Regulation of Cell Morphology and Migration by a Rho GTPase Activating Protein FilGAP

Koji Tsutsumi* and Yasutaka Ohta

Division of Cell Biology, Department of Biosciences, School of Science, Kitasato University, Sagamihara, Kanagawa, Japan
**E-mail address: k.tutumi@kitasato-u.ac.jp*

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Cell migration is a fundamental process that orchestrates embryonic development and drives cancer invasion and metastasis. Rho small GTPases (Rac, Cdc42, and Rho) regulates cell migration through remodeling of the actin cytoskeleton. Rho GTPase activating proteins (GAPs) are negative regulators of RhoGTPases. FilGAP is a GAP for Rac and binds to Filamin A. Emerging evidences suggest that FilGAP may regulate organization of the actin cytoskeleton in a spatiotemporal manner. In this paper, we describe the recent findings on the role of FilGAP in the regulation of cell morphology and migration and its regulatory mechanism.
Key words: Cell Migration, Cell Polarity, Aactin Cytoskeleton

1. Introduction

Cell migration is involved in many biological events like gastrulation, immune response, and cancer metastasis. When the cells are exposed to the extracellular stimuli such as extracellular matrices (ECM), growth factors, and cytokines, they extend the protrusions in the direction of migration. These protrusions are stabilized to the ECM to serve as traction sites for cell movement. These adhesions are disassembled in the cell rear and cell tail is retracted (Lauffenburger and Horwitz, 1996) (Fig. 1A). Rho family small GTPases (RhoGTPases) including Rac, Cdc42 and Rho are crucial regulators for cell migration (Hall, 1998). GTP bound RhoGTPases are active state and transmit the signal to the downstream effectors (Fig. 1B). Guanine nucleotide exchange factors (GEFs) activate RhoGTPases by loading GTP. GTPase-activating protein (GAP) binds to active RhoGTPases and stimulate their intrinsic GTPase activity. Active Rac stimulates actin polymerization at plasma membrane to produce lamellipodia and membrane ruffle, flat-shaped membrane protrusion. Cdc42 regulates actin-rich plasma membrane protrusion, filopodia. Rho regulates cell contraction through the formation of stress fiber and focal adhesion. In migrating cells, lamellipodia and filopodia are generated in front of the cells. On the other hand, Rho regulates the tail retraction through the acto-myosin contraction at cell rear. During cell migration, this spatial organization of the actin structures is essential for the formation of front-rear cell polarity (Fig. 1C). Rho and Rac antagonize each other, and as a result, Rac activation dominates in the front of the cell and Rho activation dominates in the rear (Guilluy *et al.*, 2011). FilGAP, a Rac specific GAP protein, is one of the molecules that mediate Rho-Rac antagonism. In this paper, we describe recent our findings on the role of FilGAP in the regulation of cell morphology and cell migration.

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2. Structure and Regulation of FilGAP

FilGAP was isolated as a binding partner of Filamin A (FLNa), an actin cross-linking protein (Ohta *et al.*, 2006). FilGAP is expressed in various tissues and shows the marked expression in kidney. FilGAP is composed of Plekstrin homology (PH) domain, RhoGAP domain and coiled-coil domain (CC) (Fig. 2). FilGAP has GAP activity against Rac and Cdc42 *in vitro*, however it specifically inhibits Rac activity in the cells (Ohta *et al.*, 2006). Generally PH domain binds to phospholipids in the membrane. PH domain of FilGAP preferentially binds to Phosphatidylinositol(3,4,5)-trisphosphate (PIP3) and also binds to another small GTPase, ADP-ribosylation factor 6 (Arf6) (Kawaguchi *et al.*, 2014). Binding of Arf6 to the PH domain activates Rac GAP activity of FilGAP. C-terminal CC domain is required for dimerization of FilGAP (Nakamura *et al.*, 2009). FLNa binds to the C-terminal region of FilGAP just close to CC domain. FilGAP localizes to FLNa-rich lamellae, but FilGAP mutant lacking C-terminus region does not. Rho associated kinase (ROCK) phosphorylates multiple serine and threonine residues located between GAP and CC domains. Phosphorylated FilGAP by ROCK is active and strongly suppresses Rac activity. Among serine and threonine residues phosphorylated by ROCK, Ser402 is a critical phosphorylation site for suppression of cell spreading on fibronectin (Morishita *et al.*, 2015). Cell adhesion to fibronectin induces dephosphorylation of Ser402 and attenuates RacGAP activity. Ser402 dephosphorylation is blocked by Calyculin A treatment, suggesting that Ser402 is dephosphorylated by PP1 or PP2A. FilGAP has close relatives (ARHGAP22 and ARHGAP25). They have the similar domain composition and functions as a GAP for Rac and show different tissue distribution from FilGAP (Aitsebaomo *et al.*, 2004; Csépányi-Kömi *et al.*, 2012).

