PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Structure and Function of Phosphatidylinositol 3-Kinase: A Potential Second Messenger System Involved in Growth Control

Michael J. Fry and Michael D. Waterfield

Phil. Trans. R. Soc. Lond. B 1993 **340**, 337-344
doi: 10.1098/rstb.1993.0076

Structure and function of phosphatidylinositol 3-kinase: a potential second messenger system involved in growth control

MICHAEL J. FRY[1] AND MICHAEL D. WATERFIELD[1,2]

[1]*Ludwig Institute for Cancer Research, 91 Riding House Street, London W1P 8BT, U.K.*
[2]*Department of Biochemistry and Molecular Biology, University College, London WC1E 6BT, U.K.*

SUMMARY

Ligand stimulation of growth factor receptors with intrinsic protein-tyrosine kinase activity initiates the assembly of multienzyme signalling complexes. This is mediated by binding of proteins with *src* homology 2 (SH2) domains to receptor autophosphorylation sites. Among the proteins involved in complex formation is phosphatidylinositol (PI) 3-kinase, a heterodimeric enzyme composed of 85 kDa and 110 kDa subunits, which binds to receptor (and non-receptor) phosphotyrosine residues through the two SH2 domains in the p85 subunit. p85 acts as an adaptor protein and possibly a regulator of the p110 catalytic subunit that phosphorylates phosphoinositides at the D-3 position of the inositol ring. p85 subunit is composed of several distinct functional domains: one SH3 and two SH2 domains, a p110 binding site and a region with homology to BCR. Expression of these domains in *E. coli* as GST-fusion proteins has allowed definition by nuclear magnetic resonance (NMR) of three-dimensional structures for the SH2 and SH3 domains. The relationship of structure to function for these domains is discussed. The p110 catalytic domain has a region of homology with vps34p of *Saccharomyces cerevisiae*, a protein involved in protein sorting to the yeast vacuole. Possible clues to the function of PI 3-kinase derived from this and other observations are presented.

1. INTRODUCTION

An important concept in receptor-mediated signal transduction has come to light whereby ligand activation of protein-tyrosine kinase (PTK) receptors, resulting in their autophosphorylation at specific sites, acts as a molecular switch which drives the assembly of multienzyme signalling complexes. Enzymes found in these complexes include key signal generating molecules such as phospholipase C$\gamma-1$ (PLCγ), the GTPase-activating protein (GAP) for ras, the cytoplasmic PTK, pp60$^{c\text{-}src}$, and phosphatidylinositol (PI) 3-kinase (reviewed in Cantley *et al.* 1991). Formation of these signalling complexes is mediated by the binding of the *src* homology (SH) 2 domains present in these proteins to specific receptor autophosphorylation sites (reviewed in Koch *et al.* 1991). We have chosen the PI 3-kinase as a model enzyme to study receptor complex formation and also the signal transduction events which are generated by this enzyme.

Since the description in 1984 of minor lipid kinase activities associated with the viral transforming proteins pp60$^{v\text{-}src}$ and p68$^{v\text{-}ros}$, PI kinases have been subjected to intense scrutiny (reviewed in Downes & Carter 1991). PI kinase activity has now been shown to associate with many PTKs including both activated oncogenes and ligand-stimulated growth factor receptors (reviewed in Cantley *et al.* 1991). In many cases PI kinase activity was shown to correlate with the presence of an associated 85 kDa phosphoprotein which was assumed to be a component of this enzyme (Courtneidge & Heber 1987; Kaplan *et al.* 1987).

A major advance was the surprise finding that the product of this enzyme was a novel phospholipid, phosphatidylinositol 3-phosphate (PI3P) (Whitman *et al.* 1988). Subsequently PI 3-kinase has been shown to phosphorylate PI4P and PI4,5P_2 to PI3,4P_2 and PI3,4,5P_3 respectively, and all three of these lipids have been identified in growth factor-stimulated or transformed cells (reviewed in Downes & Carter 1991). These phospholipids are distinct from those of the classical PI cycle and interestingly do not appear to be substrates for known phospholipases, suggesting that either the lipids themselves are the important species in signal transduction pathways or that other PLCs specific for this family of inositol lipids remain to be identified (Lips *et al.* 1989; Serunian *et al.* 1989).

