Phosphoinositide 3-kinase: the protein kinase that time forgot

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Abstract
Class I phosphoinositide 3-kinases were originally characterized as lipid kinases, although more than 10 years ago they were also found to phosphorylate protein serine residues. However, while there is a vast amount of data on the function of this lipid kinase activity, relatively little is known about the function of the protein kinase activity. We discuss the evidence that suggests that the protein kinase activity of phosphoinositide 3-kinases mediates important signalling functions in cells.

Introduction
Class I PI 3-kinases (phosphoinositide 3-kinases) are involved in a multitude of functions which are critical for the biology of the cell, including cell survival, growth, proliferation, intermediary metabolism and cytoskeletal rearrangements [1]. It is widely assumed that these functional roles are mediated by the lipid kinase activity of these enzymes, but all isoforms of class I PI 3-kinases (class IA, p110α, p110β and p110δ; class IB, p110γ) also possess an intrinsic protein serine kinase activity [2–5], the functional consequences of which have not been fully defined [6].

Autophosphorylation as a mechanism of regulation of class I PI 3-kinase activity
The first evidence for PI 3-kinase protein kinase activity was the finding that PI 3-kinase both co-purified and co-immunoprecipitated with a serine kinase activity which phosphorylated both the p85 and p110 subunits [2]. Later studies using recombinant p110α demonstrated that the PI 3-kinase itself possessed the serine kinase activity, and the sole phosphorylation site was found to be Ser508 in the inter-SH2 (Src homology 2) domain of the p85α class IA PI 3-kinase adaptor subunit [3]. In agreement with the findings of Carpenter et al. [2], autophosphorylation was found to down-regulate the lipid kinase activity. Recently, we have demonstrated that phosphorylation at Ser508 can occur in vivo and that it is stimulated by growth factors, including insulin and platelet-derived growth factor [7]. The mechanism by which the serine kinase activity is increased appears to differ from that by which lipid kinase activity is regulated, as tyrosine phosphorylated peptides stimulate the lipid kinase activity of p85α/p110α, but have no effect on their protein kinase activity [8]. However, this provides strong evidence that regulated phosphorylation of Ser508 represents a mechanism for dynamically regulating the activity of p110α in vivo.

We have found that p110β is much less efficient at phosphorylating Ser508 of p85α [7,9], and autophosphorylates instead [10]. The autophosphorylation site has recently been reported as Ser1070 [11], and autophosphorylation also results in reduced lipid kinase activity, but there are no studies of regulation of this phosphorylation by growth factors.

Autophosphorylation with a concomitant decrease in lipid kinase activity is also the case for p110δ. A single phosphorylation site has been mapped as Ser1039 at the C-terminus of p110δ, and inducible p110δ autophosphorylation at Ser1039 has been demonstrated in CD28-ligated Jurkat T cells [5].

In marked contrast with the class IA PI 3-kinase isoforms, autophosphorylation of the class IB isoform p110γ on Ser1021 does not affect its lipid kinase activity [4,11]. Furthermore, Gβγ subunits stimulate p110γ autophosphorylation in a time- and concentration-dependent manner.

PI 3-kinase serine kinase activity in the insulin and type I interferon signalling pathways
In addition to autophosphorylation, PI 3-kinase has been reported to phosphorylate exogenous protein substrates, including IRS-1 (insulin receptor substrate-1) [12]. IRS-1 was serine phosphorylated in an in vitro kinase assay containing IRS-1 and p85 immunoprecipitates from insulin-stimulated primary rat adipocytes in a wortmannin-sensitive manner. Another study demonstrated that IRS-1 competed with p85 for phosphorylation by p110, suggesting that relief of the inhibitory effect of p85 serine phosphorylation on the lipid kinase activity, caused by complexing of IRS-1 with PI 3-kinase, can be a potential regulatory mechanism of PI 3-kinase activity [13]. More recently, it has also been reported that serine phosphorylation of IRS-1 by PI 3-kinase targets the former for proteasomal degradation [14].

Another study found that, after insulin stimulation, PI 3-kinase co-immunoprecipitated with both IRS-1 and...
the insulin receptor; the lipid kinase activity was associated with IRS-1, whereas the serine kinase activity was associated with the insulin receptor [15]. Immune-complex kinase assays showed that, in addition to p85 and p110, the insulin receptor-associated kinase activity phosphorylated a 135 kDa protein which was identified as phosphodiesterase 3B. This is of interest, as it provides further evidence that the protein and lipid kinase activities of PI 3-kinase are differentially regulated.

In addition to insulin, interferon α has been shown to stimulate the serine kinase activity of PI 3-kinase [16]. Once again, the target for this serine kinase activity was IRS-1. A subsequent study [17] demonstrated that treatment with interferon α that led to PI 3-kinase activation did not result in Akt/protein kinase B activation, whereas treatment with insulin did. This finding implies that interferon signalling could be a system where PI 3-kinase mediates a function distinct from its lipid kinase activity, whereas treatment with insulin did. Further research is necessary in order to identify additional substrates and demonstrate novel functions of the protein kinase activity of PI 3-kinase.

### Dissection of the lipid kinase and protein kinase activities of PI 3-kinase

Wortmannin and LY294002 are the most widely used inhibitors of PI 3-kinases, but these inhibitors interfere equally with both the lipid kinase and protein kinase activities. More recently, we reported that certain methylxanthines, including caffeine and theophylline, inhibit the lipid kinase activity of PI 3-kinase much more potently than the protein kinase activity [10]. Thus it will be possible to devise pharmacological strategies for discriminating between the two activities.

Another strategy used to dissect the roles of the lipid kinase and protein kinase activities involves the production of forms of PI 3-kinases engineered such that they lose lipid kinase but retain protein kinase activity. A region within the conserved catalytic core of p110γ was replaced by the corresponding sequences of mTOR (mammalian target of rapamycin) [18]. The chimaeric protein was found to act only as a protein kinase; it still activated the mitogen-activated protein kinase pathway normally, but not the Akt/protein kinase B pathway. Application of the same strategy to the 110α isoform has shown that only wild-type p110α could stimulate protein kinase B activation and p70S6K phosphorylation, but ‘protein kinase only’ versions of p110α were still able to phosphorylate IRS-1 [19]. Overall, these studies clearly demonstrate distinct roles for the lipid kinase and protein kinase activities of class I PI 3-kinases.

### Concluding remarks

Despite the large volume of data regarding the features and functions of the lipid kinase activity of PI 3-kinase, the potential role of the protein kinase activity in cell signalling has received little attention. As a consequence, it is widely assumed that PI 3-kinase signals solely by generating 3′-phosphorylated phosphoinositides, which might be not the case. A series of recent studies suggest that the protein kinase activity of PI 3-kinases could be playing a wider role in intracellular signalling processes than previously thought. Further research is necessary in order to identify additional substrates and demonstrate novel functions of the protein kinase activity of PI 3-kinase.

### References


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