PI 3-Kinases and PTEN: How Opposites Chemoattract

Minireview

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Summary

Phosphatidylinositol lipids, such as $PI(4,5)P_2$ and $PI(3,4,5)P_3$, are key mediators in diverse intracellular signaling pathways. Two recent reports examine how the metabolism of these lipids by phosphatidylinositol 3-kinases and the PTEN 3-phosphoinositide phosphatase may coordinate G protein coupled signaling pathways during eukaryotic chemotaxis.

Introduction

All cells must sense and respond to signals in the extracellular environment. Many cells have the capacity to detect the direction and intensity of an extracellular chemical gradient and to respond by directed migration toward the source of the chemical. This process, called chemotaxis, is essential for a wide variety of cellular functions, including migration of single-celled organisms such as *Dictyostelium discoideum* toward nutrient sources, yeast mating, leukocyte-mediated killing of invading pathogens, wound repair, axon guidance, and cell migration during development. Eukaryotic cells of diverse origin share many common mechanisms for sensing and responding to chemoattractant gradients. These mechanisms are sensitive enough to detect gradients that differ by as little as 2% between the front and the back of the cell (Chung et al., 2001; Katanaev, 2001; Parent and Devreotes, 1999; Weiner, 2002). Many fundamental problems in this area of research center around the following question: How is a shallow external gradient of chemoattractant translated into a steep intracellular gradient of signaling responses that lead to cell polarization and accurate directional migration?

Green fluorescent protein (GFP) technology has permitted the direct examination of protein dynamics during chemotaxis in living cells (Figure 1). Initial studies on G protein coupled chemoattractant receptors in *D. discoideum* and subsequent studies in human neutrophils show that these receptors remain uniformly distributed around the plasma membrane during chemotaxis. Likewise, G protein βγ subunits do not cluster at the front of chemotaxing cells, but localize in a shallow anterior to posterior gradient that approximates receptor occupancy (Jin et al., 2000). Studies on the PH domains

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from several proteins, such as Cytosolic Regulator of Adenylyl Cyclase (CRAC) and Protein Kinase B (PKB), provided a breakthrough in understanding the dynamics of signaling during chemotaxis (Parent et al., 1998; Meili et al., 1999; Servant et al., 2000). Sudden increases in chemoattractant elicit rapid and transient translocation of PH domains from the cytosol to the activated plasma membrane. Remarkably, when *D. discoideum* amoebae or neutrophils are placed in a chemoattractant gradient, PH domains accumulate selectively at the leading edge, placing them in a position to regulate spatial sensing during chemotaxis. The PH domains of many proteins specifically bind to 3-phosphoinositides, such as $PI(3,4)P_2$ and $PI(3,4,5)P_3$. These findings have led to a model of gradient sensing in which localized 3-phosphoinositides production directs the anterior accumulation of the signaling components and machinery required for chemotaxis (Parent and Devreotes, 1999). In this model, the proper balance between the antagonist effects of 3-phosphoinositides metabolizing enzymes such as PI 3-kinase and PTEN lipid phosphatase is critical for interpretation of chemoattractant gradients.

Many laboratories have made recent progress in deciphering the roles of 3-phosphoinositides in the complex signaling pathways that lie between chemoattractant receptors and the chemotaxis hardware (Reviewed in Katanaev, 2001; Rickert et al., 2000; Stephens et al., 2002; Weiner, 2002). Here, we focus primarily on two new reports that examine the interplay between 3-phosphoinositide metabolizing enzymes in response to chemoattractants (Iijima and Devreotes, 2002; Funamoto et al., 2002 [both this issue]).

Roles of 3-Phosphoinositides in Eukaryotic Cell Polarity and Chemotaxis

Numerous reports implicate the 3-phosphoinositide synthetic enzyme, PI 3-kinase, as a central mediator linking early chemoattractant signals with downstream components of the chemotaxis response (Figure 2). Pharmacological inhibition of PI 3-kinases in *D. discoideum* amoebae and in a variety of mammalian cell types causes inhibition of chemotaxis and cell migration, to varying degrees. *D. discoideum* cells lacking the two Class I_A PI 3-kinases and leukocytes derived from mice lacking PI 3-kinase γ have defects in polarity and show reduced efficiency of chemotaxis (Chung et al., 2001; Katanaev, 2001). While these studies provide compelling evidence for a central role for PI 3-kinases during chemotaxis, other reports have given less definitive results. The disparities between these studies may arise from incomplete pharmacological inhibition, the presence of multiple PI 3-kinase isoforms, compensatory mechanisms, or nonlinear relationships between different components in the pathway. Additional studies are necessary to resolve these discrepancies and to define the precise functions of PI 3-kinases during eukaryotic chemotaxis.