Our interest in the PI 3-kinase arose from a number of observations suggesting that it might be a key substrate of several PTKs. One of the earliest identified interactions of this enzyme was with the transforming complex of middle-T (mT) antigen and pp60$^{c\text{-}src}$ from polyomavirus-transformed cells (Whitman *et al.* 1985). In all studies carried out on transformation by polyoma mT a tight correlation has

Phil. Trans. R. Soc. Lond. B (1993) **340,** 337–344
Printed in Great Britain

been observed between the presence of PI 3-kinase activity in the mT:pp60$^{c\text{-}src}$ complex and the transforming activity of mT mutants. This link has recently been strengthened by the demonstration of a mT mutant which complexes with both pp60$^{c\text{-}src}$ and the PI 3-kinase, but does not elevate cellular levels of 3-phosphorylated inositol lipids. This mutant fails to transform (Ling *et al.* 1992). Secondly, PI 3-kinase has been demonstrated to be important in normal growth factor-stimulated pathways. The PDGF β-receptor was shown to interact with the PI 3-kinase (Kaplan *et al.* 1987) and the site of association has been mapped to two tyrosine residues (Y740 and Y751; human β-receptor numbering) within the kinase insert domain (Kazlauskas & Cooper 1990). This result has recently been extended by showing that mutation of these two tyrosines to phenylalanine residues abolishes this interaction between the PDGF β-receptor and the PI 3-kinase. This leads to a mitogenically defective receptor (Fantl *et al.* 1992). However abolishing the site of interaction for rasGAP by an identical approach has no effect on growth factor-stimulated mitogenesis mediated by this receptor (Fantl *et al.* 1992). These observations pointed to PI 3-kinase as playing an important role in diverse cellular processes and as a key target for regulation by PTKs which prompted us to investigate the composition and regulation of this enzyme.

2. PURIFICATION OF AN ACTIVE BOVINE BRAIN PI 3-KINASE

Crucial to understanding the role of PI 3-kinase has been the determination of its primary structure. A conventional purification from bovine brain resulted in a 650-fold enrichment of PI 3-kinase activity (Morgan *et al.* 1990) and this preparation contained an 85 kDa protein which was a substrate for the PDGF receptor. Further conventional steps failed to increase the purity of the PI 3-kinase preparation and so an affinity step was devised, based on the known ability of the PI 3-kinase to associate with specific sequences of the PDGF β-receptor.

As noted above the major sites of interaction between the PI 3-kinase and the human PDGF β-receptor have been mapped to autophosphorylation sites at Y740 and Y751. It had been demonstrated that phosphorylation of these tyrosine residues was important for the association with the PI 3-kinase (Kazlauskas & Cooper 1990). We synthesized a 17 amino acid peptide (amino acids 742 to 758) from the kinase insert domain of the PDGF β-receptor, which included Y751 and adjacent sequences that had been suggested to be important for PI 3-kinase binding (reviewed in Cantley *et al.* 1991). This peptide was phosphorylated *in vitro* using A431 cell membranes, the phosphorylated peptide was purified by high performance liquid chromotography (HPLC) and then coupled to an Actigel matrix (Fry *et al.* 1992). When the conventionally purified material containing PI 3-kinase activity was incubated with this column only two proteins were found to bind quantitatively, one of 85 kDa and one of 110 kDa (Otsu *et al.* 1991; Fry *et al.* 1992). This result was in good agreement with data from two other laboratories who found that PI 3-kinase activity purified from rat liver and bovine thymus was associated with proteins of 85 and 110 kDa (Carpenter *et al.* 1991; Shibasaki *et al.* 1991).

3. ISOLATION OF THE 85 kDa REGULATORY SUBUNIT OF THE BOVINE PI 3-KINASE

For sequence analysis the two components of the PI 3-kinase complex were eluted from the Y751 phosphopeptide affinity column. Owing to the strong correlation between the presence of a tyrosine phosphorylated p85 protein in activated receptor preparations with associated PI 3-kinase activity (see above) our initial sequencing and cloning efforts were directed towards the 85 kDa component. Using degenerate oligonucleotides, based on several tryptic peptides, two related cDNAs were isolated. Both cDNAs contained open reading frames which could encode proteins of 724 amino acids in length and showed 62% identity at the amino acid level. We named these two proteins p85α and p85β. p85α contained sequences identical to our tryptic peptides from the sequenced bovine brain protein, while p85β was a closely related protein which was not represented in our sequenced peptides. Two other groups independently isolated p85α clones from human and murine cDNA libraries (Escobedo *et al.* 1991; Skolnik *et al.* 1991). None of these p85 proteins exhibited any recognizable kinase motifs or homology to ATP- or GTP-binding proteins. Examination of the sequences of the p85α and p85β proteins revealed a common multidomain structure. Both possess an amino-terminal SH3 domain and two SH2 domains (see figure 1*a*). The region between the SH3 domain and the amino-terminal SH2 domain displays the lowest degree of sequence similarity between p85α and p85β, but this region does possess a sequence related to that recently found in rhoGAP, n-chimaerin and BCR which has been shown to be sufficient to specify a GAP activity in the latter two proteins (reviewed in Fry 1992). However the degree of sequence similarity between the p85 proteins is not as extensive as between the other proteins in this family and to date no GAP activity has been found associated with either the p85 proteins or with the PI 3-kinase complex (Fry 1992). It is possible however that this region may provide a site for interaction with small G-protein-regulated pathways. At least one other p85-related species has been identified by Southern blotting termed p85γ, and a partial bovine cDNA suggests that this protein is closely related in structure to the other two family members (Otsu *et al.* 1991). The reason for multiple isoforms of p85 proteins is not clear. In view of the potential regulatory roles of the p85 subunit it seems logical to suppose that the different p85 subunits may confer selectivity to PI 3-kinase/receptor interactions. However, no such selectivity has been reported to date.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

