New insights into the roles of phosphoinositides and inositol polyphosphates in yeast

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Abstract
During the past half century, we have progressed from simply viewing myo-inositol-containing glycerophospholipids as quantitatively minor membrane constituents to the present, very striking, situation in which more and more important cellular functions are being assigned to a plethora of phosphorylated derivatives of inositol and phosphatidylinositol. Two such examples are discussed briefly: the activation by environmental stresses of the single phosphoinositidase C of yeast, which is related to the phospholipase Cδ so far there eukaryotes, and the involvement of PtdIns(3,5)P2 in endomembrane trafficking.

Key words: inositol hexakisphosphate, phosphatidylinositol 3,5-bisphosphate, phospholipase C, stress, yeast.

Abbreviations used: GroP, Ins(4,5)P2, glycerophosphoinositol 4,5-bisphosphate; PIC, phosphoinositidase C; PLC, phosphoinositide-specific phospholipase C.

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2Abbreviations and numbering: the numbering of monoester phosphate groups in phosphoinositides and inositol polyphosphates defines their disposition around the six-carbon myo-inositol ring. For biological clarity, the usual numbering convention treats all biologically occurring myo-inositol-containing molecules as derivatives of D-myoinositol [for relevant IUBMB recommendations, see ‘Numbering of atoms in myo-inositol’ (1989) Biochem. J. 258, 1-2]. Examples are phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2], phosphatidylinositol 3-phosphate (PtdIns3P) and inositol 1,4,5-trisphosphate [Ins(1,4,5)P3].

A plethora of eukaryotic phosphoinositides and inositol polyphosphates: how we got here

Inositol phospholipids were discovered in the 1930s [1,2], and the first hints that they might have specific regulatory functions, as well as making a general contribution to membrane structure, emerged about half a century ago. First, Jordi Folch found complex phosphoinositides containing multiple phosphate groups in the brain [3] and then Mabel and Lowell Hokin discovered that phosphoinositide metabolism is dramatically stimulated when secretory tissues are provoked to secrete [4,5]. I was introduced to these fascinating molecules by Tim Hawthorne, another pioneer of the field [1], who was also an early Morton lecturer.

About 30 years ago, my colleagues and I developed two important ideas: (i) that hydrolysis of a phosphoinositide by a phosphoinositidase C (PIC; also termed phosphoinositide-specific phospholipase C or PLC) has the properties expected of a receptor-coupled signalling reaction [6], and (ii) that this reaction might be a step in the process by which many types of receptor provoke mobilization of Ca2+ in stimulated cells [7]. We then recognized that the receptor-activated PIC primarily or exclusively attacks phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2] in vivo [9,10], but the then-prevailing view was that the low abundance of PtdIns(4,5)P2 in cells made this conclusion improbable [11]. However, this observation opened the way to discovery of the Ca2+-mobilizing ability of Ins(1,4,5)P3 [12,13], and we now know that other phosphoinositides that are less abundant than PtdIns(4,5)P2 also play pivotal roles in cell regulation.

Early pharmacological structure–function analyses showed that the Ca2+-mobilizing Ins(1,4,5)P3 receptors display an exquisite specificity for Ins(1,4,5)P3 and closely related molecules. These studies were harbingers of the recent flood of discoveries that has revealed that many of the cellular inositol lipids and/or phosphates, each of which exhibits some characteristic hydrophilic/hydrophobic balance and a particular spatial array of phosphate groups, participate in an amazing variety of stereoselective interactions with diverse target proteins and so fulfill multifarious cell functions [14–23].

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When the PIC signalling pathway was discovered, we had no appreciation of how diverse the inositol lipids and phosphates were in eukaryotic cells. Typical views then would have been that most cell membranes contain lots of PtdIns, that a cell's small complement of PtdIns(4,5)P_2 and its precursor/catabolite PtdIns4P are mainly in the plasma membrane, and that the cytosol contains a highly regulated concentration of Ins(1,4,5)P_3 (in the micromolar range) and some InsP_2 and InsP_3 as degradative intermediates. Plants were known to contain abundant InsP_4, and avian and reptilian erythrocytes contain InsP_6, but most of us saw these as 'idiiosyncratic'.

