PI5P migrates out of the PIP shadow

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The dynamic nature of membrane phospholipid turnover was first described by Hokin and Hokin during their studies on exocrine tissue stimulation (reviewed in [1]). Subsequently, phosphatidylinositol (PI) and its phosphorylated products, called phosphoinositides, were shown to have a fundamental role in regulating membrane-cytosol interfaces in several contexts, including regulating signal transduction, membrane traffic and permeability, the cytoskeleton, nuclear events and transport [1]. Seven phosphoinositides have been identified and are defined by the phosphorylation state of the inositol headgroup at the 3', 4' and 5'-position, which is regulated reversibly by several lipid kinases and phosphatases [1]. Of the seven lipid species, phosphatidylinositol-5-phosphate (PI5P) was most recently discovered by the Cantley group and remains the most enigmatic of the phosphoinositide family [2]. PI5P levels have been shown to change in response to stimuli such as thrombin, histamine, insulin and oxidative stress, and have been suggested to regulate various processes, including signalling pathways, nuclear functions, vesicular transport and cytoskeletal organization [3–5]. In a recent issue of *EMBO reports*, Oppelt and colleagues identified a new role for PI5P as a positive regulator of cell migration probably by facilitating actin cytoskeletal rearrangements [6].

Unlike other phosphoinositides (PI3P and PI4P) that can be produced through the direct phosphorylation of PI, there is no evidence that PI5P can be produced through the direct *in vivo* phosphorylation of PI. Instead, PI4P 5-kinases generate PI(4,5)P₂ from PI4P and PIKfyve generates PI(3,5)P₂ from PI3P, and the products PI(3,5)P₂ and PI(4,5)P₂ are dephosphorylated by myotubularin-related proteins (that is, MTMR3) and inositol 4-phosphatases (PI(4,5)P₂ phosphatases type I and II), respectively, to produce PI5P (Fig 1). Recent *in vivo* evidence indicates

Fig 1 | Pathways of PI5P synthesis and turnover. Pathways demonstrated *in vivo* are shown in bold text and arrows, whereas *in vitro*, putative pathways are represented in italics and broken arrows. Thick arrows highlight the PI5P production pathway responsible for cell migration as reported in reference [6]. IpgD, invasion plasmid gene D; MTM, myotubularin; PI, phosphatidylinositol; PI(3)K, phosphoinositide 3-kinase; PI5P, phosphatidylinositol-5-phosphate; PIKFyve, phosphoinositide kinase, FYVE finger containing; PTPMP1, protein tyrosine phosphatase, mitochondrial 1.

that the PIKfyve–MTMR pathway is responsible for most PI(3)P production [7]. The study from the Wesche lab shows that production of PI(3)P through the MTMR3 pathway is important for cell migration [6]. After initially observing that depletion of the class III PI(3)K Vps34 and its lipid product, PI(3)P, impairs cell migration, the authors performed a small interfering RNA (siRNA) screen to identify FYVE or PX-domain-containing PI(3)P effectors that regulate this process. In addition to factors already known to promote migration—for example, Cdc42, FGD1, FGD2 and Hrs—PIKfyve and MTMR3 were identified as new candidates. PIKfyve has a PI(3)P-binding FYVE domain and, as mentioned above, synthesizes PI(3,5)P₂, whilst MTMR3, a member of the myotubularin family containing a PI(3)P-binding PH-GRAM domain, is a phosphatase that

dephosphorylates PI(3,5)P₂ to PI5P. Cells depleted of PIKfyve or MTMR3 showed impaired cell polarization and defects in orienting actin filaments and the Golgi complex towards the growth factor stimulus. In addition, siRNA experiments and pharmacological inhibition of PIKfyve decreased migration velocity and persistence. These findings were confirmed *in vivo* when ablation of MTMR3 in border cells inhibited border cell migration in *Drosophila* during oogenesis. Taken together, the authors demonstrate that the lipid enzymes Vps34, PIKfyve and MTMR3 regulate the PI3P–PI(3,5)P₂–PI5P phosphoinositide interconversion pathway that mediates cell migration [6].