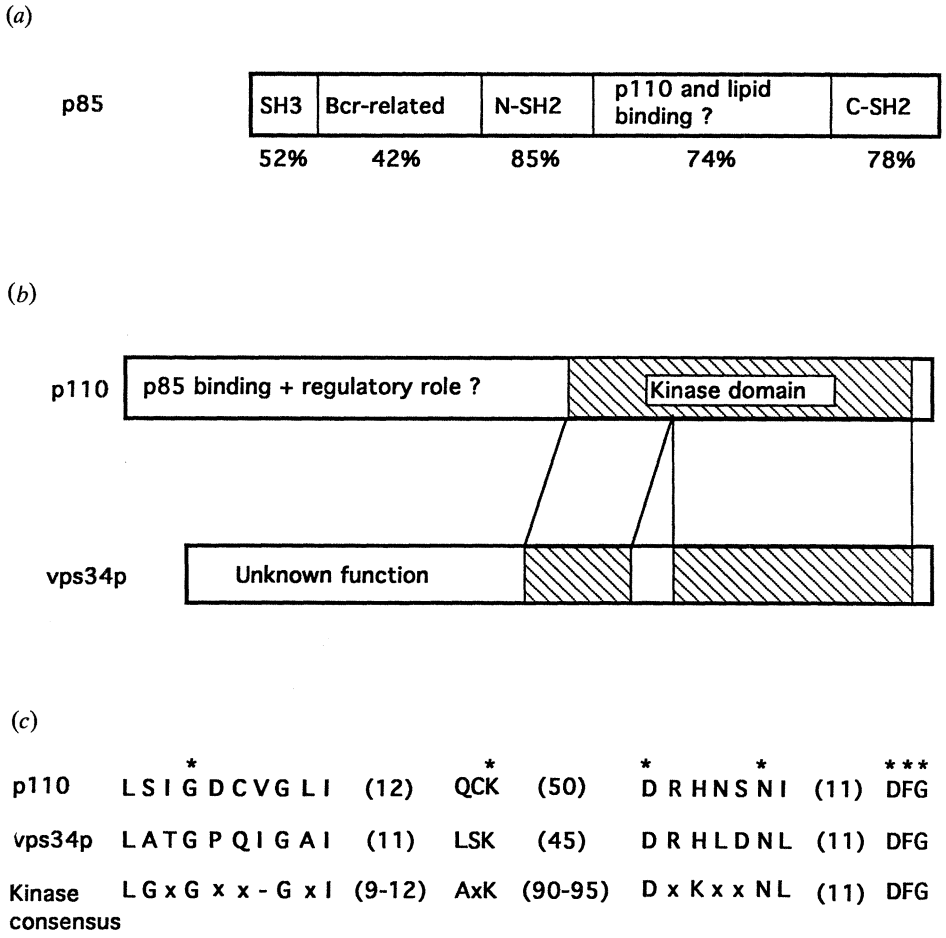

Figure 1. Structure of the components of PI 3-kinases. (*a*) p85 regulatory subunits. A schematic structure for the p85 family members is shown and the major functional domains identified are indicated. Numbers below the domains indicate the percentage identity for the domain between p85α and p85β. (*b*) PI 3-kinase catalytic subunits. Schematic of the two PI 3-kinase family members identified to date, bovine p110 and vps34p from *S. cerevisiae*. The putative kinase domain, which is conserved between the two proteins, is shaded. (*c*) An alignment between p110 and vps34p over the region most closely related to other known kinases is shown together with the consensus sequence derived from protein kinases. Residues proposed to be involved in ATP binding are marked with asterisks. Numbers in brackets indicate the number of amino acids separating the highly conserved blocks shown.

