. The family is subdivided into 8 subfamilies: Rac/RhoG subfamily, Rho subfamily, Cdc42/RhoQ/RhoJ subfamily, RhoF/RhoD subfamily, Rnd subfamily, RhoBTB subfamily, RhoH subfamily and RhoU/RhoV subfamily. These subfamilies can be classified as typical (orange circles) or atypical (purple circles) depending on the mode of regulation: typical proteins are regulated by GTP/GDP cycling, whereas atypical proteins have amino acid differences from typical proteins that alter their interactions with GTP and/or GDP. The unrooted phylogenetic tree is based on a ClustlW alignment of the amino-acid sequences of the Rho domains of 20 human Rho GTPase proteins. This does not include splice variants of Rac1 or Cdc42. The figure is modified from ⁵.

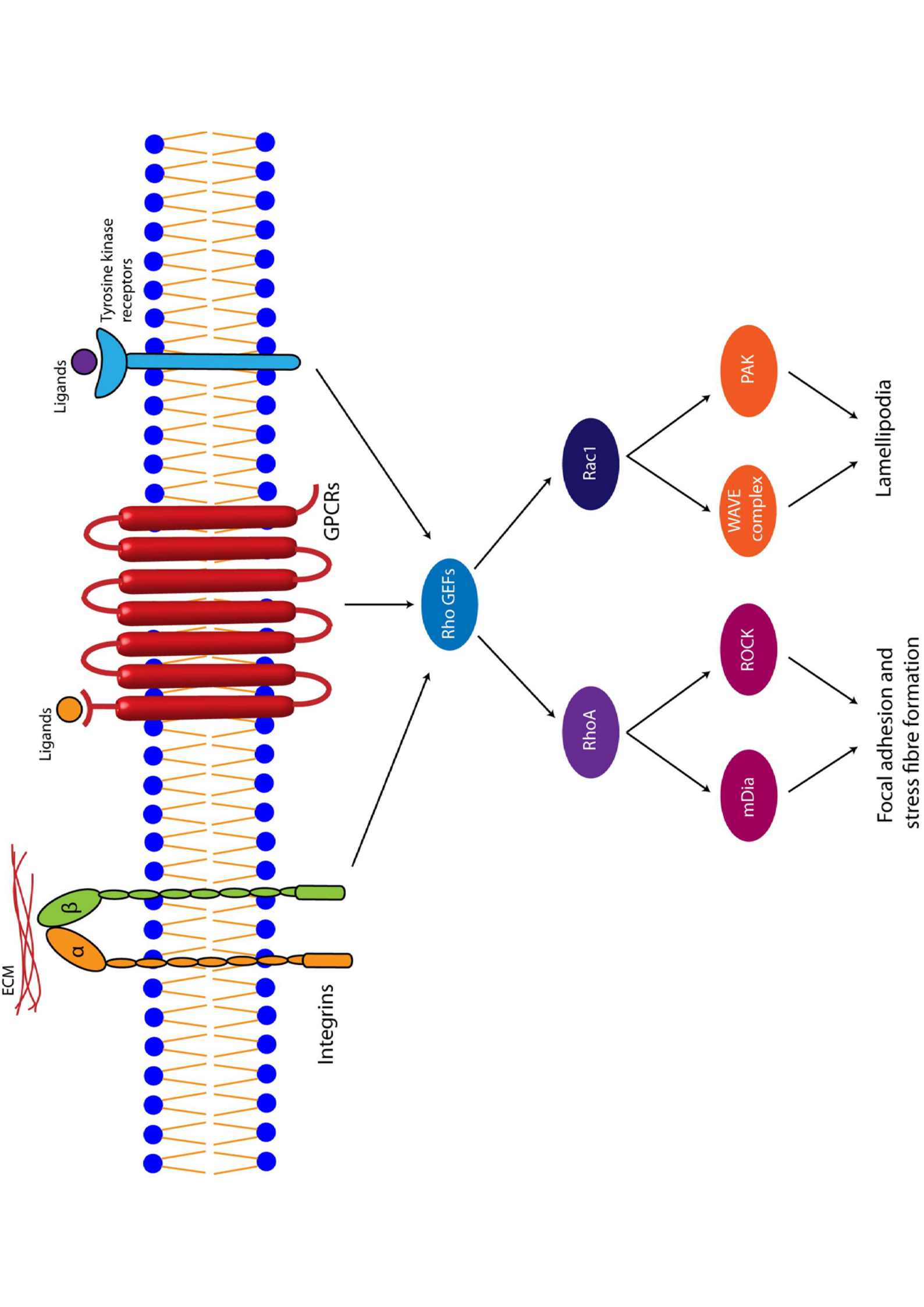
Figure 3. Regulation of Rho GTPases

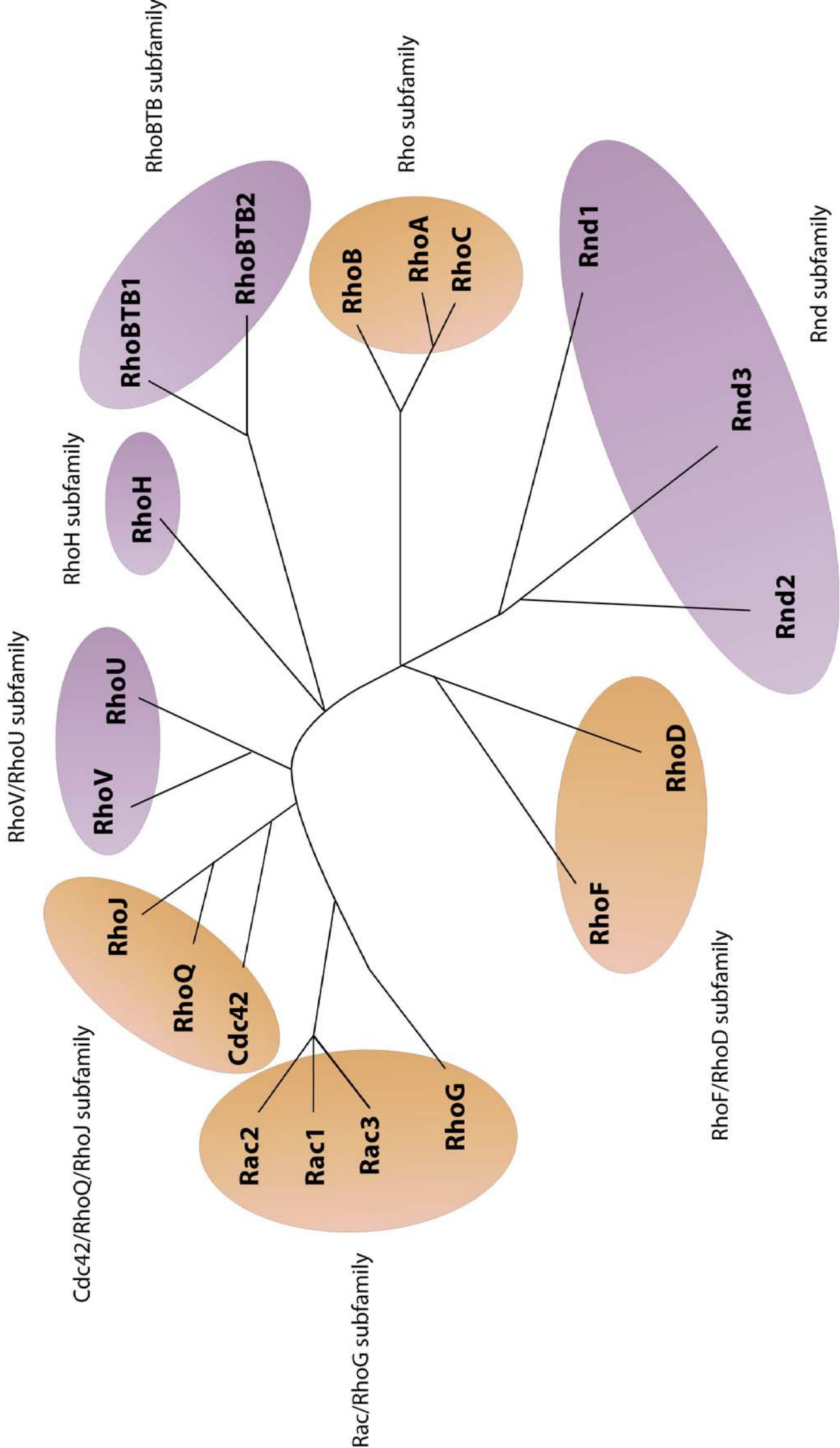
Most Rho GTPases are regulated by GEFs, GAPs and GDIs. These proteins control the cycling between the active GTP-bound form and the inactive GDP-bound form. RhoGDIs can also regulate the localisation and degradation of Rho GTPases. The expression of Rho GTPases are regulated at transcriptional and post-transcriptional (miRNA) levels. Rho GTPases are also be regulated by post-translational modifications (lipid modification, phosphorylation, ubiquitination and SUMOylation), which alter their intracellular localisation, their stability and/or their ability to signal to downstream effectors.