The low abundance, dynamic turnover and tight spatial restriction of the phosphoinositides enable them to mediate acute responses within cells [1]. Part of the initial

difficulty in identifying PI5P was due to the fact that it is the phosphoinositide of lowest abundance—approximately 1–2% of PI4P—and that earlier high-performance liquid chromatography (HPLC) techniques had erroneously measured PI5P as its PI4P isomer [8]. By using improved biochemical techniques, the authors measured phosphoinositide levels during cell migration and found specifically that levels of PI5P rise acutely in response to migratory stimulation by fibroblast growth factor 1 (FGF1). Cells treated with PIKfyve and MTMR3 siRNA failed to produce PI5P on FGF1 stimulation, again suggesting that PIKfyve and MTMR3 constitute the interconversion pathway responsible for the production of PI5P during migration. Importantly, Oppelt and colleagues showed convincingly that PI5P is a relevant signalling mediator of cell migration [6]—when exogenous PI5P was added to MTMR3 and PIKfyve siRNA-treated cells, migration was partly rescued. To further confirm this finding, the authors showed that overexpression of the *Shigella flexneri* virulence factor IpgD, a PI(4,5)P₂ 4-phosphatase that converts PI(4,5)P₂ to PI5P [9], and production of endogenous PI5P in MTMR3 siRNA-treated cells also rescue migration defects. In fact, this production of PI5P from PI(4,5)P₂ proved that PI5P is a relevant migratory signal and not merely an intermediate to the production of PI(4,5)P₂, which has been implicated previously in regulating actin dynamics and, by contrast, requires PI4P synthesis—another potential intermediate for its signalling effects [1]. Interestingly, manipulation of PI5P levels in control cells does not initiate cell migration in the absence of stimulation, suggesting that PI5P is not sufficient to promote migration; instead, the pathway works together with others to promote migration

and there is an inhibitory regulatory pathway that acts downstream from PI5P. This clearly demonstrates that it is PI5P and not PI3P, PI(3,5)P₂ or PI(4,5)P₂ that regulates cell migration.

Preliminary evidence from Oppelt and colleagues suggests that PI5P might help to regulate actin cytoskeletal remodelling during migration [6]. For instance, silencing of MTMR3 and PIKfyve resulted in actin fibres that are unable to orient properly and form knots when cell migration was stimulated. These findings complement previous work indicating that PI5P regulates cytoskeletal organization in other contexts. For instance, PI5P production in response to insulin stimulation or on overexpression of the bacterial virulence factor IpgD induces disassembly of actin stress fibres [9,10]. The molecular mechanisms underlying PI5P's regulation of actin polymerization and disassembly remain unclear. Additionally, the subcellular location of PI5P production in response to these agonists remains largely undetermined; however, membrane fractionation and HPLC analysis has localized PI5P to the nucleus, endoplasmic reticulum, Golgi and plasma membrane under basal conditions [8]. Standard tools used to study phosphoinositide localization have yet to be developed for PI5P. PI5P-binding domains are beginning to be identified and include the PHD domain of ING2, and the PH domain of Dok proteins [4], but it will be important to develop PI5P probes as well as continue to identify additional effectors to better understand PI5P function.

The PI5P field is at a naive, yet exciting, stage in which studies such as that of Oppelt and colleagues advance our understanding of PI5P multiple roles in cellular function. Although this study has concentrated largely

on identifying Vps34, PIKfyve and MTMR3 in the production of PI5P in response to migration stimuli, it will be essential to explore how the activities of these enzymes are regulated. Moreover, the mechanism of PI5P catabolism in this context and others, as well as the precise molecular basis for PI5P actions in the cellular contexts, have to be further elucidated. Finally, the crosstalk between PI5P and other phosphoinositide-mediated signalling cascades, such as the PI(3,4,5)P₃–Akt pathway, will have to be closely examined in the context of cell migration, based on previous studies showing that PI5P is a positive regulator of class IA PI(3)Ks [3].

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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