Originally identified as a tumor suppressor gene mutated in a large percentage of human cancers, the $PI(3,4,5)P_3$ / $PI(3,4)P_2$ degrading enzyme, PTEN (Phosphatase and Tensin homolog), has emerged as a key
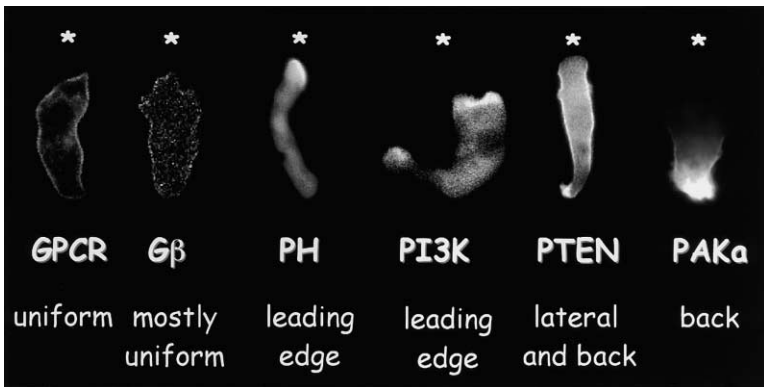
Figure 1. Montage of Fluorescent Images Revealing the Cellular Distribution of GFP-Tagged Signaling Components in *D. discoideum* Cells during Chemotaxis

Images of the G protein coupled cAMP receptor 1 (GPCR) and the β subunit of heterotrimeric G proteins (Gβ) were obtained from P.N. Devreotes. Images of the PI3K catalytic subunit, PTEN, and PAKa were obtained from R.A. Firtel. All images are used by permission. The PH domain was derived from CRAC. In each case, the gradient was established by the presence of a micropipette filled with chemoattractant. The stars represent the position of the micropipette for each image.

negative regulator of PI 3-kinase signaling (Leslie and Downes, 2002; Maehama et al., 2001; Seminario and Wange, 2002; Yamada and Araki, 2001). The PTEN protein has multiple conserved domains, including a C2 phospholipid binding domain, two potential PEST sequences, a PDZ binding domain, and a PIP_2 binding motif. Initial sequence alignments placed PTEN into the protein tyrosine phosphatase (PTP) family, and in vitro experiments directly demonstrated PTP activity. Nevertheless, the major in vivo substrates of PTEN are 3-phosphoinositides, primarily $PI(3,4,5)P_3$ and/or $PI(3,4)P_2$. Indeed, a point mutation that abrogates only the lipid phosphatase activity of PTEN (G129E) retains most of the $pten^{-/-}$ phenotype, including loss of tumor suppressor activity. Moreover, disruption of PTEN in mice causes accumulation of $PI(3,4,5)P_3$ and $PI(3,4)P_2$.

Several studies have examined the roles of PTEN in cell migration and invasiveness. Overexpression of PTEN suppresses cell migration in several cell types (Tamura et al., 1998). This decrease in migration is accompanied by a decrease in phosphorylation of the focal adhesion kinase (FAK), but there is debate about whether the PTP activity of PTEN is directly responsible

for this dephosphorylation. Fibroblasts isolated from $pten^{-/-}$ mice show a 2-fold increase in cell motility in a wound assay. The activation of Cdc42 and Rac1, which have well-established roles in cell motility (Liliental et al., 2000), may contribute to this migration defect. PTEN disruption in many cell types also increases activation of PKB, which mediates numerous $PI(3,4,5)P_3$ dependent responses.

In order for chemotactic cells to be responsive to changes in the chemoattractant gradient, the signaling pathways controlling chemotaxis must be very dynamic (Parent and Devreotes, 1999). If PI 3-kinases play a central role in the activation of chemotaxis, then PTEN is well positioned as a negative regulator in these pathways (Figure 2). The concerted action of these enzymes may therefore help to generate and maintain a polarized response to chemoattractant gradients.