These views were soon overturned, first by the discovery in animal cells of Ins(1,3,4,5)P_4 [24], Ins(1,3,4,5)P_6 [25] and a diverse array of other inositol phosphates [26–30]. And then there were several 'new' polyphosphorylated lipids: first an uncharacterized putative PtdInsP_7 [31], then a family of 3-phosphorylated phosphoinositides [32,33] and finally PtdIns5P [34] and PtdIns(3,5)P_2 [35,36].

Below we briefly outline recent information, obtained from studies of yeast, on the cellular roles of activated PIC and of PtdIns(3,5)P_2.

**PIC activation in stressed yeast**

PIC-mediated signalling downstream of activated cell surface receptors employs three families of PICs that probably occur only in metazoans, the PIC\(_ \delta\), PIC\(_ \gamma\)s and PICs [37,38], and a further type, PIC\(_ \zeta\), is essential for sperm function [39]. The only ubiquitously distributed eukaryotic PIC family comprises the PLC\(_ \delta\)s of animals, the only PICs for which there is detailed structural information, and related PICs in most or all other eukaryotes. However, there is no agreement as yet on how enzymes of the PIC\(_ \delta\) family are regulated in cells. A common expectation is that they might be responsive to activated cell surface receptors in some still unidentified manner [37,38].

Plc1p, the only PIC in yeast, is related to metazoan PLC\(_ \delta\)s, but has a longer N-terminal sequence beyond the pleckstrin homology (PH) domain. Loss of functional Plc1p prejudices several aspects of yeast biology. Among these are cell-cycle progression, cytokinesis, growth and survival in nutrient-limited environments, the switch between budding and pseudolymphal growth, sporulation, heat-tolerance and glucose-induced Ca\(^{2+}\) influx [37,40,41]. An obvious interpretation of this constellation of effects is that a lack of a normal Plc1p complement somehow makes cells less able to weather diverse environmental 'stresses', and there have long been reports that nutritional and osmotic stresses might provoke increased PIC activity in intact yeast [42–44].

Yeast lack any genes that resemble Ins(1,4,5)P_3-activated Ca\(^{2+}\) channels, so it is unlikely that the Ins(1,4,5)P_3 produced by Plc1p regulates cellular Ca\(^{2+}\) homeostasis in a 'conventional' manner. This view was reinforced when a pathway that quickly converts Ins(1,4,5)P_3 to InsP_6 was found in both Schizosaccharomyces pombe and Saccharomyces cerevisiae [45,46]. However, only since then have the first reports appeared that carefully compared the phosphoinositides and inositol phosphates of wild-type and \(\Delta plc1\) cells. These established three novel and important facts. Two inositol polyphosphate kinases (Arg82p/ArgRIII/Ipk2p and Ipk1p) rapidly phosphorylate the Ins(1,4,5)P_3 that is produced by Plc1p in intact yeast to InsP_6 [47], the synthesis of this InsP_6 is needed for mRNA export from the nucleus via the Gfe1p-dependent nucleoporin pathway [47], and protein translation controlled by the arginine-responsive ArgR–Mcm1 complex requires Plc1p-dependent PtdIns(4,5)P_2 hydrolysis and onward metabolism of Ins(1,4,5)P_3 at least to Ins(1,4,5,6)P_4 and/or Ins(1,3,4,5,6)P_5 [40,48,49]. This is the yeast pathway that was unveiled:

\[
\text{PtdIns}(4,5)P_2 \xrightarrow{\text{Plc1p}} \text{Ins}(1,4,5)P_3 \xrightarrow{\text{Arg82p/Ipk2p}} \text{Ins}(1,4,5,6)P_4 \xrightarrow{\text{Arg82p/Ipk2p}} \text{Ins}(1,3,4,5,6)P_5 \xrightarrow{\text{Ipk1p}} \text{InsP}_6
\]

It remains to be determined in what other organisms this pathway might occur and have similar roles. In yeast and other eukaryotes, some InsPs, and InsP_6 undergoes further phosphorylation to yield pyrophosphorylated derivatives, catalysed at least partly by the kinase Kcs1p [50].

We recently explored whether any or all of a variety of environmental switches that impose physical and/or metabolic stress might activate Plc1p in \(S.\ cerevisiae\). To do this, wild-type or \(\Delta plc1\) yeast were labelled to near equilibrium with \([\text{3H}]\)inositol during exponential growth, and were then subjected to hypo-osmotic shock or some other 'stress'. The cells were killed, and their inositol lipids and inositol polyphosphates were extracted and analysed by anion-exchange HPLC.

