Phosphoinositide 3-kinase signalling pathways

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Summary

Phosphoinositide 3-kinases (PI3Ks) phosphorylate the 3′-OH position of the inositol ring of inositol phospholipids, producing three lipid products: PtdIns(3)*P*, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3. These lipids bind to the pleckstrin homology (PH) domains of proteins and control the activity and subcellular localisation of a diverse array of signal transduction molecules. Three major classes of signalling molecule are regulated by binding of D-3 phosphoinositides to PH domains: guanine-nucleotide-exchange proteins for Rho family GTPases, the TEC family tyrosine kinases such as BTK and ITK in B and T lymphocytes, respectively, and the AGC superfamily of serine/threonine protein kinases. These molecules are activated by a variety of extracellular stimuli and have been implicated in a wide range of cellular processes, including cell cycle progression, cell growth, cell motility, cell adhesion and cell survival.

Keywords: Phosphoinositide 3-kinase, PH domains, Rac-1, Protein kinase B, Tec kinases

Introduction

The controlled metabolism of inositol phospholipids is fundamental for signal transduction in eukaryotic cells (Fig. 1). One well-defined pathway is mediated by phospholipases C (PLCs) that hydrolyse phosphatidylinositol (4,5) biphosphate (PtdIns(4,5)P_2) to produce inositol 1,4,5-triphosphate [Ins(1,4,5)P_3], which increases intracellular Ca^{2+} levels. PtdIns(4,5)P_2 breakdown simultaneously produces diacylglycerol, which activates serine/threonine kinases of the protein kinase C (PKC) family. A second route for inositol lipid metabolism involves phosphoinositide 3-kinases (PI3Ks), which phosphorylate the 3′-OH position of the inositol ring of inositol phospholipids, producing PtdIns(3)*P*, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3.

There are multiple isoforms of PI3Ks in mammalian cells, and these are subdivided into three classes (for reviews see; Vanhaesbroeck et al., 1997; Fruman et al., 1998; Rameh and Cantley, 1999). The class 1 PI3Ks comprise a p110 catalytic subunit and a regulatory adapter subunit. Class II PI3Ks are large (170-210 kDa) proteins that have a catalytic domain that is 45-50% homologous to class I PI3Ks. Class II PI3Ks also have a C-terminal region that has homology to the C2 domains that mediate calcium/lipid binding in classical protein kinase C isoforms. Class II PI3Ks preferentially phosphorylate PtdIns and PtdIns-4-P in vitro, but their in vivo biology is not understood. Class III PI3Ks are typified by the yeast protein encoded by the *VPS34* gene and phosphorylate only PtdIns to produce PtdIns(3)*P*; they are thought to regulate vesicle transport.

Class I PI3Ks have been the major focus of PI3K studies because it is these isoforms that are generally coupled to extracellular stimuli. The predominant substrate for receptor-coupled class 1 PI3Ks is PtdIns(4,5)P_2, and hence the primary product of their action is PtdIns(3,4,5)P_3 (Hawkins et al., 1992). This lipid is further metabolised by inositol lipid phosphatases to produce PtdIns(3,4)P_2. PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 bind to pleckstrin homology (PH) domains of proteins (Rameh et al., 1997) and can allosterically modify their activity or induce relocalisation of the protein to defined areas of the plasma membrane where activation can occur. Class 1 PI3Ks are activated by a variety of extracellular stimuli and have been implicated in a wide range of cellular processes, including cell cycle progression, cell growth, cell motility, cell adhesion and cell survival (Coelho and Leevers, 2000; Fig. 1).

Activation of PI3K

The class 1 PI3Ks comprise a p110 catalytic subunit and a regulatory adapter subunit. Four isoforms of the p110 subunit have been described (α, β, γ and δ), and three mammalian genes encode adapter subunits p85α, p85β and p55γ. The prototypical model for PI3K activation was first worked out in the context of receptor tyrosine kinases in fibroblasts. In this model, the p110 catalytic subunit is recruited to an activated receptor tyrosine kinase by the p85 adapter subunit. The interaction between p85 and the receptor complex is mediated by a high-affinity interaction between the p85 Src homology 2 (SH2) domain and specific tyrosine-phosphorylated sequences within the cytoplasmic tail of the receptor (Kazlauskas, 1994; Pawson, 1995). This process recruits the p110 catalytic subunit of PI3K to the plasma membrane, where it can phosphorylate its main substrate PtdIns(4,5)P_2 and thereby generate PtdIns(3,4,5)P_3.