4. INTERACTION OF EXPRESSED p85 WITH GROWTH FACTOR RECEPTORS

Our initial expression studies on the two p85 proteins rapidly confirmed the prediction from sequence analysis that these proteins lacked detectable PI 3-kinase activity (Otsu *et al.* 1991; Gout *et al.* 1992). The domain structure of these two proteins (SH3-bcr related-SH2-spacer-SH2) was much more reminiscent of a regulatory subunit and strongly suggested that the associated 110 kDa protein would be required for catalytic activity. Further support for this came from the work of Shibasaki *et al.* (1991) who demonstrated PI 3-kinase activity associated with a 110 kDa species from bovine thymus in the absence of any detectable p85 protein. While pursuing a cDNA encoding the p110 subunit we examined the ability of the p85 proteins to interact with a number of PTKs.

We first examined the interactions *in vivo* by co-expressing the p85 subunits together with PTKs in Sf9 cells using the baculovirus expression system. Both p85 subunits were found to be substrates for all PTKs examined, including members of the src-family ($pp60^{c\text{-}src}$, $p59^{c\text{-}fyn}$) and receptor PTKs of several subclasses (EGF receptor, Neu, CSF-1 receptor) (Gout *et al.* 1992). We also demonstrated that the p85 proteins and PTKs physically associated in tight complexes when co-expressed. After immunoprecipitation with antibodies against the receptors, the immune complexes were washed extensively and then subjected to *in vitro* kinase assays with radiolabelled ATP. Analysis by SDS-PAGE revealed phosphorylated protein bands corresponding to the receptors and the p85 proteins (Gout *et al.* 1992).

The ability of p85α and p85β to bind to the PDGF and EGF receptors, and to the polyoma middle-T/$pp60^{c\text{-}src}$ complex, was demonstrated using an *in vitro* binding assay (Kazlauskas & Cooper 1990; Otsu *et al.* 1991; Gout *et al.* 1992). This interaction was shown to require the prior phosphorylation of the receptors,

strongly arguing for the involvement of one or both of the SH2 domains of p85. An interesting difference was observed when similar experiments were performed using purified bovine brain PI 3-kinase complex instead of recombinant p85 proteins. While all receptors formed a tight complex with p85, the EGF receptor was unable to associate with the purified bovine PI 3-kinase complex. Neither enzymic activity nor a phosphorylated p85 band were observed (Otsu *et al.* 1991). In contrast, the PDGF receptor and the CSF-1 receptor were very efficient in that respect. These data suggest that when p85 is complexed with the 110 kDa protein, its specificity of binding via its SH2 domain(s) is altered towards specific receptors. This work has been extended by the use of specific phosphopeptide columns to which p85 and PI 3-kinase will bind. In these studies it was found that the purified PI 3-kinase complex would bind only to columns bearing peptides with a PTyr-X-X-Met motif, a sequence which has been demonstrated to be important for PI 3-kinase binding *in vivo*, whereas p85 proteins bound to all peptides tested as long as they contained a phosphorylated tyrosine residue (Fry *et al.* 1992). This result suggests that although the SH2 domains of p85 provide the basis for the interaction with tyrosine phosphorylated sequences the binding may be modified and the specificity enhanced by association with p110.

5. STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE SH2 AND SH3 DOMAINS OF p85

To understand better the role of p85 in the PI 3-kinase complex we have directed our attention towards defining minimal functional domains of this protein which could be studied independently, both structurally and functionally, from the whole molecule. From the studies described above it is clear that the SH2 domains are key regulatory domains in the PI 3-kinase complex. The SH2 domain was originally described as a module of approximately 100 amino acids which was present in the non-catalytic portion of the v-*fps* PTK oncogene and also in the prototype cytoplasmic PTK, pp60$^{c\text{-}src}$. This domain has since been found in many proteins possessing diverse activities and functions including PLCγ, rasGAP, v-*crk*, etc. (reviewed in Koch *et al.* 1991). Work from a number of laboratories has established that the function of these domains is to bind phosphotyrosine residues within a specific polypeptide context, thus mediating the formation of signalling complexes in response to activation of receptor PTKs (Anderson *et al.* 1990; Matsuda *et al.* 1990). We have carried out a number of structural and functional studies on the SH2 domains of p85 to try to address how these domains may operate within the context of the PI 3-kinase complex.