Coordinated Regulation of Phosphoinositide Metabolism: Implications for Chemotaxis

The Devreotes and Firtel laboratories have taken complementary approaches to investigate the interplay between PI 3-kinases and PTEN during chemotaxis of *D. discoideum* amoebae (Iijima and Devreotes, 2002; Funa-
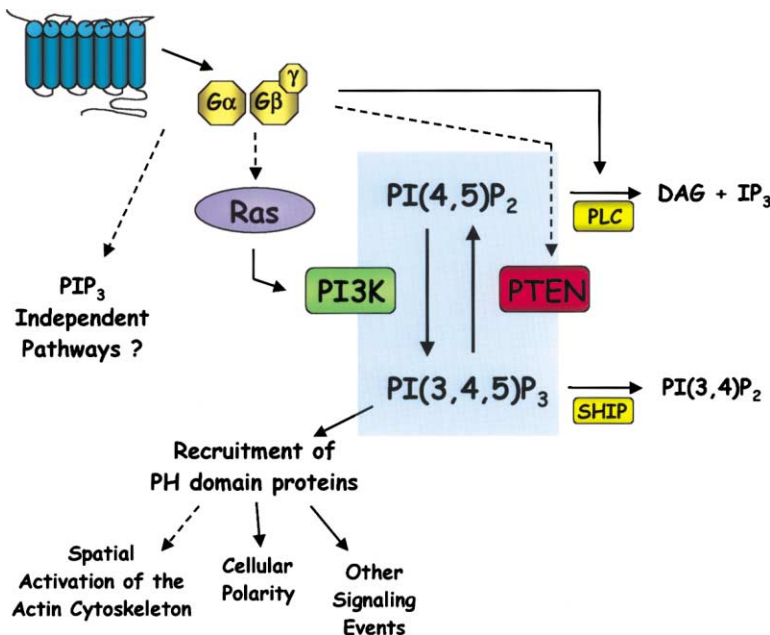

Figure 2. Model Depicting the Central Roles of PI3-Kinases and PTEN in the Regulation of Chemotaxis

The dashed lines indicate proposed pathways. When cells are placed in a gradient of chemoattractant, the delicate balance between the activation of PI3K and PTEN leads to the spatial enrichment of PIP_3 at the leading edge. This in turn selectively recruits a group of PH domain-containing proteins that act as adapters for a variety of downstream responses. PIP_2 and PIP_3 are further acted upon by PLC and SHIP, which may provide an added layer of regulation to the cascade. The fact that disruptions of PI3K and PTEN do not completely abolish chemotaxis suggests that PIP_3-independent pathways are also involved in the regulation of chemotaxis.

moto et al., 2002). The Firtel laboratory investigated the dynamics and activation of PI 3-kinases. They showed that upon uniform chemoattractant stimulation of a resting cell, PI 3-kinases rapidly translocate to the plasma membrane then dissociate with kinetics similar to that of PH domain proteins. In addition, in a chemoattractant gradient, PI 3-kinases localize to the front of chemotaxing cells (Figure 1). As with PH domains, this leading edge localization is dynamically responsive to changes in the chemoattractant gradient. The catalytic subunits of PI 3-kinases have a C-terminal lipid kinase domain, a putative Ras binding domain, and a C2 phospholipid binding domain. The *D. discoideum* PI 3-kinases do not need these domains for targeting, but only require a novel N-terminal domain for proper membrane localization. Importantly, the Firtel group found that localization and activation of PI 3-kinases are independent. Using a heterologous membrane targeting sequence, yeast two hybrid analysis, and point mutations, they identified a functional Ras binding domain and showed that it is essential for activation, but not for localization of PI 3-kinases. An important conclusion from these results is that, although PI 3-kinases may contribute to chemoattractant induced polarization and signal amplification, there must be another upstream mechanism that generates the initial asymmetry in signaling.

To investigate the other side of the 3-phosphoinositide circuit, both labs examined the effect of PTEN deficiency on various chemotaxis parameters, morphological changes, and PI 3-kinase dependent responses. The Devreotes group showed that PTEN null (*pten$^-$*) cells have both polarity and chemotaxis defects that are reciprocal to those of PI 3-kinase null (*pi3k $^{1-/2-}$*) cells (Chung et al., 2001) or wild-type cells treated with PI 3-kinase inhibitors. Whereas *pi3k $^{1-/2-}$* cells and the drug treated cells show an overall loss of polarity and decreased pseudopod formation, the *pten$^-$* cells show increased pseudopod extension and appear to have multiple competing polarity axes. Both groups observed that PH domains broadly distribute across the front half of the cell and the tips of the multiple pseudopodia during chemotaxis, suggesting that gradient sensing is impaired, but not lost, in PTEN deficient cells. The Devreotes group also found that uniform chemoattractant stimulation of resting *pten$^-$* cells results in dramatically prolonged PH domain association with the plasma membrane (6–8 min versus ∼30 s for wild-type). Although this observation demonstrates that loss of PTEN leads to a profound loss of adaptation to the chemoattractant signal, the fact that the response eventually subsides implies that there are other mechanisms to downregulate activation. The *pten$^-$* cells show a similar increase in the duration and intensity of chemoattractant-mediated actin polymerization responses. Although they are very motile, the *pten$^-$* cells are poorly chemotactic, presumably as a result of their polarity defects and their inability to spatially and temporally restrict gradient sensing. These observations confirm that cell motility is distinct from gradient sensing and chemotaxis. When the Firtel laboratory constitutively targeted PI 3-kinases to the plasma membrane, they observed defects that phenocopy the *pten$^-$* cells. The fact that loss of PTEN or loss of spatial restriction of PI 3-kinase result in similar phenotypes implies that the localized production and re-