The ability of p85 to bring the catalytic subunit to the plasma membrane is a crucial role for this adapter; constitutive membrane targeting of p110 catalytic subunits of PI3K creates a constitutively active enzyme that generates PtdIns(3,4,5)P_3 when expressed in cells (Reif et al., 1996). However, the role of p85 in PI3K regulation is a little more complicated than simply that of a vehicle to transport p110 around a cell. p85 negatively regulates the catalytic activity of its associated p110 subunit, and Parker and co-workers propose that binding of specific phosphotyrosine sequences to the p85 SH2 domain alleviates this inhibition (Kodaki et al., 1994; Woscholski et al., 1995).

There are many refinements to the prototypical model for tyrosine kinase regulation of PI3K. For example, in leucocytes, PI3K can be activated by non-receptor tyrosine kinases such as the Janus kinases (JAKs), during cytokine signalling, or Syk or ZAP70 during antigen-receptor signalling. Recruitment to the plasma membrane is not necessarily mediated by receptors; there are numerous reports of interactions between p85 and other adapters that could bring p110 to the plasma membrane. For example, a complicated signalling scaffold formed by three adapters – Shc, Grb2 and Gab2 – recruits PI3K to the plasma membrane in cells activated by haematopoietic cytokines such as interleukin 3 (IL-3) and IL-2 (Gadina et al., 2000; Gu et al., 2000).

G protein regulation of PI3K

PI3K can be activated by guanine-nucleotide-binding proteins: for example, p110γ is activated by heterotrimeric G proteins controlled by serpentine receptors such as chemokine receptors (Stephens et al., 1994). p110 catalytic subunits can also bind to the active conformation of Ras, which might stabilise the association of p110 with the plasma membrane after it has been recruited to a receptor complex by p85 and stimulate the catalytic activity of the p110-p85 complex (Rodriguez-Viciana et al., 1994; Rodriguez-Viciana et al., 1996). The crystal structure of p110γ has been solved, and the positioning of the Ras-binding domain against the catalytic domain gives some insights into how GTPases might allosterically regulate p110 catalytic activity (Walker et al., 1999; Pacold et al., 2000).

Negative feedback control of PI3K signalling

During cell activation, both positive and negative regulatory signalling cascades are vital for homeostasis. Recent studies of the consequences of deregulation of negative feedback control of PI3K have been fundamental to our understanding of the central role that PI3Ks have in mammalian cells.

At least two major routes for degradation of PtdIns(3,4,5)P_3 exist: dephosphorylation by the phosphoinositide-lipid 3-phosphatase PTEN (Cantley and Neel, 1999; Stambolic et al., 1998) and dephosphorylation by the 145-kDa SH2-containing inositol (poly)phosphate 5-phosphatase (SHIP; Fig. 1; Rohrschneider et al., 2000).

PTEN is a tumour suppressor mutated/deleted in a variety of different human tumours. Cells lacking PTEN have elevated levels of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, and exhibit constitutive activation of PI3K signalling pathways mediated by Tec family tyrosine kinases (Shan et al., 2000), the serine/threonine kinase AKT/PKB (Cantley and Neel, 1999; Stambolic et al., 1998; Alessi, 2001) and Rac/Rho GTPases (Liliental et al., 2000).