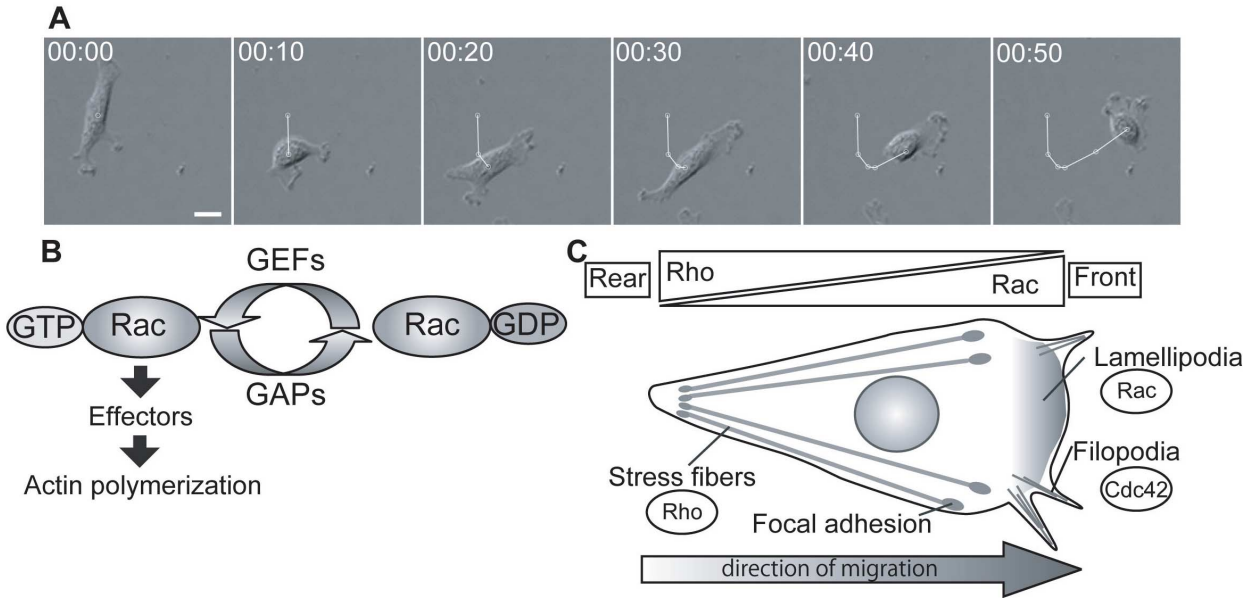

Fig. 1. Actin organization in migrating cells. (A) Time-lapse differential interference contrast images of migrating cells. A7 human melanoma cells were plated on collagen coated coverslip cells, and then treated with EGF. The images were acquired at 10 min interval. Scale bar, 20 μm. (B) Activation and inactivation mechanism of RhoGTPase. Guanine nucleotide exchange factors (GEFs) activate RhoGTPase by GTP loading. GTPase-activating protein (GAP) inactivate RhoGTPase by activating intrinsic GTPase activity. (C) A schematic representation of actin organization in a migrating cell. In front of a migrating cell, Rac and Cdc42 generate lamellipodia and filopodia, respectively. Rho regulates stress fiber formation and focal adhesion disassembly at the cell rear.

