

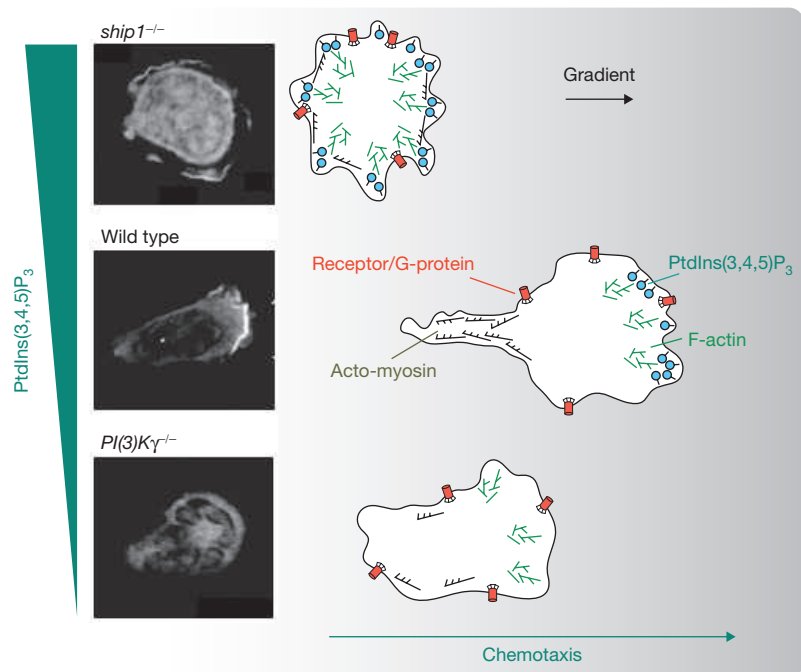
## Leading-edge research: PtdIns(3,4,5)P<sub>3</sub> and directed migration

Jonathan Franca-Koh, Yoichiro Kamimura and Peter N. Devreotes

**New studies reveal the dynamic accumulation of phosphatidylinositol (3,4,5) trisphosphate (PtdIns(3,4,5)P<sub>3</sub>) at the leading edge of primary neutrophils during chemotaxis. They also demonstrate that SHIP1, rather than phosphatase and tensin homologue (PTEN), is responsible for the degradation and localization of this lipid in neutrophils and shed light on the role of PtdIns(3,4,5)P<sub>3</sub> in directional sensing.**

Chemotaxis is the ability to move in the direction of increasing or decreasing concentrations of external signalling molecules. This process guides the spread of metastatic cancer cells towards growth factors, the coordinated cell movements that produce morphogenesis during development and the migration of immune cells to sites of infection. Research on chemotaxis by phagocytic cells, such as neutrophils, has seen significant progress in recent years. Researchers have mainly used HL-60 cells (a cell line that can be differentiated into neutrophil-like cells *in vitro*) or the amoeba *Dictyostelium discoideum* (which resemble human immune cells in their behaviour) as model systems<sup>1,2</sup>. Studies in these models have shown that the well known lipid second messenger, PtdIns(3,4,5)P<sub>3</sub>, accumulates at the front of chemotaxing cells (Fig. 1). PtdIns(3,4,5)P<sub>3</sub> is produced by phosphorylation of PtdIns(4,5)P<sub>2</sub> by a family of enzymes known as phosphatidylinositol 3-kinases (PI(3)Ks) and is known to regulate the localization of specific proteins (such as AKT/PKB; protein kinase B), by binding to their pleckstrin homology (PH) domains<sup>3,4</sup>. PtdIns(3,4,5)P<sub>3</sub> is also elevated on actin-based projections during macropinocytosis, phagocytosis and cytokinesis<sup>2</sup>. The close association of this molecule with cytoskeletal rearrangements suggested that it could act as the cell's 'compass' to translate the spatial information provided by chemoattractant gradients

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**Figure 1** The effect of PtdIns(3,4,5)P<sub>3</sub> levels on chemotaxis. The relative levels of PtdIns(3,4,5)P<sub>3</sub> in the three cell types are schematically represented on the left of the images. The images show the localization of AKT<sup>PH</sup>-GFP in neutrophils stimulated by chemoattractants and are taken from Fig. 6 of Nishio *et al.*<sup>9</sup> Compared with wild-type neutrophils, chemotaxis is inhibited by deleting either SHIP1, or to a lesser extent PI(3)K<sub>γ</sub>. This is schematically represented on the right, where the chemoattractant gradient is depicted by the grey shading and chemotactic efficiency is represented by distance along the x-axis. The accumulation of PtdIns(3,4,5)P<sub>3</sub> at the leading edge of wild-type cells is thought to promote efficient migration and polarization, possibly through positive feedback loops. However, even though PtdIns(3,4,5)P<sub>3</sub> does not concentrate at the leading edge of PI(3)K<sub>γ</sub><sup>-/-</sup> cells, loss of this protein has only slight effects on chemotaxis and polarity. Knocking out SHIP1 has much stronger effects on neutrophil chemotaxis. In these cells, PtdIns(3,4,5)P<sub>3</sub> is localized at many points around the cell periphery, leading to multiple membrane projections away from the chemoattractant source.

