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Review

# PI3Ks and small GTPases in neutrophil migration: Two sides of the same coin☆

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ABSTRACT

Cell migration is a key event in physiological processes such as embryonic development, tissue repair, angiogenesis and immune responses. Alteration of the migration program is an important component in multiple pathologies, including chronic inflammation, autoimmunity and tumor metastasis. Understanding of the precise mechanisms at the basis of cellular migration may lead to the identification of novel therapeutic approach for these diseases. Recent evidences show that the interplay between the lipid kinases phosphatidylinositol 3-kinase (PI3Ks) and small GTPases play a critical role in driving cell migration. In this review we will describe the role of these molecules and the interaction between their signal cascades in leukocyte polarization and amoeboid migration.



## 1. A way to migrate

To move toward a stimulus, cells need to achieve three different points. First, they need to detect a chemoattractant (very few cue molecules in a high background), second, they translate the information to the motile apparatus and finally, they move in the direction of the source (Insall and Weiner, 2001; Wong, 2011). To accomplish this process, cells must develop morphological and functional asymmetry. This imbalance consists in the segregation of two cellular compartments with specific properties, composition and functions: the leading edge at the front and the uropod at the rear of the cell (Janetopoulos and Firtel, 2008). The establishment of this front–back axis, also referred as cell polarity, is a condition required for ameboid migration and is dependent on actin polymerization established by a positive feedback loop occurring at the leading edge. This system amplifies an external shallow gradient of chemoattractant into high internal redistribution of molecules. This rearrangement is regulated through signal transduction events that are elicited after receptors activation by chemoattractants. Once triggered, this cascade activates the lipid kinases of the phosphatidylinositol 3-kinase family (PI3Ks) that act upstream of cytoskeletal regulators, such as the Rho family of small GTPases (Gomez-Mouton and Manes, 2007) (Fig. 1).

## 2. Chemoattractants and their receptors

Chemoattractants bind to seven-transmembrane receptors coupled to heterotrimeric G proteins (GPCRs). Despite the presence of different subclasses of trimeric G proteins, the majority of chemoattractants binds to pertussis toxin (PTx)-sensitive inhibitory G proteins (Gi). This binding triggers dissociation of the Gαβγ trimer, to generate free Gα and the Gβγ dimer. While Gα mainly mediates the control of ionic channels, the Gβγ dimer regulates the activity of effector enzymes, including PI3Ks (Neer, 1995). Upon activation of this cascade, receptor signaling needs to be switched off. This process is promoted by members of the arrestin/β-arrestin family that trigger agonist-mediated desensitization of GPCRs. In particular, β-arrestin has been described as an essential regulator of migration (DeWire et al., 2007). Noteworthy, in addition to its function as a terminator of receptor signaling, β-arrestin may also contribute to the spatial control of actin assembly by sequestering actin assembly molecules and upstream regulators of actin formation at the leading edge (reviewed in Min and Defea, 2011). This dual control on both receptor signaling and cytoskeleton remodeling could be involved in the induction of actin assembly at the leading edge and in receptor desensitization in order to facilitate gradient sensing and directional cytoskeletal reorganization.

## 3. Upon receptor stimulation. . . comes PIP3

One of the first events in chemoattractant signaling is PI3K activation. The lipid kinase activity of PI3Ks transfers the terminal phosphate of adenosine triphosphate (ATP) to phosphoinositides (PtdIns) at the 3-hydroxyl of the inositol ring, generating different 3′-phosphorylated lipids that act as second messengers (Engelman et al., 2006; Leevers et al., 1999). The primary product of class I PI3Ks is the PtdIns-3,4,5-trisphosphate (PIP3). The importance of PI3K signaling in gradient sensing and cell polarity is highlighted by studies with GFP-PH probes that selectively bind to PIP3 (Magalhaes et al., 2007). These experiments demonstrate that

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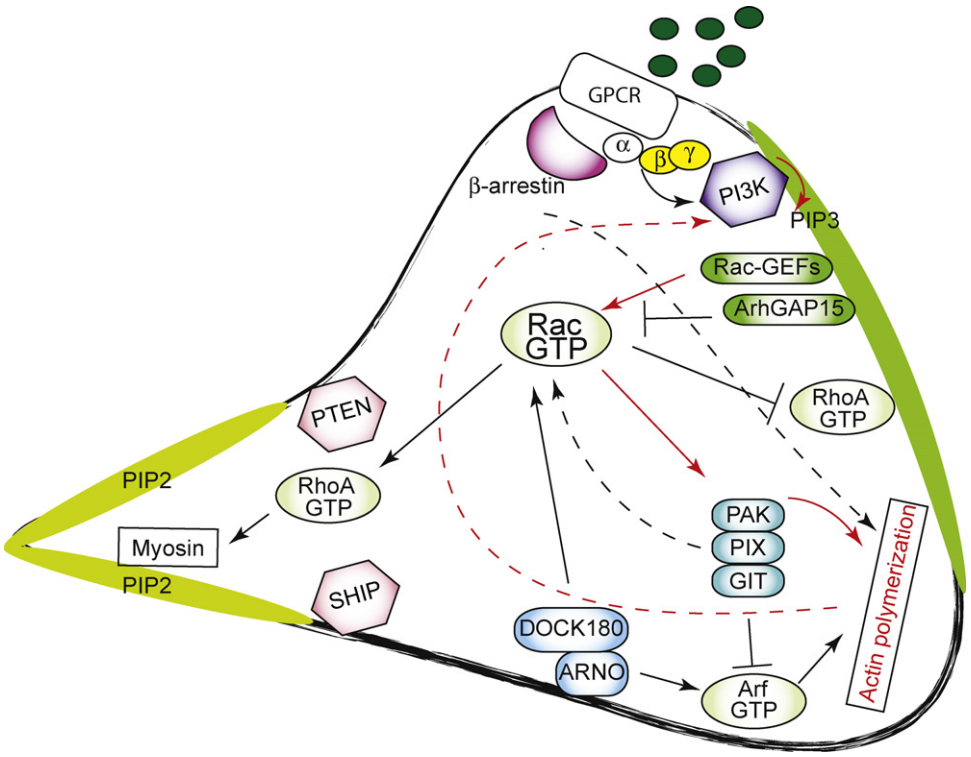