The second inositol lipid phosphatase, SHIP, mediates an important negative feedback mechanism in lymphocytes: loss of SHIP results in an unbalanced immune response, and the resultant disruption of immune homeostasis culminates in the development of autoimmunity (Helgason et al., 1998; Okada et al., 1998; Ono et al., 1997). The role of SHIP is best-characterised in B cells, in which SHIP is controlled by inhibitory receptors such as FcγRIIb (Ono et al., 1997; Sarao et al., 1998). Triggering of antigen receptors and costimulatory molecules such as CD19 in B cells activates PI3K in a response essential for lymphocyte activation and antibody production. Circulating antigen-antibody complexes then co-ligate the BCR with FcγRIIb, which results in tyrosine phosphorylation of an immune cell tyrosine-based inhibitory motif (ITIM) in FcγRIIb (Burshtyn and Long, 1997). The SH2 domain of SHIP can bind to the phosphorylated ITIM, thereby recruiting this inositol 5′ phosphatase to the BCR-FcγRIIb complex. SHIP dephosphorylates PtdIns(3,4,5)P_3 to produce PtdIns(3,4)P_2, and, accordingly, coligation of the BCR with FcγRIIb diminishes BCR elevation of intracellular PtdIns(3,4,5)P_3 levels. PI3K-controlled signalling pathways are thus the primary targets for FcγRIIb inhibition (Aman et al., 1998; Astoul et al., 1999; Bolland et al., 1998).

PI3K regulation of guanine-nucleotide-binding proteins

Important targets for the products of PI3K are the GTPases Rac and Rho. These GTPases coordinate the dynamic organisation of the actin cytoskeleton and the assembly of associated integrin structures. Rac1 and RhoA are responsible for distinct patterns of actin reorganisation: RhoA regulates the formation of actin stress fibers and focal adhesions; Rac1 controls lamellipodia formation and focal complex assembly and, subsequently, can activate Rho-mediated cytoskeletal changes.

Activation of Rac and Rho is stimulated by guanine-nucleotide-exchange factors (GEFs), which characteristically contain a catalytic Dbl-homology domain flanked by a PH domain that is critical for GEF function (Cerione and Zheng,

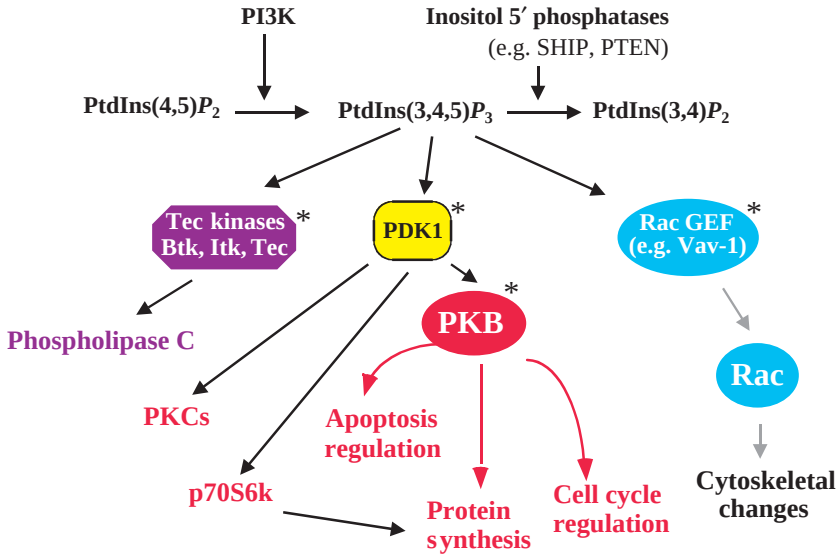

Fig. 1. A summary of the major signalling pathways initiated by the major lipid product of PI3Ks: PtdIns(3,4,5)P_3. Asterisked proteins have a PH domain that directly binds to PtdIns(3,4,5)P_3.