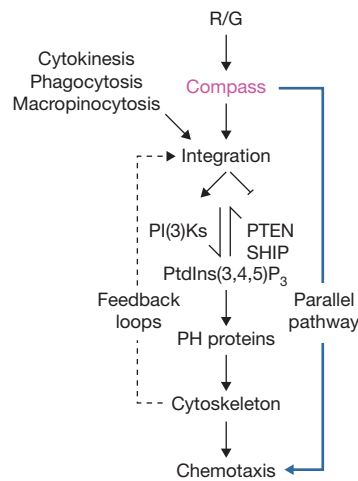
into directed cell movement<sup>5</sup>. In *D. discoideum* in particular, it has been shown that the complementary localization of PI(3)Ks and PTEN

amplifies the distribution of PtdIns(3,4,5)P<sub>3</sub> with respect to the gradient<sup>6,7</sup>. PI(3)Ks are recruited to the plasma membrane at the front

of cells, resulting in the localized production of PtdIns(3,4,5)P<sub>3</sub>. Conversely, PTEN is recruited to the back and sides of cells where it degrades PtdIns(3,4,5)P<sub>3</sub> by removing the 3-phosphate. In mammalian cells, however, the levels of PTEN at the membrane are typically too low to detect by standard techniques<sup>8</sup>. This restricted distribution of PtdIns(3,4,5)P<sub>3</sub> towards the gradient can occur in the absence of actin polymerization, indicating that cells can sense direction without moving.

On pages 36 and 86 in this issue, Nishio *et al.* and Ferguson *et al.* have extended these observations by looking at the dynamic distribution of phosphoinositides in primary neutrophils<sup>9,10</sup>. To assess the localization of these lipids, both groups studied neutrophils harvested from transgenic mice that express AKT<sup>PH</sup>-GFP (the pleckstrin homology domain of AKT fused to GFP), a biosensor that binds to PtdIns(3,4,5)P<sub>3</sub> and PtdIns(3,4)P<sub>2</sub>. Rewardingly, the expression of this construct did not interfere with chemotaxis and strongly translocated to the front of the neutrophils, as was predicted by the earlier work performed in HL-60 and *D. discoideum* cells. To determine whether PTEN played the predicted role in the neutrophil chemotaxis, Nishio *et al.* went on to investigate AKT<sup>PH</sup>-GFP localization and chemotaxis in neutrophils lacking PTEN. Homozygous *pten*<sup>-/-</sup> knockouts are embryonic lethal, therefore the Cre-Lox system was used to disrupt PTEN specifically in cells of the granulocytic lineage, which includes neutrophils. Surprisingly, *pten*<sup>-/-</sup> neutrophils did not have elevated levels of PtdIns(3,4,5)P<sub>3</sub> and were able to migrate as fast and as accurately as wild-type cells toward chemoattractants. This prompted the authors to investigate another regulator of PtdIns(3,4,5)P<sub>3</sub> degradation, the 5-phosphatase SHIP1 (SH2 domain-containing inositol 5-phosphatase 1)<sup>11,12</sup>. Strikingly, loss of this gene led to the type of defects observed in the *D. discoideum* PTEN knockouts — the *ship1*<sup>-/-</sup> neutrophils had a flat, unpolarized profile due to an increase in the number of membrane extensions labelled by AKT<sup>PH</sup>-GFP (Fig. 1). In a chemoattractant gradient, these defects prevented these cells from acquiring the characteristic polarized morphology and severely impaired the speed of migration. This work shows that different cell types can use either SHIP1 or PTEN to control the levels of PtdIns(3,4,5)P<sub>3</sub> and regulate the actin cytoskeleton.

The two studies also asked how diminished PI(3)K activity affects neutrophil chemotaxis.



