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Current Opinion in Cell Biology

Control of adhesion and protrusion in cell migration by Rho GTPases

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Cell migration is a critical process that underpins a number of physiological and pathological contexts such as the correct functioning of the immune system and the spread of metastatic cancer cells. Central to this process are the Rho family of GTPases, which act as core regulators of cell migration.Rho GTPases are molecular switches that associate with lipid membranes and act to choreograph molecular events that underpin cell migration. Specifically, these GTPases play critical roles in coordinating force generation through driving the formation of cellular protrusions as well as cell–cell and cell–matrix adhesions.Here we provide an update on the many roles of Rho-family GTPases in coordinating protrusion and adhesion formation in the context of cell migration, as well as describing how their activity is controlled to by a variety of complex signalling networks.

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Current Opinion in Cell Biology 2019, **56**:64–70

This review comes from a themed issue on **Cell architecture**

Edited by **Johanna Ivaska** and **Manuel Théry**

https://doi.org/10.1016/j.ceb.2018.09.003

Rho-family GTPases are molecular switches; most which cycle from an 'on' GTP bound state to an 'off' GDP bound state, driven by GEFs (guanine nucleotide exchange factors) and GAPs (GTPase-activating proteins) respectively. Association with lipid membranes through a lipid (farnesyl or geranylgeranyl) tail ensures Rho family GTPases signal at membrane-cytosol interfaces and exquisite control the ratio of cytosolic to membrane bound GTPase is achieved by the Rho-GDI (Rho GDP-dissociation inhibitor) family of proteins [1]. An atypical subgroup of Rho-family GTPases, known as the Rnd family are constitutively GTP bound, and instead are thought to be regulated by control of their association with lipid membranes, via 14-3-3 proteins which can bind to Rnd GTPase lipid tails [2]. Through the extensive regulation of Rho GTPase activation and localisation the cell can control the activation of Rho-family GTPases in a precise spatio-temporal manner [1]. In fact Rho-family GTPases have long been appreciated as signalling molecules that allow the cell to relay information to a variety of cellular machineries including the NADPH oxidase complex and vesicle trafficking components [3,4]. The role of Rho GTPases in controlling the actin cytoskeleton was highlighted by Alan Hall's seminal work linking RhoA, Rac1 and Cdc42 to the formation of stress fibres, lamellipodia and filopodia, respectively [5–7]. Furthermore, the discovery that RhoA drives the formation of stress fibres highlighted the importance of Rho GTPase signalling during the formation of cell–matrix adhesions [6]. This review will focus on Rho GTPase signalling in the context of cell migration, examining how these molecular switches signal to cellular protrusions and cell–matrix adhesions. Here we summarise what is known about Rho-family GTPases in the context of leading edge protrusion formation, highlighting recent studies that have helped to uncover the complexity of these fascinating molecular switches. Specifically, this review will highlight four major aspects of Rho GTPase biology: the effectors of Rho GTPases, the regulators of Rho GTPases, the role of Rho GTPases in determining cellular directionality and the importance of Rho GTPases in the context of cell–matrix adhesions. All four aspects play major roles in understanding how Rho GTPases signal during migration and all four are far from being fully understood.