**Figure 1.** A way to move: to move toward a stimulus (green dots), cells must develop morphological and functional asymmetry. After receptor–chemoattractant interaction, PI3Ks activation lead to PIP3 accumulation at the leading edge. PIP3 coordinates Rac-GEFs recruitment and in turn Rac activation that allows actin polymerization. The feedback loop among PIP3-small GTPases-actin (red arrows) allows the integration of spatial signals into directional migration. Moreover, the link between different small GTPases is needed to coordinate the formation of the leading edge and the uropod and thus cellular polarization. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

PH-containing proteins are specifically recruited to the leading edge of the migrating cell (Manes et al., 2005; Ridley et al., 2003).

The relevance of PI3K function in regulating cell migration is confirmed by studies on primary neutrophils. The absence of a specific PI3K isoform, PI3Kγ, causes defective chemotaxis and impairs migration to the site of infection (Hirsch et al., 2000; Li et al., 2000; Sasaki et al., 2000). PI3Kγ-null neutrophils show no PIP3 production upon GPCR stimulation, thus indicating that PI3Kγ is the major PI3K isoform able to produce PIP3 in this context. Neutrophils carrying a constitutively active form of PI3Kγ show significantly impaired directional migration in response to chemoattractants, because of an altered GPCR-mediated Rac activity (Costa et al., 2007). The correlation between PIP3 and cytoskeletal rearrangements suggests that this second messenger plays a key role in transforming spatial information provided by chemoattractant gradients into directional cell movement (Wang et al., 2002). The requirement of PIP3 and its spatial restriction is highlighted by the crucial role of PIP3 phosphatases like PTEN and SHIP (Nishio et al., 2007). Inactivation of SHIP1 leads to impaired neutrophil polarization and motility by regulating leading edge formation as well as polarization (Nishio et al., 2007). In particular, while SHIP is regulating the speed of migration, PTEN deficiency does not display any speed impairment. Moreover, PTEN-null neutrophils are recruited more effectively than wild type controls during infection (Subramanian et al., 2007). Additionally, Kubes et al. (Heit et al., 2008b) demonstrate that PTEN-deficient neutrophils display random migration rather than directed migration after chemokine stimulation.

## 4. Not only PIP3...

The idea that PIP3 is transmitting the spatial information to the cytoskeleton, has led to the hypothesis that PIP3 might act as a "compass" driving directional migration. Nonetheless, this view has been challenged by more recent studies. For example, Hoeller and Kay (2007) report that *Dictyostelium* lacking all type I PI3K genes is unable to generate PIP3 but is still able to chemotax in steep gradients. The analysis of PI3Kγ-null neutrophils identifies their defect not in the orientation but in the speed and proportion of cells that can move (Ferguson et al., 2007). Additionally, cells treated with PI3K pan-inhibitors show a significant delay in GPCR-dependent migration demonstrating that PI3K accelerates the initial response but that alternative pathways might replace PI3K activation over time (Heit et al., 2008a). The role of PIP3 is to contribute to orientation in shallow gradients and to enhance speed in steep chemoattractant gradients (Bosgraaf et al., 2008). PIP3 function could thus depend on the strength of the chemotactic signal (King and Insall, 2009). While PIP3 plays a role in maintaining efficient migration, its generation cannot be the sole factor determining directionality (King and Insall, 2009). Indeed, PIP3 controls pseudopodia production rate but not its direction (Andrew and Insall, 2007). In this view, PIP3 importance lies on its ability to coordinate cytoskeletal-remodeling proteins leading to a correct formation of the cell motility apparatus.

## 5. RhoGTPases

PIP3 accumulation at the plasma membrane coordinates the localization and activation of molecules able to rearrange the cytoskeleton. This observation has implied a downstream involvement of small GTPases controlling cell shape, like Rho GTPases that play specific roles in F-actin remodeling. The Rho small GTPases family is composed by three different members: Rac, RhoA and Cdc42. Furthermore, the Rac subfamily is composed by three highly homologous isoforms, Rac1, Rac2 and Rac3. Rac1 is ubiquitously