Insulin Action

Growth factor regulation of the novel class II phosphoinositide 3-kinases
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Abstract
It is well established that the class-I phosphoinositide (PI) 3-kinases play a crucial role in growth factor signalling pathways. However, evidence has recently emerged that the alpha isoform of the class-II PI 3-kinase (PI 3K-C2α) is activated by growth factors, although the consequences of this are poorly understood. Here we demonstrate that the activation of PI 3K-C2α is not associated with a change in subcellular localization. Furthermore, we provide the first evidence that PI 3K-C2β is activated by insulin, albeit with slower kinetics than activation of PI 3K-C2α. These findings suggest that both these class-II PI 3-kinase isoforms are likely to participate in insulin-signalling pathways in the cell.

Phosphorylation at the D-3 position of the inositol ring of phosphoinositides is a crucial step in many growth-factor-stimulated signalling pathways [1,2]. A family of phosphoinositide 3-kinases (PI-3Ks) have been identified [3,4], of which the class Ia PI-3Ks have been the most closely studied. These possess a catalytic subunit tightly associated with an adapter subunit containing two Src homology 2 (SH2) domains. Binding of these SH2 domains to phosphotyrosine residues in specific sequence contexts allows the recruitment of class Ia PI-3Ks into receptor-tyrosine-kinase-induced signalling complexes. This is the best-defined mechanism by which growth factors can regulate PI-3K activity.

A novel group of PI-3Ks, characterized by the presence of a C2 domain and a PX domain at the C-terminus, has recently been identified. These possess a catalytic subunit containing two Src homology 2 (SH2) domains. Binding of these SH2 domains to phosphotyrosine residues in specific sequence contexts allows the recruitment of class Ia PI-3Ks into receptor-tyrosine-kinase-induced signalling complexes. This is the best-defined mechanism by which growth factors can regulate PI-3K activity.

A novel group of PI-3Ks, characterized by the presence of a C2 domain and a PX domain at the C-terminus, has recently been identified. These were originally cloned from Drosophila [5,6] but three mammalian isoforms of class II PI-3K have also been cloned. PI-3K-C2α [6-8] and PI-3K-C2β [9,10] are widely expressed, whereas the expression of a third isoform, PI-3K-C2γ, is restricted to liver [11-13]. The lipid product of the class II PI-3Ks in vivo remains to be established, although in vitro they display a strong substrate preference for PtdIns, with a much lower efficiency towards PtdIns4P and almost no detectable activity towards PtdIns(4,5)P2. This suggests that PtdIns3P might be the main lipid product of the class II PI-3Ks in vivo. The class II PI-3K isoforms can be distinguished pharmacologically from each other and from class I PI-3Ks by their differential sensitivities to the two most widely used PI-3K inhibitors: the relative amounts of wortmannin required for inhibition are class I PI-3Ks = PI-3K-C2α > PI-3K-C2β; those of LY 294002 are class I PI-3Ks < PI-3K-C2β < PI-3K-C2α [7,8,10].

Little is known about the role of class II PI-3Ks in the cell. Some hint of function might come from understanding cellular localization. Although PI-3K-C2β is reported to associate largely with membranes [10], there are now several reports that PI-3K-C2β is associated with clathrin-coated pits [14,15], suggesting that the latter isoform might have a role in regulating endocytosis and vesicle trafficking. In support of this, a direct association between the N-terminal portion of PI-3K-C2α and clathrin has recently been reported [16]. This interaction causes an increase in the catalytic activity of PI-3K-C2α, at least in vitro. These findings point to a role for the class II PI-3Ks in regulating endocytosis.

There is a growing body of evidence to suggest that the activation of class II PI-3Ks might be involved in agonist-mediated regulation of cellular function. The chemokine macrophage chemoattractant protein 1 induces a transient activation of PI-3K-C2α via a Gα-linked mechanism in monocytes [17]. For PI-3K-C2β, stimulation with epidermal growth factor causes recruitment to the intracellular portion of the receptor [18], whereas platelet aggregation induces a transient activation of PI-3K-C2β [19].