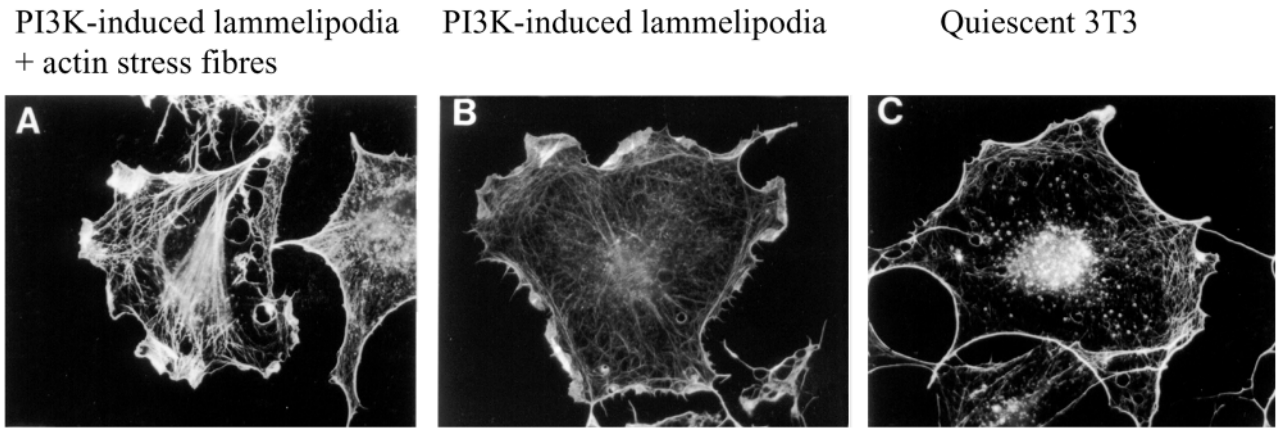

Fig. 2. The data show 3T3 cells stained with phalloidin to reveal polymerised actin structures. Panel A shows a cell microinjected with a membrane targeted constitutively active PI3K (Reif et al., 1996) and reveals the ability of PI3K to induced lammelipodia formation and actin stress fibres. Compare the PI3K-activated cell in (A) with the quiescent cell in (C). Panel B shows a cell microinjected with active PI3K plus *Clostridium Botulinum* C3 transferase, a selective inhibitor of the RhoA GTPase. In these cells, PI3K-mediated formation of actin stress fibres is suppressed, which reveals more dramatically the ability of active PI3K to initiate membrane ruffling or lammelipodia formation. If Rac function is inhibited by coexpression of dominant negative Rac mutants, then all PI3K-mediated changes in the actin cytoskeleton are lost (Reif et al., 1996). Figure reproduced, with permission, from Reif et al., 1996.

1996). One well-studied Rac GEF is Vav-1, catalytic activity of which depends on tyrosine phosphorylation by Src kinases. In vitro models have suggested that PtdIns(3,4,5)P_3 binding to the PH domain of Vav-1 potentiates tyrosine-kinase-mediated activation of this enzyme (Han et al., 1998). The in vivo relevance of these data is not known, although they provide a tempting model for how PI3K might regulate Rac and Rho GTPases.

The evidence for PI3K control of Rac- and Rho-mediated changes in actin dynamics is overwhelming and supported by experiments using pharmacological inhibitors of PI3K, studies of receptor mutants that have signalling defects that prevent PI3K activation and experiments using constitutively active PI3K mutants. These latter studies show quite dramatically that active PI3K mutants are sufficient to induce Rac- and Rho-mediated cytoskeletal responses of membrane ruffling and actin stress fibre formation (Arrieumerlou et al., 1998; Reif et al., 1996; Fig. 2). Moreover, recent chemotaxis studies have shown that there is dynamic local accumulation of 3′ phosphoinositides at the leading edge of migrating cells (Servant et al., 2000). This has resulted in the proposal that changes in local concentrations of PtdIns(3,4,5)P_3 allow cells to polarise and distinguish the front of the cell from the back.

Rac and Rho have quite pleiotropic functions in cells (Bishop and Hall, 2000), and their actions are not limited to the control of the actin cytokeleton but include the regulation of the stress-activated MAP kinases p38 and JNKs and activation of transcription factors. Interestingly, PI3K induces only a subset of Rac/Rho-mediated cellular responses that control the cortical actin cytoskeleton and is not sufficient to stimulate the full range of Rac- or Rho-pathways that couple them to MAP kinases or transcription factor activation (Reif et al., 1996). The mechanism for this restricted action of PI3K on Rac/Rho effector pathways is not understood, but the hypothesis is that architectural organisation of Rac and Rho effector molecules by multivalent adapter, anchoring or scaffold proteins ensures that discrete subpopulations of Rho family GTPases are linked to different effector pathways. The lipid products of PI3K thus activate Rac effector pathways close to the plasma membrane but may well not be capable of activating the entire cellular pool of Rac and Rho.

