SPOTLIGHT

MapPIng PI inside cells brings new light to polyphosphoinositide biology

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It is unclear how phosphatidylinositol (PI), the precursor of polyphosphoinositides, is distributed within cell membranes. Pemberton et al. (2020. *J. Cell. Biol.* https://doi.org/10.1083/jcb.201906130) and Zewe et al. (2020. *J. Cell. Biol.* https://doi.org/10.1083/jcb.201906127) describe new approaches to map the subcellular PI abundance and clarify how polyphosphoinositide metabolism relates to PI distribution.

Phosphatidylinositol (PI) accounts for ∼10% of glycerophospholipids ([1]), which form the bulk of eukaryotic cell membranes. PI is synthesized in the ER and supplied to other organelle membranes and the plasma membrane (PM). What makes PI unique is its role as a precursor for a group of seven key signaling lipids called polyphosphoinositides (PPIn). Made in trace amounts (1% of total lipids) in a highly accurate manner, these lipids regulate processes— signaling pathways, vesicular trafficking, cytoskeletal dynamics, ion transport, and lipid exchange—in different cellular regions. Two studies, by Pemberton et al. ([2]) and Zewe et al. ([3]), present innovative approaches to detect PI in living cells, shedding new light on PI distribution and availability for PPIn production.

PI consists of a glycerol backbone that bears two hydrophobic acyl-chains and an inositol ring as the polar head. PPins are derived from PI via the concerted action of PPIn kinases and phosphatases that add or remove phosphate groups to a specific (3-, 4-, or 5-) position of the inositol ring. The enzymology of PPIn synthesis and turnover is well understood ([4]), but a question still stands: Does the generation of PPIns also rely on a regulation of PI availability? Notably, two PPIn species, phosphatidylinositol-4-phosphate (PI4P) and PI 4,5-bisphosphate (PI(4,5)P$_2$) are particularly enriched in the PM, likely representing 4–7% of PM glycerophospholipids. Therefore, one expects a large PI pool to exist

in that membrane but, surprisingly, recent lipidomic analyses suggest the opposite ([5]). This might indicate that, in some organelles, the control of PI pools is much tighter than previously anticipated and might tune PPIn generation. Exploring this question requires a better quantification of PI levels in different compartments, which is not an easy task.

Indeed, one problem is that the organelle membranes and the PM are composed of two leaflets whose lipid composition can be very different; this is especially the case for the PM. Second, PPIn synthesis occurs exclusively in the leaflet that faces the cytosol. Thus, it is necessary to specifically measure how much PI is present in this cytosolic leaflet. Cellular subfractionation coupled to lipid analysis can reveal the lipid composition of organelle membranes, but not of their individual leaflets. Fluorescent derivatives of PI (Fig. 1 a), reassessed by Zewe et al. ([3]), may be fair reporters of PI distribution between but not within cell membranes.

Other approaches could be useful to probe PI intracellularly while keeping topological information. A popular one relies on the use of genetically encoded lipid biosensors, i.e., lipid-binding domains, derived from natural proteins, fused to fluorescent proteins (e.g., GFP; [6]). Once expressed in the cytosol, a biosensor goes onto organelle surfaces that expose the targeted lipid. Using fluorescent microscopy, it has been possible to track

different glycerophospholipids, including PPINs, with high spatiotemporal resolution. Unfortunately, no PI-binding domain was identified to design a faithful sensor for visualizing the intracellular PI distribution.

Pemberton et al. ([2]) and Zewe et al. ([3]) solved this issue by reengineering bacterial enzymes called PI-specific phospholipase C (PI-PLC), which bind to lipid membranes and convert PI into diacylglycerol (DAG), releasing inositol-1-phosphate. One strategy was to abolish the catalytic activity of the PI-PLC to get a mere PI-binding protein that, once fused to GFP, was amenable to intracellularly detect PI (Fig. 1 b; [2]). A second approach, based on a previous work ([7]), was to use activatable PI-PLCs to produce DAG in the cytosolic side of a given organelle and, by measuring how much DAG is produced, to indirectly evaluate PI levels ([2]). For this, a PI-PLC variant, deficient in binding lipid surfaces, was fused to a FK506-binding protein (FKBP) and coexpressed in cells with a FKBP–rapamycin binding (FRP) domain anchored to the organelle. Addition of rapamycin, which induces FKBP–FRB heterodimerization, leads to the recruitment of PI-PLC to this organelle and triggers PI hydrolysis (Fig. 1 c). The production of DAG is measured by fluorescent microscopy or Bioluminescence Resonance Energy Transfer (BRET) using a specific biosensor.