The most straightforward results came from pre-labelled cells that were equilibrated in hypertonic medium (0.5 M NaCl for 2 h, then 0.9 M NaCl for 2 h) and quickly diluted so as to impose an acute hypo-osmotic stress. The \([\text{3H}]\)PtdIns(4,5)P_2 complement was quickly depleted, to a minimum at approx. 2 min, and this decline was roughly matched by an accumulation of \([\text{3H}]\)InsP_6 (Figure 1). There was little or no accumulation of PtdIns4P or of any isomers of InsP_3, InsP_4 or InsP_5, and no GroPIns(4,5)P_2 (glycerophosphoinositol 4,5-bisphosphate) was formed. The \([\text{3H}]\)PtdIns(4,5)P_2 complement soon started to recover towards the starting value (Figure 1). Isogenic \(\Delta plc1\) yeast did not show this response, but cells lacking the PtdIns(4,5)P_2 phosphatase Inp51p showed the same response as wild-type cells. The \([\text{3H}]\)PtdIns(4,5)P_2 that was hydrolysed by Plc1p was synthesized by Stt4p (one of two or three \(S.\ cerevisiae\) PtdIns 4-kinases) and Mss4p (PtdIns4P 5-kinase). When \(ARG82\) or \(IPK1\) was inactivated, PtdIns(4,5)P_2 depletion still occurred normally, but other inositol polyphosphates accumulated instead of InsP_6. These were primarily InsP_4, InsP_5 and a pyrophosphate derivative of InsP_3 (PP-InsP_3) in \(\Delta ipk1\) cells. A molecule tentatively identified as the major Ins(1,4,5)P_3 metabolite Ins(1,4)P_2 accumulated in \(\Delta arg82\) cells.

These results provide compelling evidence that an acute hypo-osmotic shock rapidly activates the PIC activity.
of Plc1p, but how this activation occurs remains to be determined. The liberated Ins(1,4,5)P$_3$ is then quickly and quantitatively converted into InsP$_6$ by the pathway shown above, and some InsP$_6$ is further phosphorylated to PP-InsP$_5$. Despite the substantial evidence that PICδs are sometimes nuclear-localized and the known function of Arg82 in nuclear gene-regulatory complexes, all of our experimental information can be accommodated by a simple model in which activated Plc1p hydrolyses PtdIns(4,5)P$_2$ at the plasma membrane and Ins(1,4,5)P$_3$ is converted into InsP$_6$ in the cytosol. The cellular consequences of stimulating this pathway may include both local effects of PtdIns(4,5)P$_2$ depletion at the inner face of the plasma membrane, for example on cytoskeletal organization, and also intracellular effects of the liberated inositol polyphosphates in one or more cell compartments.

We also have evidence that Plc1p is activated, to varying degrees and sometimes less quickly, by exposing wild-type yeast to heat, by the re-admission of a nitrogen source to nitrogen-starved cells and by re-admission of glucose to glucose-starved cells. However, in each of these situations, the Plc1p-initiated PtdIns(4,5)P$_2$ depletion and InsP$_6$ accumulation are elements of a more complex set of metabolic changes in the status of cell phosphoinositides and inositol phosphates. For example, the first relevant study of glucose re-admission suggested that this causes PIC activation [43]. However, a later study indicated that this might be a misinterpretation of a situation in which deacylation is the major route of PtdIns(4,5)P$_2$ metabolism, with the liberated GroPIns(4,5)P$_2$ released into the medium [44] in the same manner as was reported for GroPIns many years earlier [51,52]. Our results point to a combination of these events, with glucose readmission causing (i) a modest and transient activation of the PIC activity of Plc1p, and (ii) a substantial and sustained deacylation of PtdIns(4,5)P$_2$, with the liberated GroPIns(4,5)P$_2$ released into the medium. Unexpectedly, ∆plc1 cells displayed neither response, indicating that Plc1p activation must occur if the PtdIns(4,5)P$_2$ deacylation and GroPIns(4,5)P$_2$ release is to proceed.