PI3K and tyrosine kinases

Members of the TEC family of tyrosine kinases (ITK, TEC and BTK) require phosphorylation within their activation loop by Src kinases for activation (Rawlings et al., 1996; Schaeffer and Schwartzberg, 2000). ITK, TEC and BTK have an N-terminal PH domain that binds to PtdIns(3,4,5)P_3 and controls their association with the plasma membrane and thus with Src kinases (Bolland et al., 1998; Shan et al., 2000). The importance of the PH domains of these kinases is illustrated by BTK: mutations in the BTK PH domain cause X-linked agammaglobulinaemia in humans and a similar X-linked immunodeficiency in mice (Li et al., 1995; Schaeffer and Schwartzberg, 2000). The importance of BTK for B cell development and function stems from the essential role of this tyrosine kinase in the control of PLC$\gamma_{1/2}$ activity (Schaeffer and Schwartzberg, 2000; Scharenberg et al., 1998). Antigen receptor coupling to PLC$\gamma_{1/2}$ results in the hydrolysis of PtdIns(4,5)P_2 and the consequent production of Ins(1,4,5)P_3, which initiates increases in intracellular calcium levels, and diacylglycerol, which activates PKCs. The essential role of BTK in PLC$\gamma_{1/2}$ regulation puts essential calcium/PKC signalling pathways under the control of PI3K and is a clear demonstration of the type of signalling crosstalk that controls inositol lipid metabolism in mammalian cells (Schaeffer and Schwartzberg, 2000). The phenotype of mice lacking p85α provides genetic evidence for the importance of PI3K in B lymphocytes: these mice show profound defects in B cell function (Suzuki et al., 1999). This phenotype is consistent with that of BTK-deficient mice, which provides strong genetic evidence for the link between PI3K and BTK and the physiological relevance of PI3K for B cell biology.

PI3K and serine/threonine kinases

A key serine/threonine kinase that mediates PI3K action is protein kinase B (PKB, also known as AKT; Burgering and Coffer, 1995; Datta et al., 1996). PtdIns(3,4,)P_2 and PtdIns(3,4,5)P_3 bind to the PH domain of PKB, recruiting the kinase to the plasma membrane. Expression of active PI3K is sufficient to trigger PKB activation (Astoul et al., 1999; Reif et al., 1997); suppression of PtdIns(3,4,5)P_3 production by inositol phosphatases such as SHIP prevents PKB activation (Aman et al., 1998; Astoul et al., 1999). Moreover, in PTEN-null cells, PKB is constitutively active and uncoupled from mitogenic stimuli (Cantley and Neel, 1999; Stambolic et al., 1998). At the plasma membrane, PKB is phosphorylated on Thr308 in the kinase activation loop and on Ser473 in a hydrophobic region of the C-terminus (Alessi and Cohen, 1998; Bellacosa et al., 1998). The upstream kinase that targets Thr308 is a 67-KDa ubiquitously expressed phosphoinositide-dependent protein kinase, PDK1 (Alessi et al., 1997a; Alessi et al., 1997b; Stephens et al., 1998). The importance of PDK1 for PI3K action was first established in the context of PKB. However, PDK1 is also important for the regulation of many AGC superfamily kinases (Williams et al., 2000) and phosphorylates key residues in the activation loops of PKC isoforms (Dutil et al., 1998; Le Good et al., 1998) and the ribosomal S6k and, S6K1 kinases (Toker and Newton, 2000; Vanhaesebroeck and Alessi, 2000).

PDK1 has an N-terminal catalytic domain and a C-terminal PH domain that binds to PtdIns(3,4,5)P_3 with high affinity. The role of D-3 phosphoinositides in controlling PDK1 activity and subcellular localisation is very controversial (Anderson et al., 1998; Currie et al., 1999; Toker and Newton, 2000). One view is that PDK1 actions are regulated primarily by substrate conformation (Flynn et al., 2000). In these models the regulatory impact of the lipid products of PI3K occurs because of their actions on substrate availablity rather than any direct action on PDK1. For example, the binding of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 to the PKB PH domain is proposed to relieve auto-inhibition of the active site, allowing PDK1 to phosphorylate Thr308 – the key step in PKB activation.