As an alternative approach, Zewe et al. ([3]) coexpressed N- and C-terminal lobes of a

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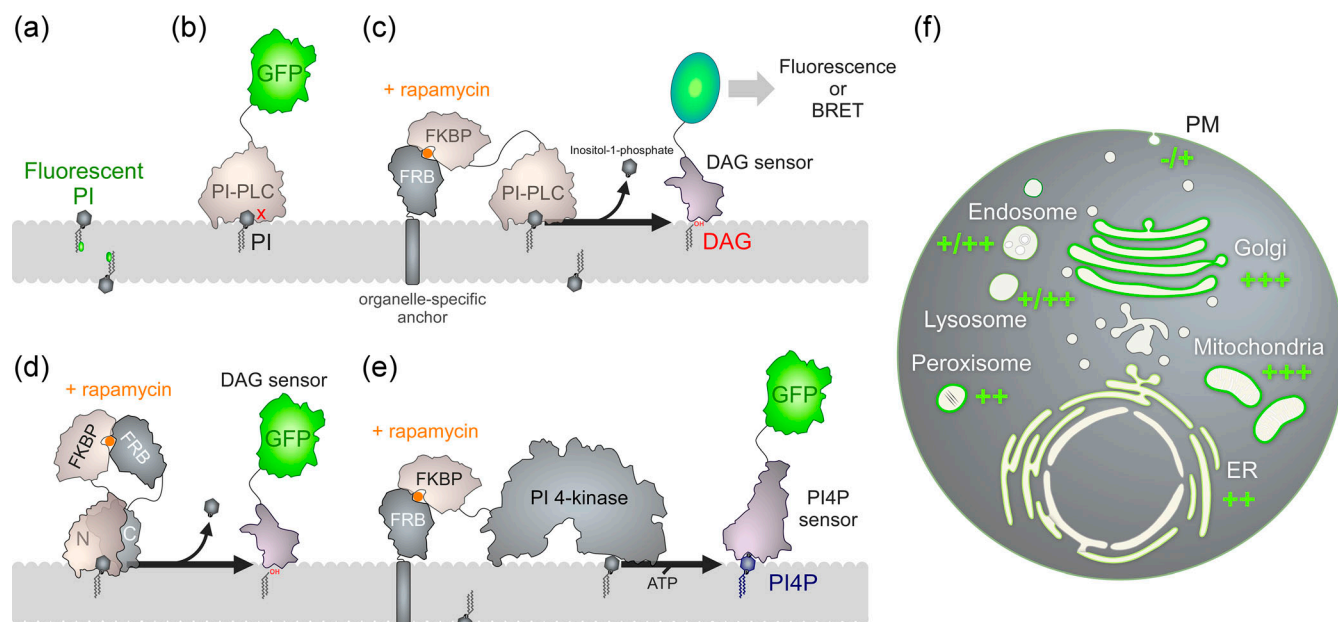
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Figure 1. **A toolkit to map the PI abundance inside living cells. (a–e)** The main tools are a fluorescent PI to evaluate PI distribution in the cell (a); a catalytically dead PI-PLC in tandem with GFP to detect PI (b); an activatable PI-PLC that produces a local DAG pool only upon adding rapamycin (c); a split PI-PLC that becomes active once reassembled by rapamycin (d); and a PI 4-kinase that produces PI4P upon rapamycin addition (e). DAG or PI4P are detected using specific fluorescent or BRET biosensors to indirectly quantify PI. **(f)** Combined, these tools provide a new map of PI abundance in the cytosolic leaflet of cell membranes. The relative PI levels are illustrated by − and + symbols.

PI-PLC, fused to FKBP and FRB, respectively. Adding rapamycin reassembles the two halves of the enzyme, eliciting its membrane-binding and catalytic aptitudes to probe the relative PI levels between membrane compartments (Fig. 1 d). One of the lobes can be anchored to a specific organelle for local analysis. An additional way to quantify PI was to detect its conversion into PI4P by recruiting on organelles a catalytic fragment of PI 4-kinase via the rapamycin-based strategy (2,3; Fig. 1 e).

The use of these tools, combined with knowledge of PPIn metabolism, membrane features, and lipid dynamics, provides a new map of PI distribution inside cells (Fig. 1 f). Collectively, the two studies indicate that the cytosolic leaflets of the Golgi and outer mitochondrial membranes contain substantial amount of PI. A little less PI is detected in the ER, the endosomes, which harbor phosphatidylinositol-3-phosphate (PI3P), the lysosomes, and peroxisomes. Importantly, both studies conclude that the steady-state PI level in the cysotolic leaflet of the PM is low. Remarkably, the amount of DAG generated from PI(4,5)P$_2$ by phospholipase-C-dependent signaling pathways largely exceeds the one resulting from the digestion of PI by PI-PLC, demonstrating the prominence of PI(4,5)P$_2$ over PI in the PM. Only treatments that block

or counteract PPIn phosphorylation at the PM can create an observable PI pool. Another key observation is that the levels of PI4P in the Golgi and PM and of PI3P in endosomes are lowered by a distant depletion of PI at the ER using PI-PLC (2).

These studies have important implications. First, they strengthen the model that PI-specific lipid transfer proteins (LTPs) from different families (PI-transfer proteins, Sec14p, SMP-containing proteins [8, 9]), control PPIn generation by channeling PI from the ER toward other regions. Second, the observation that some organelles, rich in PPIn, are almost devoid of PI reinforces a long-standing idea: that these LTPs directly present PI to the PI kinases to boost their activity instead of simply delivering PI into the membrane (10). The use of PI biosensors could help to directly interrogate in vivo this disputed concept. Third, they describe tools that should also permit to better dissect at the ER/PM interface the functional interplay between PI-transfer proteins (e.g., Nir2/Nir3) and other LTPs that exchange PI4P with lipids made in the ER and likely regulate PI and PPIn levels at the ER and PM, respectively (8). This would allow better defining the role of LTPs in the regulation of PPIn-mediated cellular signaling. Last, they confirm that PI pools, accessible for PPIn synthesis, exist in peroxisomes and mitochondria; this motivates

future studies aiming to explore whether these organelles harbor functionally relevant PPIn pools.

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