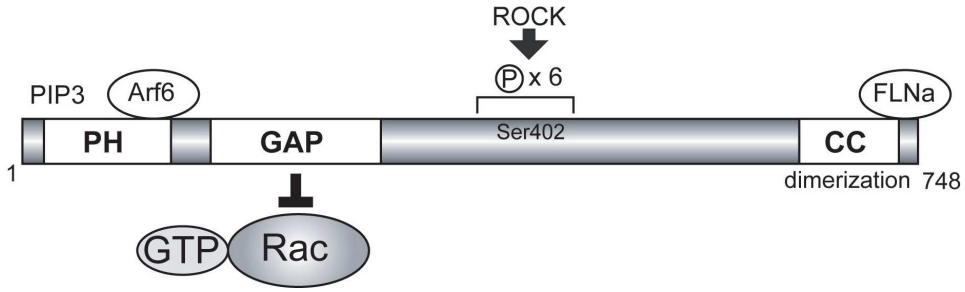

Fig. 2. Domain structure of FilGAP. FilGAP is consisted of Plekstrin homology (PH) domain, GAP domain, and Coiled-coli (CC) domain. There are multiple phosphorylation sites between GAP domain and CC domain. Among them, Ser402 is critical phosphorylation site to regulate RacGAP activity. PH domain binds to Phosphatidylinositol(3,4,5)-trisphosphate (PIP3) and ADP-ribosylation factor 6 (Arf6). Filamin A (FLNa) binds to C-terminus of FilGAP.

3. Regulation of Cell Morphology and Migration by FilGAP in 2D and 3D Environment

FilGAP regulates cell morphology and migration through the inactivation of Rac. Depletion of FilGAP by siRNA increases Rac-dependent lamellae formation in melanoma cells (Ohta *et al.*, 2006). Overexpression of FilGAP suppresses Rac-dependent lamellae formations induced by Epidermal growth factor. Because Rac and Rho antagonize each other, activation of FilGAP often stimulates Rho activity. In some cells, forced expression of FilGAP stimulates membrane blebbing, spherical protrusions driven by acto-myosin contraction (Fig. 3). Membrane blebbing is distinct protrusions from lamellipodia and utilized for cell migration *in vivo* or in 3D environment (Blaser *et al.*, 2006; Charras and Paluch, 2008). Arf6 binds to PH domain of FilGAP and stimulates membrane blebbing by activating Rac GAP activity of FilGAP (Kawaguchi *et al.*, 2014).

Depletion of FilGAP in lymphocyte increased cell polarization and migration speed induced by SDF-1 (stromal cell-derived factor 1α) (Iida *et al.*, 2016). SDF-1 stimulated FilGAP depleted lymphocytes show more persistent migration than control cells because of more stable lamellae formation. Depletion of FilGAP in normal epithelial MDCK (Madin Darby canine kidney) cells also increased the migration speed and cell scattering induced by Hepatocyte growth factor (Nakahara *et al.*, 2015). ARHGAP25 is expressed primarily in hematopoietic cells and regulates leukocyte migration (Csépányi-Kömi *et al.*, 2016). ARHGAP22 suppresses EGF-induced lamellae formation and cell spreading on fibronectin (Mori *et al.*, 2014) and regulates cell migration downstream of Akt signaling in fibroblasts (Rowland *et al.*, 2011). Thus FilGAP and its close relatives regulates 2D cell migration by suppressing the Rac-dependent lamellae formation in front of the migrating cells and stimulating activation of RhoA in the rear of the cells.

Many studies of cell migration have been performed using plastic or glass bottom dish because it is easy-to-handle.