Although the borders of the SH2 domain had been defined by sequence alignment it was unclear whether further sequences might be required for proper folding and function. We therefore began these studies by defining a functional SH2 domain by limited proteolysis of recombinant p85α (Panayotou *et al.* 1992). A fragment of p85α containing the N-terminal SH2 domain (covering residues 301–439 as determined by N-terminal sequencing and mass analysis by electrospray mass spectroscopy), was obtained by digestion with V8 protease. This fragment was capable of high affinity binding to the 17 amino acid Y751 phosphopeptide column described earlier. Isolated p85α SH2 domains of similar size to this fragment have been demonstrated to be sufficient for binding to growth factor receptors (McGlade *et al.* 1992). We have also demonstrated that this N-terminal SH2 domain of p85 shows differential affinities for binding to phosphopeptides containing either Y740 or Y751 (G. Panayotou, unpublished observations). Circular dichroism and fluorescence spectroscopy techniques indicated that phosphopeptide binding to this SH2 domain-containing fragment resulted in a change in its shape (Panayotou *et al.* 1992). This is of interest following recent indications that PI 3-kinase activity can be activated simply by binding to phosphopeptides (Backer *et al.* 1992; M. J. Fry, unpublished observations).

The N-terminal SH2 domain of p85α was expressed in bacteria, purified and subjected to structural analysis by multidimensional nuclear magnetic resonance (NMR) spectroscopy. The structure derived from these studies is characterized by a central region of β-sheet flanked by two α-helices, with a highly flexible loop close to functionally important residues identified by site-directed mutagenesis (Booker *et al.* 1992). Similar structures for SH2 domains from c-*abl* by NMR (Overduin *et al.* 1992), and for v-*src* crystalized with tyrosine phosphorylated peptides (Waksman *et al.* 1992), have been obtained and have allowed the assignment of key residues involved in binding to the phosphotyrosine moiety. However, the phosphopeptides used in the latter study exhibited only low-affinity binding and further studies performed with high-affinity binding peptides will be required to determine other specific interactions which occur between the SH2 domain and the other residues in the binding site motif.

We have also examined the other well defined structural module of p85 proteins, the SH3 domain. This is a small module, approximately 60 amino acids in length, which was initially identified as a conserved sequence in the N-terminal non-catalytic region of pp60$^{c\text{-}src}$ (reviewed in Musacchio *et al.* 1992*a*). The function of this protein domain is unclear but its presence in a number of proteins that associate with membranes led initially to the suggestion that it might have a role in subcellular localization, possibly via interaction with cytoskeletal components (reviewed in Koch *et al.* 1991). However, recent preliminary findings suggest that this domain may function in the regulation of small G-proteins (reviewed in Pawson & Gish 1992).

Both a structural and functional approach to the analysis of this domain has been adopted. With respect to determining the function of SH3 domains, a protein which binds to the SH3 domain of c-*abl* has recently been described (Cicchetti *et al.* 1992). We

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

have taken a similar approach to identify proteins which bind to the SH3 domains of the p85 proteins. GST-SH3 fusion proteins were prepared and screened for their ability to specifically bind proteins from a bovine brain extract. Several promising candidate proteins were isolated by this method and they are currently being subjected to sequence analysis (I. Gout, unpublished observations). For a structural approach we again turned to NMR analysis of an isolated p85α SH3 domain expressed in bacteria. The analysis of this domain is not yet complete, however our preliminary data would suggest that the solution structure for this domain is essentially the same as the recently published crystal structure of the spectrin SH3 domain, with a few interesting minor differences (Musacchio *et al.* 1992*b*). The basic fold for this domain is a β-barrel made up of five antiparallel β-strands. New insights into SH3 domain structure and function are forthcoming and it is likely that the next couple of years will prove as enlightening for SH3 function as the previous two have for SH2 domains.

6. ISOLATION OF THE 110 kDa CATALYTIC SUBUNIT OF THE BOVINE PI 3-KINASE

Peptide sequence data generated from the 110 kDa protein was used to clone its cDNA from a pSV3neo-transfected bovine adrenal cortex zona fasciculata cell line cDNA library. A full-length cDNA clone was obtained by a combination of conventional hybridization screening together with 5′ RACE (rapid amplification of cDNA ends) -PCR to obtain sequences encoding the extreme N-terminus of the protein (Hiles *et al.* 1992). The largest clone isolated coded for a protein of 1068 amino acids in length with a calculated relative molecular mass of 124 247. When the p110 amino acid sequence was compared with sequences in the Swiss-prot and NBRF data bases only one significantly similar protein, vps34p, was identified (see figure 1*b*). This was a low abundance, 100 kDa protein of unknown activity from *Saccharomyces cerevisiae*, which had been isolated on the basis of its involvement in the sorting of proteins into the yeast vacuole (Herman & Emr 1990). p110 is 33% identical with vps34p over a stretch of approximately 450 amino acids that constitute the C-terminal two-thirds of both proteins. Allowing for conservative substitutions the degree of similarity rises to 55%. The possible significance of this observation is discussed in more detail later. Comparison of the p110 sequence with that of other kinases showed that several of the amino acids conserved within the active site are present in the C-terminal half of p110 (Hiles et al. 1992; see figure 1*c*). Equivalent residues can also be found in the related region of vps34p and suggest a common ancestry for both protein kinases and this family of lipid kinases.

