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Review

Detection and manipulation of phosphoinositides ☆

Olof Idevall-Hagren [a,*], Pietro De Camilli [b,*]

[a] Department of Medical Cell Biology, Uppsala University, BMC Box 571, 75123 Uppsala, Sweden
[b] Department of Cell Biology, Howard Hughes Medical Institute and Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT 06510, USA

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ABSTRACT

Phosphoinositides (PIs) are minor components of cell membranes, but play key roles in cell function. Recent refinements in techniques for their detection, together with imaging methods to study their distribution and changes, have greatly facilitated the study of these lipids. Such methods have been complemented by the parallel development of techniques for the acute manipulation of their levels, which in turn allow bypassing the long-term adaptive changes implicit in genetic perturbations. Collectively, these advancements have helped elucidate the role of PIs in physiology and the impact of the dysfunction of their metabolism in disease. Combining methods for detection and manipulation enables the identification of specific roles played by each of the PIs and may eventually lead to the complete deconstruction of the PI signaling network. Here, we review current techniques used for the study and manipulation of cellular PIs and also discuss advantages and disadvantages associated with the various methods. This article is part of a Special Issue entitled Phosphoinositides.

1. Introduction

Reversible phosphorylation of phosphatidylinositol at the 3, 4 and 5 positions of its inositol head group by phosphatidylinositol kinases and phosphatases gives rise to the seven different phosphoinositides (PIs) and the heterogeneous distribution of these lipids contributes to cellular membrane identity (Fig. 1). PIs are versatile signaling molecules important for diverse cellular functions such as signal transduction, transport across membranes, membrane trafficking, regulation of the cytoskeleton, cell migration and proliferation [1,2]. Consistent with the fundamental roles of these lipids, the network of enzymes responsible for their synthesis and degradation are largely conserved from yeast to mammals, although the genes encoding several of these enzymes have undergone duplications during evolution. Mutations in PI-metabolizing enzymes are associated with the development of diseases, including psychiatric and neurological disorders, cancer, diabetes and allergy [3] (De Matteis, this volume). This has spurred the development of techniques both for the detection and for the manipulation of these lipids. Biochemical detection techniques now allow quantification of all seven PIs, and the use of fluorescently tagged PI-binding domains enables real-time visualization of most of them in intact cells [4,5] (see Balla, this volume). Together, these and other methods now allow us to examine the dynamics of the seven PIs at different levels, from global changes in cells and tissues down to changes in a specific PI in a cellular subcompartment. Parallel to the development of detection techniques, new techniques for the chronic or acute, cell-wide or spatially localized manipulation of PIs have been developed. In this Review we summarize and discuss available methodology for the analysis and manipulation of PIs, compare the strengths and weaknesses of different methods and also suggest future directions for this field of PI biology.

2. Measuring PI levels

Several excellent reviews on techniques for PI detection have been previously published [5–8] (see also Balla in this volume). Briefly, these techniques can be divided into biochemistry- and microscopy-based methods.

2.1. Biochemical detection of PIs

PIs present in tissue and cell lipid extracts are typically identified and quantified by thin layer chromatography (TLC) or by ion-exchange HPLC separation of their glycerophosphoinositol moieties following deacylation [6,7]. As PIs represent minor species in cellular lipid extracts, their detection requires previous metabolic labeling (optimally equilibrium labeling) with [^{3}H]inositol or [^{32}P]inorganic phosphate. Nonradioactive detection of HPLC separated PIs is also possible using HPLC followed by suppressed conductivity measurements [9,10]. This method efficiently detects only PIP and PIP_2 without discriminating between the phosphorylated positions on the inositol ring. However, since PI4P and $PI(4,5)P_2$ are the predominant PIs in cells of high eukaryotes,

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* Corresponding authors.
E-mail addresses: olof.idevall@mcb.uu.se (O. Idevall-Hagren), pietro.decamilli@yale.edu (P. De Camilli).