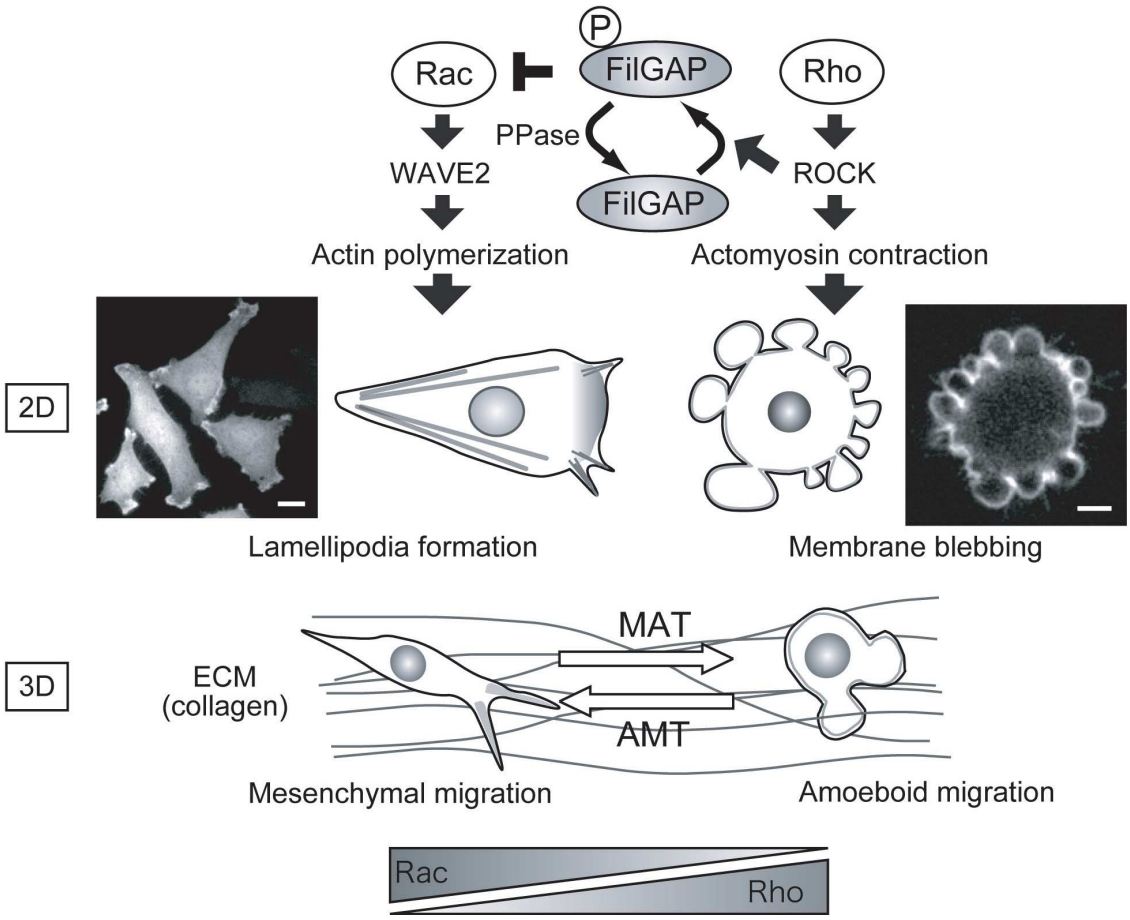

Fig. 3. FilGAP regulates cell morphology and migration by mediating the Rho-Rac antagonism. FilGAP is phosphorylated by ROCK downstream of Rho. Phosphorylated FilGAP is in an active state and suppresses Rac. Protein phosphatase (PPase) dephosphorylate FilGAP and inactivate FilGAP. In 2D environment, FilGAP suppresses Rac-dependent lamellae formation. Because Rac and Rho antagonize each other, inhibition of Rac results in activation of Rho. Rho stimulates membrane blebbing through acto-myosin contraction. Rac-dependent actin polymerization observed in membrane ruffles is visualized by lifeact-EGFP (F-actin marker, left) and membrane blebbing is visualized by fusionRed-f-mem (membrane marker, right). Scale bar, 20 μm (left) and 5 μm (right). In 3D ECM, tumor cells migrate using two types of migration mode, mesenchymal and amoeboid movement. In mesenchymal migration, cells show elongated morphology, extend thin protrusions. In amoeboid migration, cells show round morphology. In ECM, tumor cells plastically switch mesenchymal migration and amoeboid migration according with their surround microenvironment. Rac and Rho promotes mesenchymal and amoeboid migration respectively. FilGAP promotes amoeboid movement downstream of Rho.

However, it may only reflect some aspects of the cell migration *in vivo*. In 3D ECM, cells move switching the several migration modes depending on extracellular environment (Petrie and Yamada, 2016). Tumor cells in 3D ECM often switch two types of cell migration mode, mesenchymal cell migration and amoeboid cell migration (Sanz-Moreno *et al.*, 2008) (Fig. 3). The mesenchymal cell migration is characterized as an elongated cell shape and high Rac activity. In amoeboid migration, cells have round morphology and high Rho activity. Depletion of FilGAP in breast carcinoma cell (MDA-MB-231) induced Rac-dependent elongated mesenchymal morphology in collagen fibers (Saito *et al.*, 2012). Forced expression of FilGAP induced round amoeboid morphology. Importantly induction of round amoeboid morphology induced by FilGAP required the phosphorylation by ROCK. Depletion of FilGAP inhibits invasion to the collagen matrices, suggesting FilGAP may mediate Rho-dependent contraction and contribute to cell invasion to ECM. ARHGAP22 also induces amoeboid morphology in melanoma cells by inhibiting Rac (Sanz-Moreno *et al.