To determine whether this cDNA encoded the p110 subunit and whether it was sufficient for PI 3-kinase activity it was expressed using the baculovirus system. Immunoprecipitation of Sf9 cells infected with the p110 virus, using a peptide antiserum raised against the predicted sequence of p110, revealed a novel protein of 110 kDa that co-migrated with the 110 kDa protein purified from bovine brain. This 110 kDa protein was absent from cells infected with wild-type virus. These immunoprecipitates containing the p110 protein were found to possess high levels of PI kinase activity, and HPLC analysis confirmed that this activity specifically catalysed the addition of phosphate to the 3 position of the inositol ring (Hiles *et al.* 1992). These results clearly demonstrated that the p110 subunit alone was sufficient for catalytic activity as had been suggested by Shibasaki *et al.* (1991). This p110 subunit was however unable to associate directly with activated receptors when co-expressed either *in vivo* or in an *in vitro* association assay. Co-expression with either p85 subunit resulted in the formation of an active heterodimeric complex which could be immunoprecipitated with antibodies specific for either component. Moreover, these complexes were able to bind to tyrosine phosphorylated receptors such as that for CSF-1 (Hiles *et al.* 1992).

7. ACTIVATION AND REGULATION OF PI 3-KINASE: A GENERATOR OF NOVEL SECOND MESSENGERS?

The composition and structure of the PI 3-kinase is now quite well understood, but relatively little is known about the mechanism of its activation following stimulation of cells by various factors. Clearly the SH2 domains play a role both in translocating the enzyme to a subcellular location in close proximity to membranes where its substrates are found, and also possibly in transmitting a conformational change following binding to phosphotyrosine-containing sequences which further activates this enzyme (Backer *et al.* 1992). Our preliminary observations suggest that this latter effect may be mediated by an increase in affinity for its substrate following phosphopeptide binding to the SH2 domains (M. J. Fry, unpublished observations). Tyrosine phosphorylation of components of the PI 3-kinase may also play a regulatory role, but to date there is no firm evidence to support this. Finally there is the possibility of an interaction with G-protein-mediated signalling pathways, either directly via the BCR-related domain (Fry 1992), or, in light of recent suggestions, indirectly via the SH3 domain (Pawson & Gish 1992). Clearly this is an area requiring further investigation.

A growing body of evidence suggests that the polyphosphoinositides have direct regulatory roles in addition to their well characterized role as precursors of the intracellular second messengers, diacylglycerol and inositol 1,4,5-trisphosphate. There is still some controversy as to which of the 3-phosphorylated lipids is the putative second messenger, but based upon steady state measurements $PI3,4,5P_3$ seems the most likely candidate. The other critical issue which remains unanswered is the nature of the target with which this putative second messenger might interact. A number of candidates have been suggested including components of the actin cytoskeleton and MAP kinases (see figure 2; reviewed in Downes & Carter 1991) but no firm target has been identified. Another possibility is that the generation of large amounts of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