To summarize, evidence accumulated during the 1990s indicates that yeast possesses a single catalytically active PIC, Plc1p, and that this PICδ-like enzyme is an essential part of the apparatus by which yeast weather environmental variations. It has recently emerged that at least some of the effects of the produced inositol polyphosphates are on gene transcription and on mRNA movement from nucleus to cytosol. It has always been implicit in these studies that the PIC activity of Plc1p might be environmentally regulated, but we have lacked unequivocal evidence on this point. The studies described above establish beyond doubt that rapid environmental changes can indeed provoke rapid changes in the catalytic activity of Plc1p in intact yeast. However, these effects are complex and varied: each stress evokes a different and characteristic set of changes in the metabolism of phosphoinositides and inositol polyphosphates. The next task is to understand how Plc1p is activated and to define what additional regulatory processes interact with this pathway in order to produce a variety of outcomes.
PtdIns(3,5)P₂, a ‘novel’ phosphoinositide implicated in membrane trafficking

PtdIns(3,5)P₂, which is probably ubiquitous in eukaryotic cells, was discovered as a minor polyphosphoinositide in labelled mammalian fibroblasts [35] and in yeast and plant cells [36]. It was immediately of interest because hyperosmotic stress, with salt or a non-ionic solute, very quickly stimulates its synthesis in S. cerevisiae or S. pombe in a manner that is independent of the slower gene-regulatory Hog1 pathway [36]. PtdIns(3,5)P₂ is made by a family of Type III PtdInsP kinases (PIPKins) of which the S. cerevisiae protein Fab1p is the prototype [53–59]. The Type III PIPKins are large proteins, found in all eukaryotic genomes scanned to date, that include an N-terminal FYVE domain, a central Type II [CCT (chaperonin-containing Tcp1/Tcp1/thermosome-like) chaperone-like domain (see [60]) and a C-terminal catalytic PIPK domain [55].

FAB1 was discovered as a gene that is implicated in vacuole function and is needed for proper chromosome and nuclear segregation during the budding of new cells [61]. Genetically, it was grouped with VAC7 and VAC14 as a Class III ‘vacuolar inheritance’ gene, a lack of which causes cells to have very enlarged and un-lobed vacuoles that do not acidify correctly [62]. This vacuolar phenotype was interpreted as a failure of vacuole membranes to undergo vesicle fission, so destroying the normal balance of membrane delivery to the vacuole and retrograde traffic of vacuolar membrane to other membrane compartments. Inhibition of the murine Fab1p homologue (also known as PIKfyve) induces vacuolation in mammalian cells, which suggests that a need for PtdIns(3,5)P₂ synthesis may be a fairly general requirement for normal retrograde membrane traffic from eukaryotic lysosomal compartments.

Recent work has confirmed a direct functional relationship between Fab1p, Vac14p and Vac7p, with both Vac7p and Vac14p serving as upstream activators of the PtdIns3P 5-kinase activity of Fab1p [63–66], and genetic evidence suggests that the phosphatase Fig4p may be responsible for PtdIns(3,5)P₂ hydrolysis in vivo [63]. Vac14p orthologues are widely distributed [65,66], so probably serve as Fab1p regulators in many species, whereas Vac7p is uniquely a yeast protein. This is the probable pathway:

\[
PtdIns \xrightarrow{\text{PtdIns3P}} PtdIns(3,5)P_2 \xrightarrow{\text{Fab1p}} \xrightarrow{\text{Vac7p/Vac14p}} \xrightarrow{\text{Fig4p}} \xrightarrow{\text{PtdIns3P}} \xrightarrow{\text{PtdIns(3,5)P2}} \text{Effectors}
\]

Studies of the effects of ablating or mutating, in three types of yeast, either Fab1p or its homologues or Fig4p have suggested that maintenance of a normal cellular complement of PtdIns(3,5)P₂ may be necessary for a variety of cell functions other than vacuole acidification and retrograde membrane trafficking from lysosomes/vacuoles. These include resistance to heat stress [61], trafficking of proteins to the vacuole through multivesicular bodies [65], correct processing and secretion of mating pheromones [58], formation of normal buds and mating projections [67,68] and pseudohyphal growth in some environments [59].

Analysis of these phenotypes suggests that these processes are not all similarly sensitive to PtdIns(3,5)P₂ availability, so several different PtdIns(3,5)P₂ effector proteins may be needed for cells to execute all of the intracellular functions of PtdIns(3,5)P₂. The screen that previously allowed us to identify a Fab1 activator that turned out to be Vac14 [65] has also yielded candidate PtdIns(3,5)P₂ effector proteins. These include at least one protein that binds PtdIns(3,5)P₂ with very high affinity and selectivity, the lack of which causes a fab1-like swollen vesicle phenotype, and which includes none of the currently known phosphoinositide-binding domains (S.K. Dove, unpublished work).

References

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