We have recently demonstrated that PI-3K-C2α is acutely activated by insulin in cells expressing large numbers of insulin receptors [20]. We find that the insulin-stimulated activation is accompanied by a shift in apparent molecular...
mass of PI-3K-C2α, which seems to be due to a phosphorylation event. Further we find that, after stimulation with insulin, PI-3K-C2α associates with an unknown tyrosine-phosphorylated protein of molecular mass 160 kDa. Regulation of subcellular location is one mechanism by which insulin could be regulating PI-3K-C2α function. We find that the cellular PI-3K-C2α is distributed between fractions soluble and insoluble in Triton X-100 (Figure 1), the presence in the insoluble fraction being consistent with a location in clathrin-coated vesicles. However, stimulation with insulin for 5 min does not cause redistribution between these fractions (Figure 1), despite the fact that PI-3K-C2α is maximally activated in this time [20]. This suggests that cellular redistribution is not the major mechanism involved in the acute regulation of PI-3K-C2α.

In our early studies of regulation of class I1 PI-3Ks by insulin we found that acute stimulation with insulin caused very little stimulation of PI-3K-C2β. However, in subsequent studies we have found that longer stimulations with insulin do cause a significant activation of PI-3K-C2β in transiently transfected Chinese hamster ovary (CHO)-IR cells (Figure 2). This is accompanied by a small but appreciable bandshift in the PI-3K-C2β isofrom (Figure 3). These findings indicate that PI-3K-C2β might also have a role in insulin signalling, although probably not in the regulation of acute metabolic responses such as increased glucose transport and modulation of glycogen synthase.

In summary, although the role of the class I1 PI-3Ks is currently poorly understood, there is

Figure 2
Insulin activates PI 3K-C2β in CHO-IR cells

CHO-IR cells were transiently transfected with Glu-tagged PI-3K-C2β, serum-starved for 3 h, stimulated with 100 nM insulin for the indicated durations, lysed, then immunoprecipitated with anti-Glu antibody. PI-3K activity was assayed in the immunoprecipitates with PtdIns as a substrate. Results show the fold increase in activity compared with basal.

Figure 3
Insulin stimulation induces a bandshift in PI 3K-C2β in CHO-IR cells

CHO-IR cells were transiently transfected with Glu-tagged PI-3K-C2β, serum-starved for 3 h, stimulated with 100 nM insulin for the indicated time, lysed, then immunoprecipitated with anti-Glu antibody. Immunoprecipitates were separated by SDS/PAGE and Western blotted with anti-(PI-3K-C2β) antibody.
Growing evidence that they have a role in growth factor signalling and perhaps also in endocytosis and in regulating vesicle traffic from the trans-Golgi network. Defining their true cellular roles will require establishing the nature of their lipid products in vitro and clearly identifying their binding partners in vitro and the mechanisms regulating these interactions.

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Abstract

Glucose uptake into muscle and its subsequent storage as glycogen is a crucial factor in energy homeostasis in skeletal muscle. This process is stimulated acutely by insulin and is impaired in both insulin-resistant states and in type 2 diabetes mellitus. A signalling pathway involving protein kinase B and glycogen synthase kinase 3 seems certain to have a key role in stimulating glycogen synthesis but other signalling pathways also contribute, including a rapamycin-sensitive pathway stimulated by amino acids. Although glycogen synthesis is one of the classical insulin-regulated pathways, it is also regulated in an insulin-independent manner; for example glycogen synthesis in muscle is stimulated significantly after strenuous exercise, with much of this stimulation being independent of the involvement of insulin. Evidence suggests that glucose and the glycogen content of the muscle have a key role in this stimulation but the molecular mechanism has yet to be fully explained.

Introducing

Glycogen is the major energy store in both liver and muscle: the glycogen in liver is a glucose reservoir for the whole body (including muscle), whereas that in muscle serves as a store for muscle...