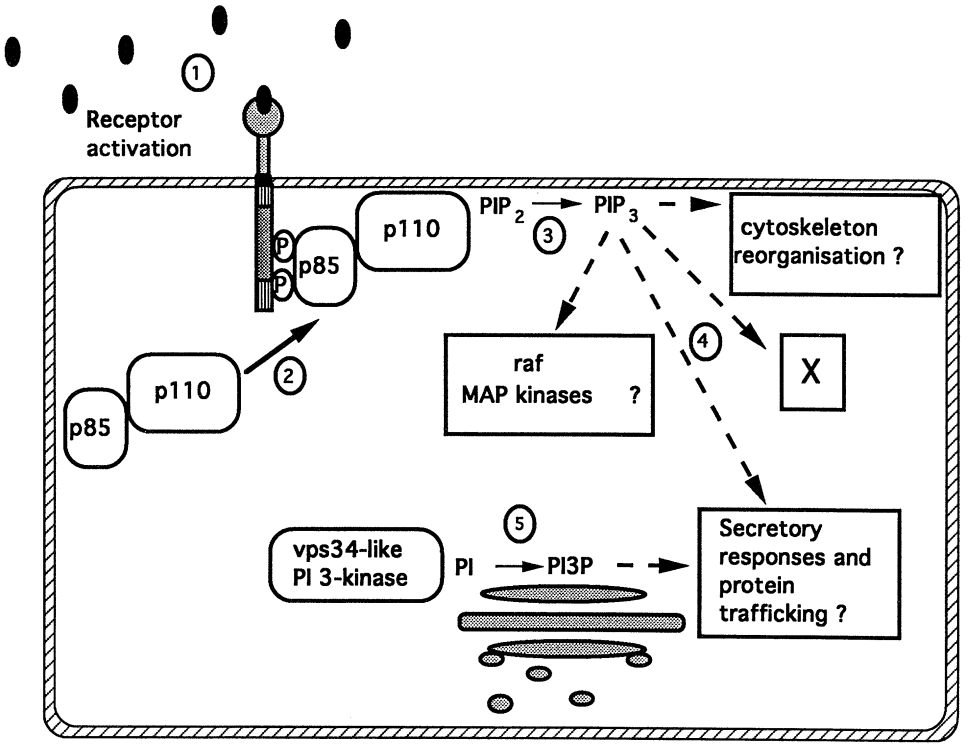

Figure 2. Possible functions of mammalian PI 3-kinases. Ligand-induced receptor activation (1) leads to recruitment of the PI 3-kinase complex from the cytosol (2). This results in the activation of PI 3-kinase and the production of 3-phosphoinositides (3). These lipids presumably interact with as yet unidentified target molecules (4). The current candidate pathways are indicated. The lower portion of the figure indicates that a distinct vps34-related PI 3-kinase may exist which is involved in secretory pathways and/or protein trafficking (5). Dashed lines indicate putative interactions whereas solid lines indicate known interactions or chemical conversions.

these highly phosphorylated lipids might result in physical changes in the membrane bilayer (Herman *et al.* 1992). However, this latter possibility seems less likely in light of the relative low abundance of PIP_3 even in stimulated cells, unless they turn out to be highly localized in specific organelles.

As described earlier, the cDNA for p110 shows significant sequence identity to a yeast protein, vps34p (Herman & Emr 1990). Mutations in the VPS34 gene cause defects in vacuole protein sorting in *S. cerevisiae* and extreme temperature-sensitive growth defects. We have recently demonstrated that vps34p possesses an intrinsic PI 3-kinase activity. PI 3-kinase activity is readily detectable in wild-type yeast strains, while strains deleted for VPS34, or containing VPS34 point mutations in the putative kinase domain, possess undetectable levels of PI 3-kinase activity and exhibit a severe vacuole protein sorting defect (Herman *et al.* 1992; Schu *et al.* 1993). This observation strengthens the earlier suggestion that the region of homology between p110 and vps34p defines a lipid kinase domain. Does this homology provide any clues to possible functions of the mammalian PI 3-kinase? It is probably too early to say whether this similarity will shed light on the role of PI 3-kinase in mammalian cells, but vps34p appears to have distinct properties from p110 in terms of its substrate specificity (M. J. Fry, unpublished observations). The major *in vivo* 3-phosphorylated lipid in *S. cerevisiae* appears to be PI3P, which is present at levels similar to PI4P (Auger *et al.* 1989; M. J. Fry, unpublished observations), rather than $PI3,4P_2$ or $PI3,4,5P_3$, which are thought to be the key signalling lipids in higher organisms. The suggested involvement in vacuole protein sorting would also seem to suggest a role distinct from typical mitogenic pathways in which the PI 3-kinase has been implicated. However, precedents exist for such a suggestion: mammalian PI 3-kinase is strongly activated by thrombin in platelets and by fMLP in neutrophils. Both of these cell types are terminally differentiated and unable to proliferate in response to mitogenic stimuli. However, common to the cellular responses illicited by thrombin and fMLP is the fusion of vesicular structures, actin re-organization and morphological changes. Similar responses are seen following growth factor stimulation of cells during a mitogenic response and it is tempting to speculate that PI 3-kinase might be involved in these processes. A second possibility exists that vps34p may define a novel PI 3-kinase family member which may have its own distinct homologue in mammalian cells (see figure 2). Experiments are in progress to see if such a cDNA can be isolated.

In conclusion, it is currently unclear whether the apparent relationship between p110 and vps34p will resolve the question of the function of the mammalian PI 3-kinase, but it seems likely that it will generate a better understanding of *S. cerevisiae* vacuole protein

sorting. The pieces in this puzzle are starting to fit together and the emerging picture would seem to suggest that PI 3-kinase interacts with receptors in many pathways and is a key regulatory switch in seemingly diverse physiological processes.

REFERENCES

Anderson, D., Koch, C.A., Grey, L., Ellis, C., Moran, M.F. & Pawson, T. 1990 Binding of SH2 domains of phospholipase Cγ1, GAP, and *src* to activated growth factor receptors. *Science, Wash.* **250**, 979–982.

