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SPOTLIGHT

TAPPing into PIPs: A new reporter reveals the origin of plasma membrane PI(3,4)P_2

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The membrane lipid phosphatidylinositol-3,4-bisphosphate (PI(3,4)P_2) is an important signaling effector, controlling both anabolic pathways and membrane trafficking. In this issue, Goulden et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201809026) report a new PI(3,4)P_2 probe and show that plasma membrane PI(3,4)P_2 is a product of PI(3,4,5)P_3 dephosphorylation.

Phosphoinositide lipids direct membrane trafficking and coordinate the timing and localization of cellular signaling. The lipid products of phosphatidylinositol 3-kinase (PI3K), PI(3,4,5)P_3 and PI(3,4)P_2, are important regulators of signaling pathways that couple growth factor receptors to cellular anabolic pathways. Their accumulation due to mutations in PI3K or inactivation of the counteracting lipid phosphatases, such as PTEN, leads to hyperactivation of the downstream kinases Akt and mTORC1 and cellular transformation. While PI(3,4,5)P_3 had long been considered to play the major role in PI3K signaling, accumulating evidence indicates that PI(3,4)P_2 has distinct functions in the control of Akt (1) and mTORC1 activation (2), endocytosis (3), the formation of invadopodia, and cancer metastasis (4). Unlike PI(3,4,5)P_3, however, the cellular sources, dynamics, and functions of PI(3,4)P_2 remain poorly understood, largely due to a lack of tools to detect this rare lipid in cells. In this issue, Goulden et al. report the development and characterization of a genetically encoded fluorescent PI(3,4)P_2 reporter and propose that the major population of PI(3,4)P_2 at the plasma membrane derives from PI(3,4,5)P_3 dephosphorylation (5).

Lipid reporters are typically based on specific protein binding domains, tagged with a fluorescent protein. The probe reported by Goulden et al. is a triple tandem of the PI(3,4)P_2-specific C-terminal lipid-binding pleckstrin homology (cPH) domain of the protein TAPP1 (5). Such probes are often optimized for avidity to their lipid ligands, for example, through mutagenesis or by fusion to additional protein modules serving to enhance their recruitment to the endocytic vesicles (6). Yet, an avidity that is too high could result in unspecific binding, prevent lipid degradation, and/or lead to dominant-negative effects on downstream signaling. Goulden et al. set a golden standard by carefully designing and characterizing the specificity, selectivity, and diffusion and off-rates of their probe, to ensure that it faithfully reports the true kinetics of PI(3,4)P_2 in a cell (5). Further, as an additional control, they developed an orthogonal optogenetic system to demonstrate that PI(3,4)P_2 is both necessary and sufficient for recruitment of the probe to the cellular compartments where it is produced.

With this sensitive, genetically encoded reporter, Goulden et al. (5) examined the kinetics and cellular sources of PI(3,4)P_2 in cells. Three synthetic routes could lead to PI(3,4)P_2 production: (a) direct phosphorylation of PI(4)P by class I PI3Ks; (b) direct phosphorylation of PI(4)P by class II PI3Ks; and (c) dephosphorylation of PI(3,4,5)P_3 by lipid 5-phosphatases. Chemical inhibition of class I PI3Ks resulted in a nearly complete depletion of PI(3,4)P_2, indicating that the latter likely derives from PI(3,4,5)P_3 and arguing against the involvement of class II PI3Ks. Consistently, depletion of PI(4,5)P_2 and PI(3,4,5)P_3 pools by recruitment of a 5-phosphatase INPP5E followed by direct phosphorylation of the resulting PI(4)P by class I PI3K resulted in weaker and transient PI(3,4)P_2 production, suggesting that the plasma membrane pool of PI(3,4)P_2 is predominantly a product of direct PI(3,4,5)P_3 dephosphorylation by a 5-phosphatase, like SHIP1/2.

These results are satisfyingly consistent with those of a recent study that used a monomeric TAPP1 cPH domain conjugated to a hydrophobic environmentally sensitive organic fluorophore as a reporter for PI(3,4)P_2 (1). Distinct emission spectra of this fluorophore in the aqueous and lipid environments enable ratiometric detection of PI(3,4)P_2 upon introduction of the probe into cells by microinjection. While the tool reported by Goulden et al. has all the advantages of the genetically encoded reporter (5), the solvatochromic probe additionally offers a convenient way to calibrate the signal for precise quantitation of PI(3,4)P_2 molar percentage on subcellular structures. Both studies demonstrate transient PI(3,4,5)P_3 kinetics and a more sustained PI(3,4)P_2 accumulation, which was linked to differential recruitment of Akt1 and Akt2 isoforms (1). Inhibition of SHIP2 significantly reduced PI(3,4)P_2 levels (1) and suppressed PI(3,4)P_2-dependent cell invasion (4), well in line with the model proposed by Goulden et al. (5).

What are the cellular degradation routes for PI(3,4)P_2? The lipid 4-phosphatases INPP4A/B were proposed to specifically inactivate PI(3,4)P_2 in various models (reviewed in 7), yet, unlike the 3-phosphatase PTEN, INPP4A/B deficiency alone was never sufficient to cause cellular

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transformation. Data by Goulden et al. provide further support for the exciting new model where $PI(3,4)P_2$ is directly degraded by the PTEN phosphatase (4, 8), which would explain the high transformation and metastatic potential of PTEN deficiency.

While the set of tools developed by Goulden et al. offers important new insights into $PI(3,4)P_2$ dynamics in cells (5), as is often the case in science, many new intriguing questions arise. Several previous studies have reported localization-specific functions for $PI(3,4)P_2$ on various endosomal populations (1–3, 6); yet the cPH triple tandem probe reported by Goulden et al. is conspicuously absent from any vesicular structures (5). While accumulation of $PI(3,4)P_2$ on endosomes could well be cell type specific, it is tempting to speculate that $PI(3,4)P_2$ generated through class II PI3Ks could also have distinct intracellular kinetics. Indeed, knockout of class II *PI3K-C2γ*, which is recruited to Rab5/Appl1-positive endosomes in response to insulin, led to significantly shorter Akt phosphorylation in primary hepatocytes (9). Conversely, fibroblasts lacking INPP4 showed sustained Akt phosphorylation in response to EGF, which typically induces only transient Akt activation (10), suggesting that the interplay between class I and II PI3K enzymes and lipid phosphatases at the plasma membrane and endosomal vesicles could define the cellular $PI(3,4)P_2$ dynamics and orchestrate downstream signaling.

Equally exciting is the question of the functional role of $PI(3,4)P_2$ in membrane trafficking and cell migration. $PI(3,4)P_2$ has been implicated in clathrin-mediated endocytosis, lamellipodia dynamics, and the formation of invadopodia (3, 4). It would be interesting to test if these processes are coupled to intracellular trafficking of specific receptors and examine how exactly they are regulated by different PI3K enzymes. The new tools reported by Goulden et al. will undoubtedly spur new insights into how cells use lipids, such as $PI(3,4)P_2$, to organize signaling processes in space and time (5).

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