Figure 4. Roles of Rho GTPases in single cell migration

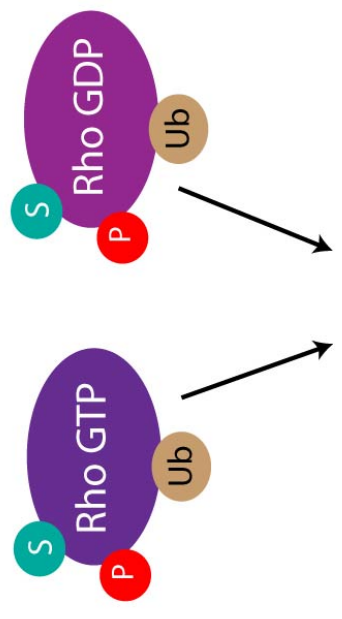
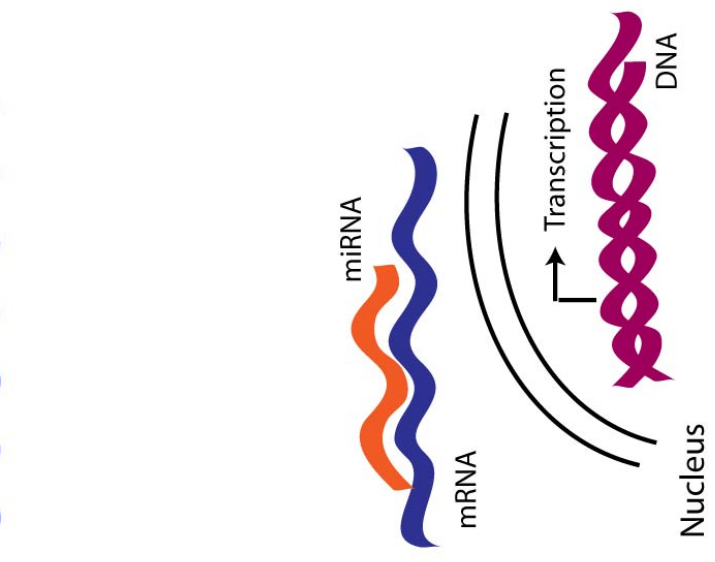
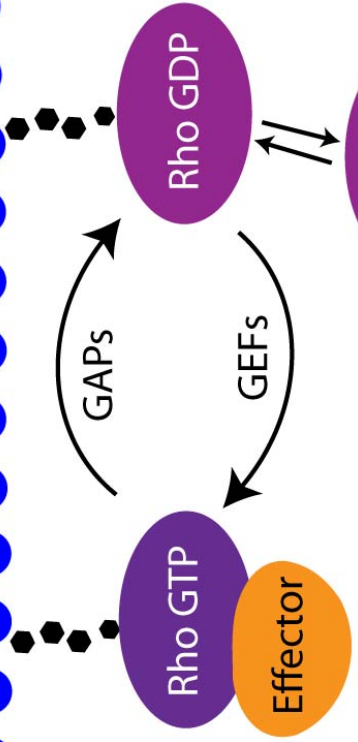
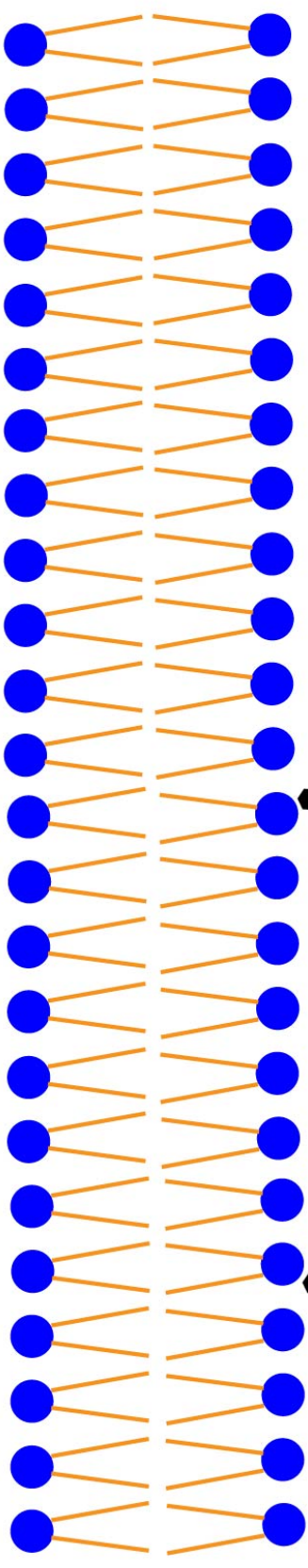
Single cell migration is a multistep process in which cells extend membrane protrusions at the cell front (lamellipodia, filopodia and/or membrane blebs), form new adhesions, contract the cell body and detach the cell rear from the surrounding environment. In lamellipodium-based migration (left), lamellipodium and filopodium formation involves new actin