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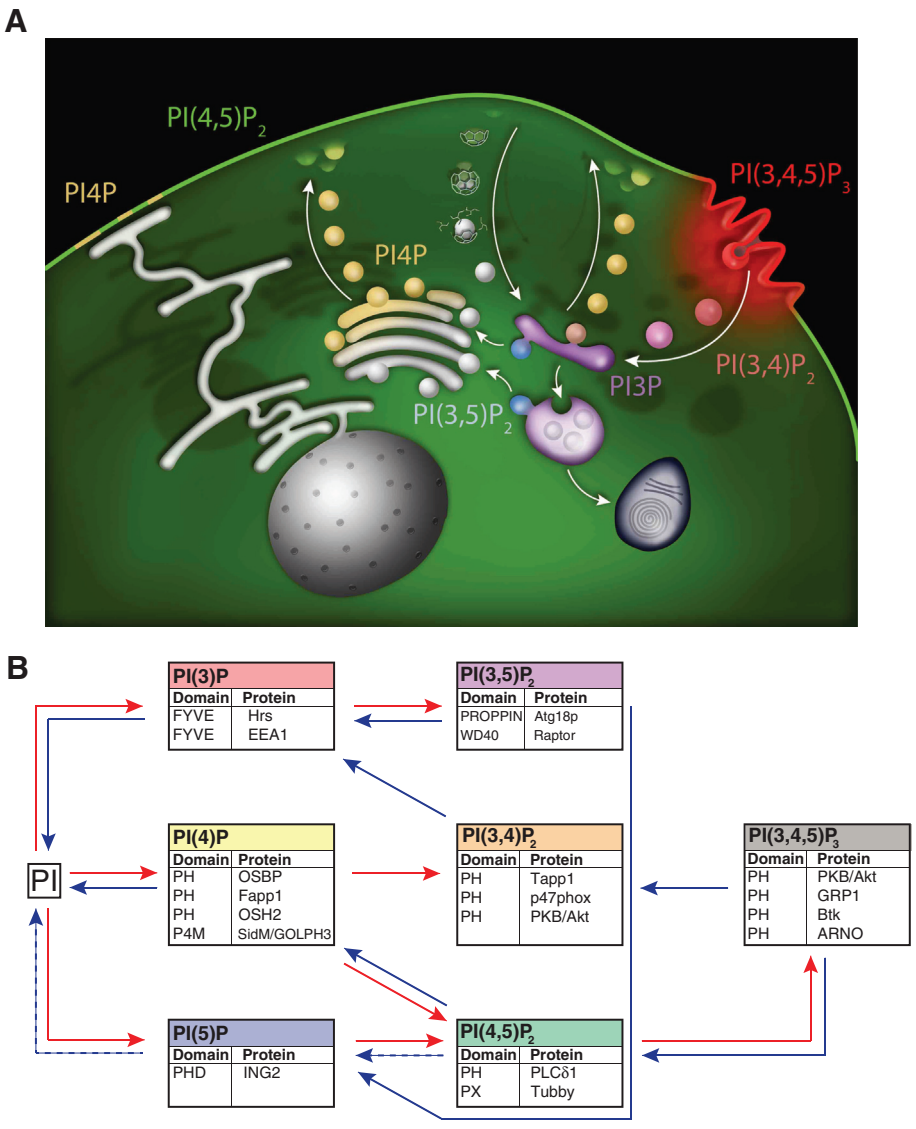

Fig. 1. The PI network. A. Heterogeneous distribution of PIs in subcellular membranes. The cartoon depicts the predominant localizations of different PI species. It should be noted that small, but physiologically important, PI pools that do not fit this simplified view (for example 3-phosphorylated PIs at sites of clathrin-mediated endocytosis [91]) also occur in cells. Arrows indicate membrane traffic directions thus illustrating the coupling of membrane transport reactions to PI conversion. B. The illustration shows the seven PIs and the enzymatic steps involved in their synthesis (red) or dephosphorylation (blue). Below each PI are indicated protein modules typically used for the detection of that PI, as well as the proteins from which they are derived. Metabolic reactions that have not been well characterized are indicated with dashed arrows.

levels of PIP and PIP_2 roughly reflect the levels of these two PIs. Mass spectrometry methods can also be used. Mass spectrometry has great sensitivity and also allows identification of the fatty acid chains and not just of the head group. Combining chromatographic separation with mass spectrometry improves both sensitivity and specificity of detection without need for radiolabeling [4,11,12].

A limitation inherent to biochemical detection is the poor temporal resolution, as it provides a snapshot of the PI composition of cells but does not give information about dynamic changes in PI levels. Moreover, the metabolic labeling required to detect minor PI species precludes experiments in whole organisms due to the problems associated with the use of radioactive tracers. Another limitation of biochemical detection is the lack of information about the distribution of PIs within cells, as subcellular fractionation prior to lipid extraction results, at least to a large extent, in their dephosphorylation. An important part of the signaling power embedded in the phosphoinositide code comes from spatial segregation of different PI species inside cells [2] (see Fig. 1A). Recent developments in imaging mass spectrometry are pushing the resolution of this technique into the micrometer range, which may allow biochemical detection of individual PI species with subcellular resolution in the near future [13]. A major advantage with biochemical detection techniques is that they allow the simultaneous detection of all PIs, which in turn enables the identification of compensatory changes in lipid levels associated with the manipulation of the intricate PI network.

2.2. Microscopy-based detection of PIs

Microscopic detection of PIs utilizes the specific interaction of different PIs with protein domains or antibodies that can be labeled with fluorescent probes. This method allows determining the intracellular location of specific PIs and their relative levels in different membranes. Importantly, light microscopy-based live imaging of cells expressing PI binding modules fused to fluorescent protein permits us to monitor changes in PI levels or distribution in response to physiological or experimental perturbations. Most commonly, the detection is done using a regular wide-field epifluorescence microscope or a confocal microscope, which provides high-resolution images of optical sections of

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