Rho Rocks PTEN

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Many biological processes require the movement of cells in response to guidance cues. Small GTPases and phosphoinositides are key mediators of the underlying cytoskeletal rearrangements, and a new study establishes that the localization and activation of the phosphoinositide phosphatase PTEN at the rear of chemotaxing neutrophils is mediated by the small GTPase RhoA.

Chemotaxis of cells requires three key processes: cell alignment along the chemoattractant gradient; cell polarization; and protrusion and retraction of cytoskeletal elements leading to net movement towards the attractant source. Many of the mechanisms controlling these processes have been defined using two experimental systems — mammalian neutrophils and the amoebae *Dictyostelium* — and the underlying mechanisms in both systems are similar. G-protein-coupled chemoattractant receptors initiate the process, and Rho family GTPases are the key effectors in all three processes. In neutrophils, Rac mediates protrusion of the leading edge, or pseudopod, by stimulating F-actin assembly; Rho controls retraction of the posterior via actin-myosin contractility; and Cdc42 guides the direction of the response at the front[1–3]. Production and localization of the lipid signalling molecule phosphatidylinositol 3,4,5 trisphosphate (PtdIns(3,4,5)P_3) at the leading edge is also important for directed migration. The PtdIns(3,4,5)P_3 phosphatase PTEN, a tumour suppressor that is an essential regulator of growth responses[4], was known to restrict PtdIns(3,4,5)P_3 localization in chemotaxing *Dictyostelium* cells[5,6]. Now, on page 399 of this issue, Li *et al.*[7] determine that Rho and Cdc42, in addition to their roles in cytoskeletal regulation, control the localization and activity of PTEN in mammalian cells.

In *Dictyostelium*, PTEN is associated with the plasma membrane in resting cells. During chemotaxis, PTEN delocalizes from the leading edge and is present only at the back and lateral sides of the cell and thereby restricts PtdIns(3,4,5)P_3 accumulation to the front of the cell. Loss of PTEN leads to elevated, unregulated PtdIns(3,4,5)P_3 production along the entire plasma membrane and defective directional movement[5,6]. Similarly, in migrating mammalian neutrophils, PTEN is localized to

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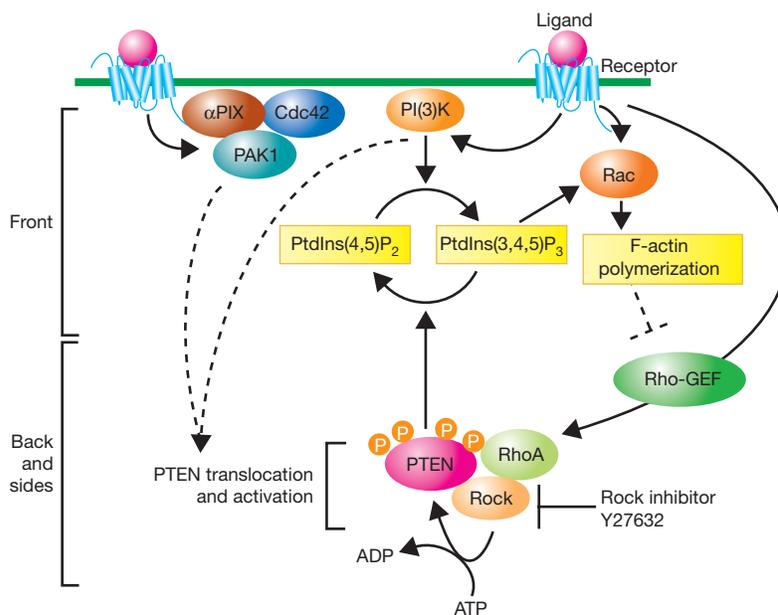e-mail: rafirtel@ucsd.edu

Figure 1 PTEN localization and activity is restricted by the small GTPases RhoA and Cdc42 in chemotaxing leukocytes. The study from Li *et al.* provides new insights into the spatial regulation of PTEN. First, chemoattractant activates Rock via Rho-GEF (guanine nucleotide-exchange factor) and RhoA. Activated Rock then binds and phosphorylates PTEN, which localizes with RhoA and Rock to the back and sides of polarized, motile cells. The activation of PI(3)K and Cdc42/PAK1/αPIX complex at the leading edge further increases PTEN activity, possibly through the restriction of RhoA to the cell's posterior. Dashed lines indicate a linkage by as yet unknown mechanisms.

the posterior of the cell[2]. However, in mammalian cells there seems to be an additional layer of regulation because in resting cells PTEN is found in the cytoplasm, whereas its substrates are membrane lipids. Furthermore, although Cdc42 function was shown to be required for PTEN localization in leukocytes[2], the mechanisms by which PTEN is restricted to the cell's posterior were not known. PTEN activity also seems to be controlled by phosphorylation: previous analysis has revealed several phosphorylation sites on PTEN that inhibit activity[8], but no phosphorylation events resulting in activation had been previously described. In their new study, Li *et al.* provide evidence that RhoA/RhoA-associated kinase (Rock)-mediated phosphorylation of PTEN controls PTEN's localization and its activation.