polymerization, and requires actin nucleators such as the Arp2/3 complex that are activated by Rac and Cdc42. Actin polymerization in lamellipodia can also be mediated by formins after RhoA activation. Formation of integrin-based focal adhesions by Rho/ROCK signalling stabilizes lamellipodia and mediates interaction with the extracellular matrix. Actomyosin-mediated contractility and detachment of the cell rear are controlled by Rho/ROCK signalling. In bleb-based migration (right), actomyosin contractility driven by RhoA and/or RhoC activation of ROCKs leads to the extension of blebs, which are protrusions of the cell membrane caused by actomyosin contraction of the cell cortex. Bleb-based migration is less dependent on integrin-mediated adhesion than lamellipodium-based migration.



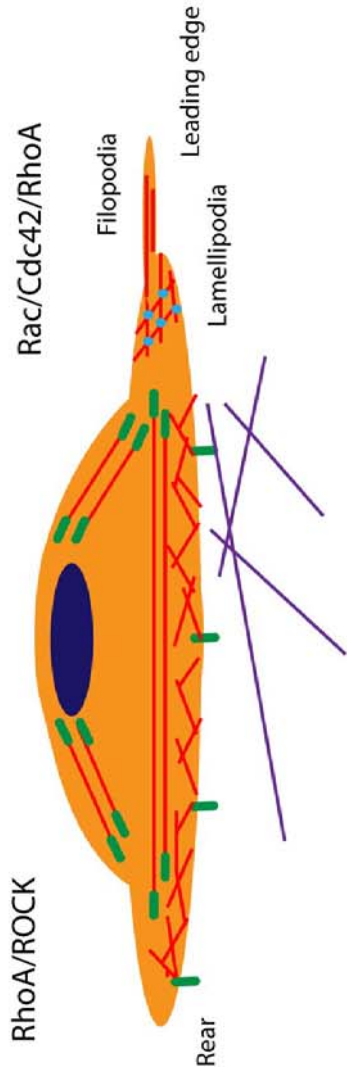


Plasma membrane

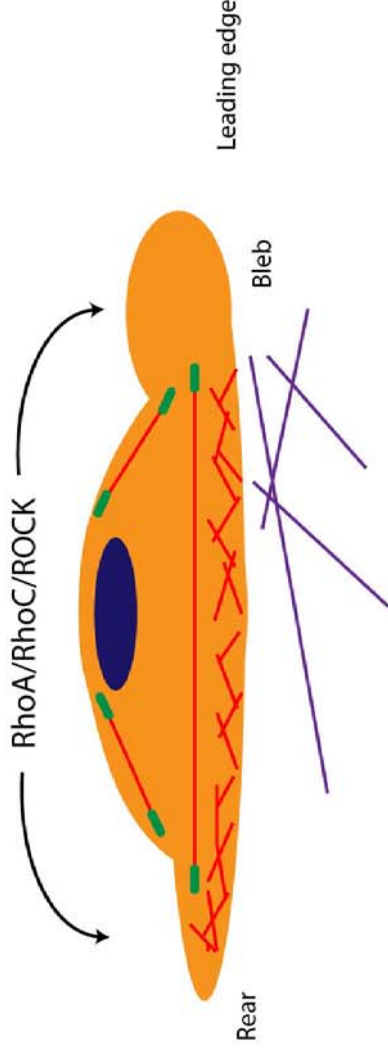


- Ub ubiquitination
- P phosphorylation
- S SUMOylation
- Lipid modification prenylation and/or palmitoylation

Lamellipodium-based motility



Bleb-based motility



- Arp2/3 complex
- Focal adhesion
- F-actin
- Extracellular matrix