**Figure 2** Schematic representation of a model for directional sensing. We propose that the data from Nishio *et al.*, Ferguson *et al.* and previous studies that have investigated the role of PtdIns(3,4,5)P<sub>3</sub> in chemotaxis can be best explained by invoking the existence of a parallel pathway downstream of the compass. In this model, receptor signalling through heterotrimeric G-proteins (R/G) orients the cell's compass and activates both the PtdIns(3,4,5)P<sub>3</sub> pathway and at least one other, unknown, parallel pathway that can regulate chemotaxis. Phosphoinositide signalling is also regulated by other events (such as cytokinesis, phagocytosis and macropinocytosis) and we suggest that signals from the compass may be integrated with these inputs before regulating PI(3)K, PTEN and SHIP activity. The accumulation of PtdIns(3,4,5)P<sub>3</sub> may regulate the cytoskeleton by recruiting PH-domain containing proteins, and positive feedback loops may promote migration and polarization. When phosphoinositide levels are reduced (such as in the PI(3)K<sup>-/-</sup> cells), signalling through the parallel pathway can compensate for this defect and may explain why only moderate effects on chemotaxis are observed. However, when PtdIns(3,4,5)P<sub>3</sub> levels are elevated (such as in *ship1*<sup>-/-</sup> cells), the chemotaxis defects are much more severe as overactive phosphoinositide signalling overrides signals from the parallel pathway.

Nishio *et al.* report that neutrophils from PI(3)K<sup>-/-</sup> mice migrated at about half the speed of wild-type cells on albumin coated surfaces (Fig. 1)<sup>9</sup>. Ferguson *et al.* did not observe any chemotaxis defects on uncoated glass. In their hands, PI(3)K<sup>-/-</sup> neutrophils or wild-type cells treated with isoform specific inhibitors of PI(3)Ks caused many cells to become immobile when studied on surfaces coated with fibrinogen<sup>10</sup>. In this context, Ferguson *et al.* showed that PI(3)K<sup>-/-</sup> neutrophils have a strong adhesion defect that is probably due to the failure of these cells to upregulate certain integrins. They also found that although the initial phase of chemoattractant-induced

polarized actin polymerization was normal in the PI(3)K<sup>-/-</sup> knockouts, the proportion of cells that remain polarized over longer time courses was reduced. This is consistent with previously published data showing that PI(3)K inhibitors prevent differentiated HL-60 cells maintaining long-term polarity, and that *D. discoideum* cells with reduced PI(3)K activity have a defect in the second phase of actin polymerization<sup>13,14</sup>. These data suggest a role for PtdIns(3,4,5)P<sub>3</sub> in a positive-feedback loop that promotes persistent actin polymerization.

Taken together, these two studies validate the results from model systems that suggest PtdIns(3,4,5)P<sub>3</sub> influences cell motility by positively regulating the actin cytoskeleton, but they also add to the somewhat confusing series of reports on the role of PI(3)K signalling in chemotaxis<sup>8,15,16</sup>. Although its distribution and regulation strongly suggest that PtdIns(3,4,5)P<sub>3</sub> is part of the cell's compass, it is also clear that whether this second messenger is depleted or in excess, cells still can move towards chemoattractants. Nishio *et al.* argue that, in neutrophils, the main role of PtdIns(3,4,5)P<sub>3</sub> is to augment an initial polarization set-up by some other receptor-mediated event<sup>9</sup>, whereas Ferguson *et al.* conclude that PtdIns(3,4,5)P<sub>3</sub> is not a major component of the compass<sup>10</sup>. However, these results do not exclude PtdIns(3,4,5)P<sub>3</sub> as a mediator of directional sensing if there is also a parallel pathway. We propose a scheme to attempt to integrate all of these findings (Fig. 2). In this scheme, the 'compass' is upstream of PtdIns(3,4,5)P<sub>3</sub> synthesis. This can be inferred, not only from the studies reported here, but also from the observation that *D. discoideum* cells, in the absence of PI(3)K activity, can asymmetrically localize PI(3)K protein, PTEN and Ras activity in gradients<sup>6,7,17</sup>. Information from the compass may then be integrated with signals from other processes to regulate the localization of PtdIns(3,4,5)P<sub>3</sub>. The compass would also signal through a parallel pathway to direct chemotaxis. In this model, perturbations that lower PtdIns(3,4,5)P<sub>3</sub> production would only have minor effects on chemotaxis due to the compensatory pathway. Mislocalized or increased levels of phosphoinositides, however, would have severe effects as excess amounts of PtdIns(3,4,5)P<sub>3</sub> would disrupt polarity and directed migration by promoting the extension of lateral pseudopodium, as has been observed. Our scheme does raise the question: what are the parallel mediators of chemotaxis? Stay tuned. □