Another view of PI3K regulation of PDK1 is that D-3 lipids initiate PH-dependent relocalisation of PDK1 from the cytosol to the plasma membrane (Anderson et al., 1998; Filippa et al., 2000). PtdIns(3,4,5)P_3 could also modulate the activity of PDK-1 in cells. For example, Filippa et al. suggest that the PDK1 PH domain acts as a negative regulator of PDK1 activity and that PtdIns(3,4,5)P_3 binding relieves this autoinhibition (Filippa et al., 2000). However, there is neither universal acceptance of the idea that PtdIns(3,4,5)P_3 modulates the intrinsic catalytic activity of PDK1 nor any generally accepted mechanism. Nevertheless, the recognition that PDK1 is a critical regulator of several kinases with quite diverse functions explains how PI3K can regulate so many distinct cellular processes. It should be emphasised that, for some of these pathways, PDK1 operates a simple on/off switch for catalytic activity – for example, in the case of PKB (Alessi and Cohen, 1998; Bellacosa et al., 1998). In contrast, in other situations, such as the regulation of classical PKCs, PDK-1 phosphorylation at the activation loop is a priming step that promotes the autophosphorylation required to generate a catalytically competent form of PKC that needs only diacylglycerol/calcium binding and relief from autoinhibition to allow phosphorylation of substrates (Parekh et al., 2000).

The PDK1-regulated kinases have essential functions: for example, one target for PI3K/PDK1 is p70S6K1, a kinase that is important for the regulation of protein synthesis and cell growth (Alessi et al., 1997b; Pullen et al., 1998; Williams et al., 2000). Similarly, PKB plays an important role in cell survival (Datta et al., 1997; Del Peso et al., 1997; Kauffmann-Zeh et al., 1997; Zha et al., 1996). Moreover, a pathway induced by PI3K and mediated by PKB and S6K1 can stimulate the activity of E2F transcription factors, important components of the mechanisms that control the mammalian cell cycle (Brennan et al., 1999; Brennan et al., 1997). PKB also phosphorylates and inactivates GSK3 (Cross et al., 1995), an enzyme initially identified as a regulator of glycogen metabolism that also has broader functions: interactions between GSK3 and β-catenin regulate activity of the T cell factor lymphoid enhancer factor (Tcf/Lef; Rubinfeld et al., 1996). GSK3 can also control the nuclear export of nuclear factor of activated T cells (NFAT), a group of transcription factors involved in cytokine gene induction (Beals et al., 1997).

Transcription factors directly phosphorylated by PKB include members of the Forkhead family of transcription factors FKHR, FKHRL and AFX (Medema et al., 2000). The phosphorylation of FKHR, FKHRL and AFX by PKB promotes their export from the nucleus to the cytoplasm, where they form a complex with 14-3-3 proteins that effectively retains them in the cytoplasm away from their transcription factor targets. Such targets include the cyclin inhibitor $p27^{kip1}$ and the cell death receptor Fas (Medema et al., 2000). A discussion of the substrates of PKB is beyond the scope of this article but is a predominant concern for the field (see Vanhaesebroeck and Alessi, 2000).

The functions of different PI3K isoforms

The multiple isoforms of PI3K are frequently coexpressed in cells and have very similar substrate specificities for lipids; yet it has been proposed that they have unique functions within a cell (Hooshmand-Rad et al., 2000; Vanhaesebroeck et al., 1999b). The biological roles of different PI3K isoforms are beginning to be unravelled by gene targeting: mice lacking p110γ are viable and fertile but have defective neutrophil and lymphocyte function (Li et al., 2000; Sasaki et al., 2000; Hirsch et al., 2000). In contrast, mice lacking p85α are not viable, and lymphocyte repopulation studies using p85α-null stem cells have shown that B cells lacking p85α are functionally deficient whereas p85α-null T lymphocytes seem completely normal (Fruman et al., 1999; Suzuki et al., 1999). Moreover, mast cells lacking p85α have a defect in stem-cell factor (SCF) responses but normal signalling by the high-affinity receptor for IgE (Lu-Kuo et al., 2000). Another example of different roles of PI3K isoforms comes from in vitro experiments using neutralising antibodies in macrophages, which suggest that p110α but not p110β or p110δ is required for mitogenic responses triggered by colony-stimulating factor 1 (CFS-1). In contrast, p110β and p110δ are necessary for CSF-1-induced modulation of actin dynamics (Vanhaesebroeck et al., 1999b).