Li *et al.* now demonstrate that, as in *Dictyostelium*[1,5,6,9], PTEN is required for

directional movement during leukocyte chemotaxis. PTEN localizes to the back of chemotactic neutrophils, colocalizing there with activated Rho, but is absent from the front where Cdc42 is active. This prompted the authors to test the hypothesis that Rho activity positively regulates PTEN localization to the cell's posterior whereas Cdc42 excludes PTEN from the area of its activity. As with other cells, neutrophils polarize spontaneously when presented with a uniform concentration of chemoattractant and the authors make use of this property to test their model. They examined the role of Cdc42 and RhoA in PTEN localization. They find that PTEN no longer localizes to the back of the cell either in αPIX-null cells (which cannot activate Cdc42 (ref. 2)) or after inhibiting the RhoA effector kinase Rock[10], suggesting that activation of both GTPases is essential for

PTEN's localization in polarized, migrating cells. Their finding in this present manuscript that Cdc42 activity is required for proper RhoA localization could explain Cdc42's role in PTEN localization. The authors go on to investigate the mechanism of the RhoA effect in detail. They demonstrate that expression of a constitutively active mutant of RhoA induces PTEN translocation to the cortex of HEK cells, indicating that the mechanism regulating PTEN cortical localization is not exclusive to leukocytes. They did not observe this localization when they used green fluorescent protein (GFP)-tagged PTEN, which might suggest that both the amino and the carboxyl termini of PTEN participate in the localization process. This is consistent with the observation that the N-terminal membrane-binding domain is required for PTEN's membrane localization in *Dictyostelium*[11].

To follow PTEN phosphatase activity, the authors measured the phosphorylation of Akt, which depends on PtdIns(3,4,5)P$_3$ levels. Their results suggest that PTEN is activated when it translocates to the posterior cortex and that PTEN is inactive in resting cells. PTEN activation by constitutively active RhoA was confirmed using an *in vitro* phosphatase assay. Furthermore, they demonstrated that Rock can directly phosphorylate PTEN *in vitro* and that Rock, PTEN and activated RhoA form a complex *in vivo*. Inhibition of Rock was known to affect neutrophil polarity and motility[3,10,12,13]. This suggests a mechanism by which activated RhoA in the back of the cell activates Rock, which then forms a complex with and phosphorylates PTEN. Mass spectrometry identified four sites in the C2 domain of PTEN that were phosphorylated in chemoattractant-stimulated cells. Mutant PTEN with alanine substitutions at all four sites was unable to rescue the

inhibition of Jurkat cell chemotaxis by *PTEN* siRNA. In addition, unlike wild-type PTEN, mutant PTEN did not reduce Akt phosphorylation when co-overexpressed in HEK cells with constitutively active RhoA, indicating that the mutant PTEN was not activated. Thus, Rock-mediated phosphorylation is required for PTEN activation.

While it has long been recognized that Cdc42 has a function in defining the leading edge of the cell, and RhoA has a function in defining the rear, the link with PI(3)K signalling has been unclear. The authors convincingly demonstrate a link from RhoA through Rock to PTEN. Such a pathway agrees with the previously observed effect of Rock inhibitors on leukocyte polarity and chemotaxis[3,10,12,13]. Activation of PI(3)K at the front of cells seems to be mainly mediated by direct binding of the p101 regulatory subunit to Gβγ, and activated Ras to the p110 catalytic subunit after receptor activation[14,15] and, as a consequence, increasing PtdIns(3,4,5)P$_3$ levels there. Activation of Cdc42 occurs via a parallel pathway involving a αPIX/PAK1/Cdc42 complex[2]. These two pathways cooperate to activate F-actin polymerization via Rac. The authors observe that activated Cdc42 seems to be required for localization of activated RhoA to the back of the cell. It will be interesting to understand the mechanism by which Cdc42 regulates RhoA. Further experiments will also be needed to determine whether Rock directly phosphorylates PTEN at the identified phosphorylation sites *in vivo*, whether phosphorylation of PTEN regulates its catalytic activity, or whether it simply serves to recruit PTEN to its substrate. The authors' demonstration that RhoA/Rock-mediated PTEN recruitment into a complex in the back of leukocytes as a consequence of phosphorylation of PTEN by Rock provides

a mechanistic insight into how leukocytes restrict PI(3)K function to their leading edge. What is missing is a biochemical confirmation that the activated PTEN resides on the cortex. It would also be interesting to know where the non-phosphorylatable PTEN mutant localizes in chemotaxing cells. When constitutively active components are overexpressed in non-chemotactic cells, PTEN localizes to the cortex, suggesting that the mechanism of PTEN localization may function in a wide range of cell types. It remains to be seen how general this mode of PTEN activation is. PTEN is an important tumour suppressor[4], but it seems that its function during chemotaxis is unrelated to its role in cell growth. The link is solely via PTEN's antagonistic effect on PI(3)K activity, which is required to downregulate growth and survival signals in one case, and to establish front-back polarity in the other. The study by Li *et al.* adds an important piece to the puzzle of how the PI(3)K/PTEN pathway is controlled during leukocyte chemotaxis and directional sensing. □

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