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## Sculpting a fly leg: BMP boundaries and cell death

Marco Milán

**Bone morphogenetic proteins (BMPs) shape vertebrate limbs and define digits by inducing programmed cell death in interdigital tissues. Recent findings show that *Drosophila* legs are also sculpted by programmed cell death. In this case, rather than the absolute activity of BMP, it is the sharp discontinuity of BMP signalling that is required for forming the leg joint.**

The elimination of interdigital cells in vertebrate limbs with free digits constitutes a well-studied example of inductive morphogenetic-programmed cell death or apoptosis. BMPs, a family of secreted proteins that belongs to the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily, are expressed in the interdigital zones of the developing limbs, where they trigger apoptotic signals<sup>1</sup>. In *Drosophila*, programmed cell death is activated through a different pathway in the developing compound eye to remove excess cells between ommatidia — in this situation, the epidermal growth factor (EGF) receptor provides cells with a survival signal that is antagonized by the activity of the Notch pathway to induce apoptosis in excess cells<sup>2</sup>. The acquisition of the final shape and size of *Drosophila* adult legs and wings has classically been associated with the control of cell proliferation.

On page 57 of this issue, Manjón *et al.* demonstrate that apoptosis is also required for the morphogenesis of adult legs<sup>3</sup>. Fly legs are morphologically subdivided into segments by flexible structures called joints (Fig. 1a) and the study shows that joint morphogenesis relies on programmed cell death. Interestingly, in this case, the authors provide evidence that cell death is caused by the generation of a sharp boundary in the activity of Dpp, another member of the BMP family involved in morphogenetic apoptosis.

In the embryonic ectoderm of *Drosophila*, limb primordia are set aside as discrete groups of cells (known as imaginal discs) that proliferate during subsequent larval stages to give rise, after metamorphosis, to adult appendages. The leg primordium is subdivided into an anterior and a posterior compartment by the activity of the homeodomain transcription factor Engrailed, which is found in posterior cells. Engrailed activates the expression of the secreted molecule Hedgehog, which signals to nearby anterior cells and induces the expression of the long-range organizing molecules Wingless (Wg) and Dpp (Fig. 1a). Wg and Dpp form overlapping activity gradients that specify cell fates along the proximo-distal axis of the leg by regulating the expression of transcription factors that define several domains<sup>4</sup>. Each of these genes is required for the formation of specific regions of the leg (Fig. 1a), however, their expression domains do not correspond precisely to the future segments of the adult leg. The manner in which the activity of these genes leads to the precise induction of the segment boundaries and subsequently the joints, is a complex process that is now beginning to be elucidated<sup>5</sup>.

Segmentation can already be visualized in the developing leg primordia as folds of the epithelium and is initiated by the activity of the Notch pathway in a pattern of rings, each corresponding to the distal end of each forming segment<sup>6–8</sup>. Loss of Notch activity leads to disruption of segment boundaries, absence of adult joints and reduced growth of the flanking regions. The expression of the

pro-apoptotic gene *reaper* also follows a ring pattern, indicating its presence in the distal end of each forming segment<sup>3</sup>. This drew the attention of Manjón *et al.* to the function of programmed cell death in joint formation. The *Drosophila* proteins Reaper, Hid and Grim, together with the mammalian functional orthologue Smac/Diablo, belong to the class of cell-death activators that contain a conserved amino-terminal RHG motif, and that induce caspase-dependent cell death through the repression of inhibitor of apoptosis proteins (IAPs)<sup>9</sup>. The authors show that *reaper* expression in *Drosophila* legs correlates with increased apoptosis in these regions and activation of the JNK pathway (known to be required for the removal of ‘unwanted’ mutant or slow proliferating cells in limb primordia<sup>10</sup>). Inhibition of apoptosis by expressing the apoptotic inhibitor P35, or by removing the three RHG pro-apoptotic genes *reaper*, *hid* and *grim*, compromises the formation of the folds in the leg primordium and of the adult joints. On the basis of these observations, the authors conclude that apoptosis is required in this process. A crucial and unanswered question is how apoptosis of *reaper*-expressing cells leads to the epithelial folding that occurs in presumptive joints. It is interesting to note that a similar *reaper*-dependent mechanism is also used in the fly embryo to sculpt the head segments and to generate the epithelial folds at their boundaries<sup>11</sup>.

BMPs are expressed in the interdigital zone of developing vertebrate limbs, where they induce apoptosis to generate a free-digit limb<sup>1</sup>. The BMP orthologue Dpp has previously been reported to be expressed at the

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