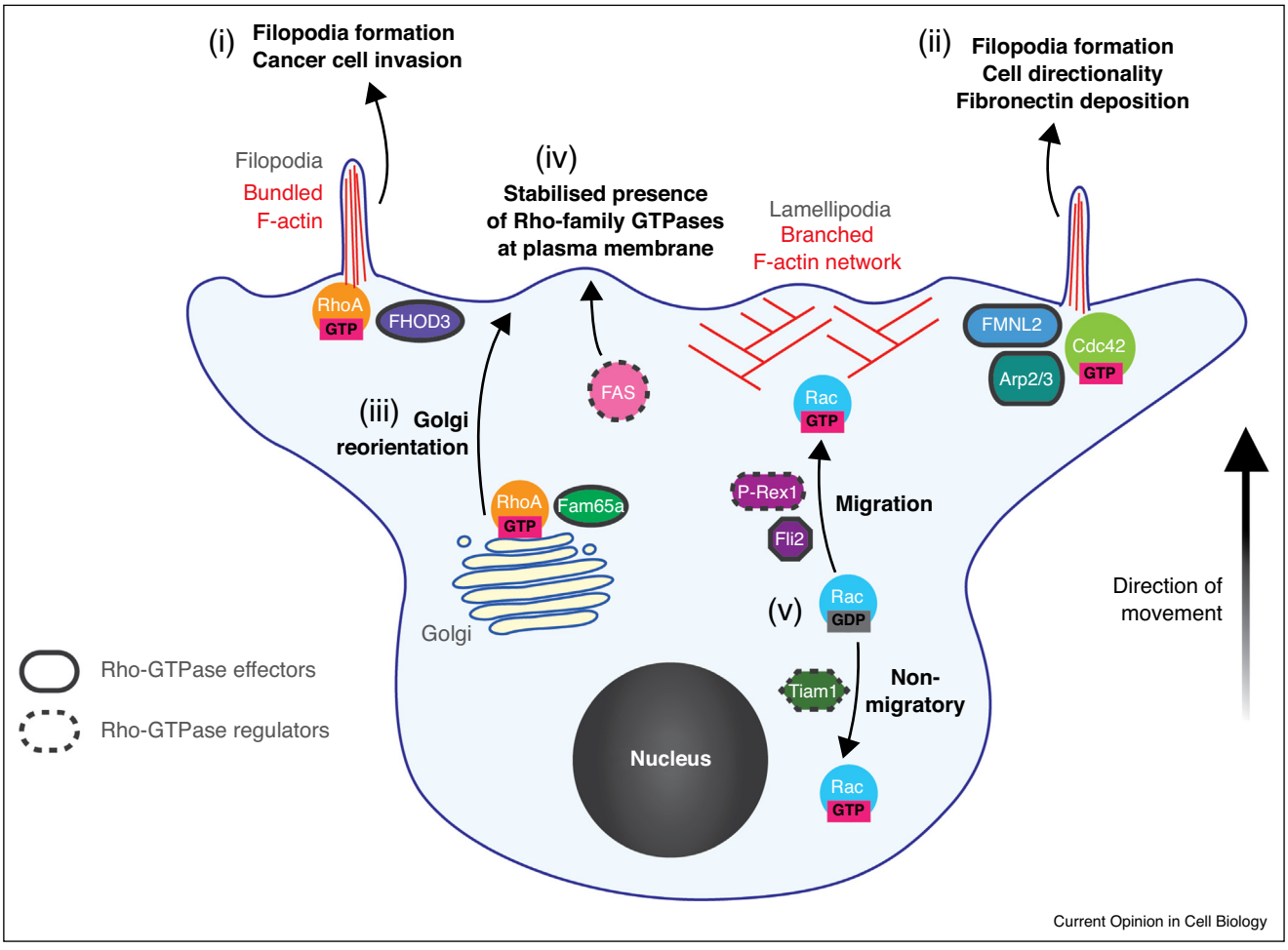
Rho-family GTPase effectors

Following the discovery that Rac1 and Cdc42 stimulate the formation of lamellipodia and filopodia respectively, numerous factors were identified that enable these GTPases to build a protrusive leading edge. Of key importance are the proteins that enabled Rac1 and Cdc42 to drive actin nucleation. These included the Arp2/3 activators of the WAVE and WASP family for both Rac1 and Cdc42 respectively [8,9]. The discovery of these proteins led to the concept, based on 2D cell culture studies that Rac1 and Cdc42 signalling to the Arp2/3 complex is essential for the establishment of the leading edge. However this concept was extended and challenged by the direct observation of RhoA signalling at the leading edge of mouse fibroblasts and human cancer cells migrating in 2D cell culture [10–13]. Furthermore knockout studies of Arp2/3 complex components in fibroblasts migrating in 2D demonstrated that Arp2/3 is not a universal requirement for movement on such surfaces, although defects in lamellipodia formation

and directional migration in both haptotaxis and chemotaxis have been observed [14–16]. The universal requirement for Arp2/3 in migration was also challenged by the discovery of amoeboid migration which utilises RhoA signalling at the leading edge of the cell to disrupt cortical actin, allowing the cell to control the number and size of plasma membrane-based blebs that drive the cell's movement through gaps in 3D extracellular matrix [17–19]. Therefore, it is not surprising that studies continue to identify proteins that act downstream of Rho-GTPases to facilitate protrusion formation and couple such formation to the motility of the rest of the cell. An example of such work includes the identification of FAM65A as a RhoA effector. By binding to Golgi associated FAM65A, RhoA is thought to re-orientate the Golgi network towards the leading edge, facilitating efficient migration of single cells in 2D [20]. FMNL2 has recently been identified as a formin that localises to the leading edge of cells in 2D and promotes filopodia formation downstream of Cdc42 [21]. Furthermore RhoA activation at the leading edge of cells in 3D matrix promotes filopodia formation and invasive migration through ROCK-mediated activation of the formin FHOD3 [22,23] (Figure 1).

Given the complexity of the leading edge of migrating cells and the refinement of methodologies being developed to study it, it seems likely that the list of proteins known to act down stream of Rho GTPases will continue

Figure 1



Rho GTPases in protrusion formation summary. (i) RhoA can signal to the formin FHOD3, via the ROCK family kinases, to promote the invasion of cancer cells into 3D fibronectin rich ECM. This form on invasive migration occurs downstream of the upregulated endocytic recycling of the $\alpha 5\beta 1$ integrin, and does not require the action of the Arp2/3 complex. (ii) Cdc42 can drive the formation of filopodia by activating the formin FMNL2 and/or Arp2/3. (iii) RhoA-FAM65 interaction can re-orientate the Golgi apparatus towards the leading edge of the cell in 2D environments, facilitating efficient migration. (iv) Fatty acid synthesis alters the biochemical and biophysical properties of the plasma membrane, stabilising the presence of Rho GTPases in the membrane. This may have important implications for understanding how the metabolic state of a cell may affect its ability to migrate. (v) Different GEFs can promote differential Rac1 signalling, either promoting a migratory output by ensuring Rac1 binds to FLI2 (P-Rex1) or preventing a migratory output (Tiam1).