*, 2008). ARHGAP22 also induce amoeboid morphology downstream of ROCK, however it is not phosphorylated by ROCK. ARHGAP22 shows different cellular localization from FilGAP (Mori *et al.*, 2014), it may be activated by another mechanism downstream of ROCK. Recently, Thuault *et al.* reported that ARHGAP25 positively regulates invasion to the ECM downstream of ROCK in Rhabdomyosarcoma (Thuault *et al.*, 2016). Thus all FilGAP family proteins appear to mediate Rho-Rac antagonism and regulate invasion to the ECM downstream of ROCK in different type of tumor cells.

4. Conclusions and Perspective

We've analyzed the role of FilGAP in 2D and 3D cell morphology and migration. However there remain several problems to be solved. PH domain of FilGAP binds to PIP3 *in vitro*, suggesting its involvement in phospholipids signaling mediated by PI3K (Kawaguchi *et al.*, 2014), however its role in the cell migration is unclear. PIP3 is produced in front of the migrating cells and stimulates actin polymerization through the activation of Rac GEFs (Rossman *et al.*, 2005). PIP3 binding to PH domain of GEFs not just re-

cruit it to the plasma membrane but also change the GEF activity. PIP3 binding to PH domain of FilGAP may also regulate not only its cellular localization but also RacGAP activity. Phosphorylation of FilGAP by ROCK increases its RacGAP activity in the cells but not *in vitro* (Ohta *et al.*, 2006). Non-phosphorylated FilGAP localizes to actin filaments and FilGAP is released from actin filament by phosphorylation (Morishita *et al.*, 2015). Phosphorylation may be required for the release of unidentified inhibitory protein from FilGAP. Now we are trying to identify FilGAP-binding protein and succeeded to identify several candidate proteins (Kawaguchi *et al.*, 2014; Yamada *et al.*, 2016; and unpublished data). Some of them are involved in membrane transport. Microtubule-based membrane transport is also important for the polarity formation in cell migration (Watanabe *et al.*, 2005). FilGAP may regulate cell morphology and migration through the coordination of actin cytoskeleton and membrane transport.

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