The key question is how does a cell recognise that PtdIns(3,4,5)P_3 has been produced as a consequence of p110α activation rather than p110β or p110δ stimulation? The answer

may reside in the fact that each isoform has a different mechanism of activation and hence will not be coordinately activated and will operate with different spatial and temporal characteristics. For example, p110γ is typically activated by receptors coupled to heterotrimeric G proteins, whereas p110α, p110β and p110δ are associated more with activation by tyrosine kinases. The kinetics of G protein and tyrosine kinase activity can be quite different, meaning that p110γ and p110α/δ will operate at different times during a cells lifetime. It is also likely that the exact subcellular localisation of a heterotrimeric G protein or an activated tyrosine kinase will be different, and so the precise point in the cell at which PI3K activity occurs will be different.

It is increasingly recognised that the plasma membrane is not uniform but divided into signalling sub-compartments. In the context of PI3K signalling, different membrane subdomains probably contain different repertoires of PH-domain-containing proteins, the direct targets of the lipid products of PI3K. In this context, there is some debate as to whether the regulatory/adapter subunits of PI3K, such as p85α and p85β, play roles as scaffolds that target PI3K effectors to the sites of PI3K activity. If they do, then this could generate signalling heterogeneity. In addition, the availability of PI3K effectors might be regulated by other signalling pathways triggered by a particular receptor, hence giving quite a different biological outcome. For example, recent studies have identified a novel adaptor protein, dual adaptor for phosphotyrosine and 3-phosphoinositides (DAPP1), that possesses an SH2 domain and a PH domain that can bind to PtdIns(3,4,5)P_3 with high affinity. DAPP1 is tyrosine phosphorylated by Src kinases at Tyr139 (Dowler et al., 2000). This low-molecular-weight adapter molecule can thus integrate at least three different signals, and there is clearly the potential for its subcellular localisation to be modulated by its SH2 domain as well as by its PH domain.

One final comment on the unique roles of different PI3K isoforms: the fact that the catalytic subunits of PI3K have serine/threonine kinase activity is frequently overlooked (Vanhaesebroeck et al., 1999a). The lipid substrate specificities of different PI3K catalytic subunits are similar, but differences in their protein kinase activities have been noted at least in vitro. As yet, no study has explored the relevance of the serine/threonine kinase function of the classical PI3Ks in vivo. Moreover, Reif et al. have observed serine/threonine phosphorylation of p110 catalytic subunits and noted differences in serine/threonine phosphorylations induced by p85α and p85β during lymphocyte activation (Reif et al., 1993). The regulatory significance of these phenomena is not known, but the potential influence of serine/threonine phosphorylation on protein conformation and as determinants of protein interactions means they may hold the keys to understanding the unique biological roles of different members of the PI3K family.

Perspectives

The future for PI3K studies will undoubtedly involve more genetic analysis of the functions of PI3K and its effectors in both vertebrates and invertebrates. There is already a diverse array of targets for the lipid products of PI3K, but our knowledge of the functions of these is very sketchy; it is based mostly on studies of fibroblasts, and we know little about targets in other, physiologically relevant cell systems. For some targets, such as DAPP1, the challenge is simple: find a function! For serine kinases such as PKB/Akt or the Tec tyrosine kinases, the challenge is to identify the critical substrates in each cell type. Probably one of the most important aspects of PI3K signalling research will be to work out the spatial dynamics of the processes. There are examples of differences in the localisation of the PI3K effector PKB in different cell types: in epithelial cells, active PKB has been reported to reside at the plasma membrane at cell-cell contact zones (Watton and Downward, 1999), whereas activated PKB rapidly moves to the cytosol and nucleus of activated lymphocytes (Astoul et al., 1999). The differential localisation of PKB opens up the possibility for phosphorylation of different substrates. There is already evidence that membrane-targeted PKB has a wider signalling capacity than cytosolic PKB – for example, plasma-membrane-targeted active PKB can stimulate p70SK1 and GSK3, whereas a cytosolic active PKB can activate only GSK3 and not p70S6K1 (Dufner et al., 1999). There have been many advances in imaging techniques in the past few years, and it is the refinement of these techniques and their application to studies of different PI3K isoforms and effectors that will give the key insights into PI3K biology.

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