striction of 3-phosphoinositides are important for efficient interpretation of chemoattractant gradients.

Examination of PTEN localization and dynamics provides additional clues to the role of 3-phosphoinositide metabolism during chemoattractant elicited signaling responses. Both groups showed that a fraction of PTEN is localized to the plasma membrane in the basal state. Upon uniform chemoattractant stimulation, PTEN delocalizes from the activated membrane within a few seconds and then relocalizes within 30–60 s, the opposite behavior of PI 3-kinases and PH domain proteins. Perhaps the most exciting finding from these reports is that PTEN localization is reciprocal to that of PI 3-kinases during chemotaxis (Figure 1). Both groups found that PTEN localizes to the sides and posterior of chemotaxing cells, while it is excluded from the leading edge. As with PH domains and PI 3-kinases, PTEN localization is dynamically responsive to changes in the chemoattractant gradient. The Devreotes report shows that deletion of a putative PI(4,5)P$_2$ binding motif results in a loss of membrane binding and an inability to rescue the *pten$^-$* phenotype, suggesting that membrane localization is essential for PTEN function. Taken together, these findings suggest the existence of a feedback loop. In this scenario, PI 3-kinase activity at the front of a chemotaxing cell activates downstream signaling responses and also excludes PTEN, resulting in greater persistence of 3-phosphoinositides at the leading edge. Meanwhile, PTEN activity on the sides and the posterior generates its own binding sites and also restricts the PI 3-kinase signal to the leading edge. The spatial restriction and the concerted action of these two enzymes could significantly amplify the signals emanating from G protein coupled chemoattractant receptors.

Future Challenges

The many remaining questions about how 3-phosphoinositide biology and chemotaxis are linked will provide a fertile area of investigation for several years to come. Perhaps the greatest difficulty in interpreting the roles of 3-phosphoinositide metabolism in eukaryotic chemotaxis is the complexity of the signaling pathways elicited by chemoattractants. G protein coupled chemoattractant receptors initiate multiple downstream physiological responses, but the identities of effectors immediately downstream from G proteins are largely unknown. Many key players have been identified, such as those described here, but the interrelationships between them and precisely how they are linked to G protein activation is unclear. Furthermore, some of these components associate with chemoattractant-stimulated membranes almost simultaneously. Although there are linear relationships between some components, it is likely that there are alternate, parallel, or synergizing pathways that may be partially interdependent. Indeed, synergy between multiple pathways may explain the fact that disruptions of PI 3-kinase or PTEN do not abolish chemotaxis. In support of this notion, a recent report described a new guanine nucleotide exchange factor (GEF) for Rac called P-Rex1 (Welch et al., 2002). P-Rex1 is subject to synergistic activation by both PI(3,4,5)P$_3$ and G protein βγ subunits, suggesting a role in signal integration between these two pathways. Such a GEF and its associated GTP binding protein may be a nexus be-

tween G protein activation and downstream responses such as PI 3-kinase activation.

A fundamental question remains: what is the initial amplification signal that translates a shallow extracellular chemoattractant gradient into a steep intracellular gradient of signaling responses? Since PI 3-kinases and PTEN become polarized in response to chemoattractant, there must be a polarizing signal upstream of these enzymes. Nevertheless, PI 3-kinases and PTEN may contribute to a feedback loop that amplifies the initial polarizing signal. Results from both the Firtel and Devreotes labs suggest that the spatial restriction of positive PI 3-kinase signals at the front and negative PTEN signals at the sides and back amplifies the initial signal. Additional circuits comprising a similar feedback loop may constitute the initial mechanism of spatial interpretation of chemoattractant gradients.

There is still much to learn about the regulation of phosphoinositide metabolizing enzymes during chemotaxis. Discovery of how PI 3-kinases localize to the leading edge of chemotaxing cells may identify the initial polarizing signal. It is important to understand how both spatial restriction and Ras mediated activation of PI 3-kinase may regulate 3-phosphoinositide signaling during chemotaxis. Studies on how PTEN may regulate its own localization by producing $PI(4,5)P_2$ binding sites will provide further insight into the spatial control of the PI 3-kinase response. It is also important to know whether chemoattractant receptor activation regulates the enzymatic activity as well as the spatial restriction of PTEN. In addition to PTEN and PI 3-kinases, many reports suggest a role for 5-phosphoinositide phosphatases (Figure 2), such as SHIP, in modulating $PI(3,4,5)P_3$ dependent responses (Field et al., 2002). Since many PH domains bind to $PI(3,4)P_2$, in addition to $PI(3,4,5)P_3$, SHIP may provide additional physiologically important signaling intermediates.

There are also gaps in our understanding of downstream responses. The 3-phosphoinositide-dependent activation of Rac and Cdc42 suggests key roles for these lipids during the actin cytoskeleton remodeling required for pseudopod extension and the establishment and maintenance of polarity. Indeed, the Devreotes lab showed that disruption of PTEN alters chemoattractant mediated actin polymerization, suggesting a link between 3-phosphoinositide metabolism and actin remodeling. Although this review has focused on signaling events at the leading edge, it is also important to consider how these responses coordinate with activity at the back of the cell. The 3-phosphoinositide dependent activation and localization of the *D. discoideum* p21 activated kinase (PAKa) to the posterior of chemotaxing cells (Chung et al., 2001) directs the cortical accumulation of myosin II, which is required for posterior retraction (Figure 1). This observation demonstrates that there is anterior to posterior coordination during chemotaxis.

The challenges for the future are typical of the postgenomic era: we know many of the players, but not the complex relationships between them. Studies such as those presented here are rapidly advancing our understanding of the complex signal integration required for gradient sensing and chemotaxis.

Selected Reading

Chung, C.Y., Funamoto, S., and Firtel, R.A. (2001). Trends Biochem. Sci. *26*, 557–566.

Field, S.J., Lamia, K.A., Rameh, L.E., and Cantley, L.C. (2002). Dev Cell *2*, 407–410.

Funamoto, S., Meili, R., Lee, S., Parry, L., and Firtel, R.A. (2002). Cell *109*, this issue, 611–623.

Iijima, M., and Devreotes, P. (2002). Cell *109*, this issue, 599–610.

Jin, T., Zhang, N., Long, Y., Parent, C.A., and Devreotes, P.N. (2000). Science *287*, 1034–1036.

Katanaev, V.L. (2001). Biochemistry *66*, 351–368.

Leslie, N.R., and Downes, C.P. (2002). Cell Signal *14*, 285–295.

Liliental, J., Moon, S.Y., Lesche, R., Mamillapalli, R., Li, D., Zheng, Y., Sun, H., and Wu, H. (2000). Curr. Biol. *10*, 401–404.

Maehama, T., Taylor, G.S., and Dixon, J.E. (2001). Annu. Rev. Biochem. *70*, 247–279.

Meili, R., Ellsworth, C., Lee, S., Reddy, T.B., Ma, H., and Firtel, R.A. (1999). EMBO J. *18*, 2092–2105.

Parent, C.A., and Devreotes, P.N. (1999). Science *284*, 765–770.

Parent, C.A., Blacklock, B.J., Froehlich, W.M., Murphy, D.B., and Devreotes, P.N. (1998). Cell *95*, 81–91.

Rickert, P., Weiner, O.D., Wang, F., Bourne, H.R., and Servant, G. (2000). Trends Cell Biol. *10*, 466–473.

Seminario, M.C., and Wange, R.L. (2002). Semin. Immunol. *14*, 27–36.

Servant, G., Weiner, O.D., Herzmark, P., Balla, T., Sedat, J.W., and Bourne, H.R. (2000). Science *287*, 1037–1040.

Stephens, L., Ellson, C., and Hawkins, P. (2002). Curr. Opin. Cell Biol. *14*, 203–213.

Tamura, M., Gu, J., Matsumoto, K., Aota, S., Parsons, R., and Yamada, K.M. (1998). Science *280*, 1614–1617.

Weiner, O.D. (2002). Curr. Opin. Cell Biol. *14*, 196–202.

Welch, H.C., Coadwell, W.J., Ellson, C.D., Ferguson, G.J., Andrews, S.R., Erdjument-Bromage, H., Tempst, P., Hawkins, P.T., and Stephens, L.R. (2002). Cell *108*, 809–821.

Yamada, K.M., and Araki, M. (2001). J. Cell Sci. *114*, 2375–2382.