Auger, K.R., Carpenter, C.L., Cantley, L. & Varticovski, L. 1989 Phosphatidylinositol 3-kinase and its novel product, phosphatidylinositol 3-phosphate, are present in *Saccharomyces cerivisiae J. biol. Chem.* **264**, 20181–20184.

Backer, J.M., Myers, Jr, M.G., Shoelson, S.E. *et al.* 1992 Phosphatidylinositol 3′-kinase is activated by association with IRS-1 during insulin stimulation. *EMBO J.* **11**, 3469–3479.

Booker, G.W., Breeze, A.L., Downing, A.K. *et al.* 1992 Structure of an SH2 domain of the p85α subunit of phosphatidylinositol-3-OH kinase. *Nature, Lond.* **358**, 684–687.

Cantley, L.C., Auger, K.R., Carpenter, C. *et al.* 1991 Oncogenes and signal transduction. *Cell* **64**, 281–302.

Carpenter, C.L., Duckworth, B.C., Auger, K.R., Cohen, B. Schaffhausen, B.S. & Cantley, L.C. 1990 Purification and characterization of phosphatidylinositol 3-kinase from rat liver. *J. biol. Chem.* **265**, 19704–19711.

Cicchetti, P., Mayer, B., Thiel, G. & Baltimore, D. 1992 Identification of a protein that binds to the SH3 region of *abl* is similar to Bcr and GAP-rho. *Science, Wash.* **257**, 803–806.

Courtneidge, S.A. & Heber, A. 1987 An 81 kd protein complexed with middle T antigen and pp60$^{c\text{-}src}$: a possible phosphatidylinositol kinase. *Cell* **50**, 1031–1037.

Downes, C.P. & Carter, A.N. 1991 Phosphoinositide 3-kinase: a new effector in signal transduction? *Cell. Signal.* **3**, 501–513.

Escobedo, J.A., Navankasattusas, S., Kavanaugh, W.M., Milfay, D., Fried, V.A. & Williams, L.T. 1991 cDNA cloning of a novel 85 kd protein that has SH2 domains and regulates binding of PI 3-kinase to the PDGF β-receptor. *Cell* **65**, 75–82.

Fantl, W.J., Escobedo, J.A., Martin, G.A. *et al.* 1992 Distinct phosphotyrosines on a growth factor receptor bind to specific molecules that mediate different signalling pathways. *Cell* **69**, 413–423.

Fry, M.J. 1992 Defining a new GAP family. *Curr. Biol.* **2**, 78–80.

Fry, M.J., Panayotou, G., Dhand, R. *et al.* 1992 Purification and characterization of a phosphatidylinositol 3-kinase complex from bovine brain by using phosphopeptide affinity columns. *Biochem. J.* **288**, 383–393.

Gout, I., Dhand, R., Panayotou, G. *et al.* 1992 Expression and characterisation of the p85 subunit of the PI 3-kinase complex and a related p85β protein using the baculovirus expression system. *Biochem. J.* **288**, 395–405.

Herman, P.K. & Emr, S.D. 1990 Characterization of VPS34, a gene required for vacuolar protein sorting and vacuole segregation in *Saccharomyces cerevisiae*. *Molec. cell. Biol.* **10**, 6742–6754.

Herman, P.K., Stack, J.H. & Emr, S.D. 1992 Essential role for a protein and lipid kinase complex in secretory protein sorting. *TICB.* **2**, 363–368.

Hiles, I.D., Otsu, M., Volinia, S. *et al.* 1992 Phosphatidylinositol 3-kinase: structure and expression of the 110 kd catalytic subunit. *Cell* **70**, 419–429.

Kaplan, D.R., Whitman, M., Schaffhausen, B. *et al.* 1987 Common elements in growth factor stimulation and oncogenic transformation: 85 kd phosphoprotein and phosphatidylinositol kinase activity. *Cell* **50**, 1021–1029.

Kazlauskas, A. & Cooper, J.A. 1990 Phosphorylation of the PDGF receptor β subunit creates a tight binding site for phosphatidylinositol 3-kinase. *EMBO J.* **9**, 3279–3286.

Koch, C.A., Anderson, D., Moran, M.F., Ellis, C. & Pawson, T. 1991 SH2 and SH3 domains: elements that control interactions of cytoplasmic signalling proteins. *Science, Wash.* **252**, 669–674.

Ling, L.E., Druker, B.J., Cantley, L.C. & Roberts, T.M. 1992 Transformation-defective mutants of polyomavirus Middle T antigen associate with phosphatidylinositol 3-kinase but are unable to maintain wild-type levels of PI 3-kinase products in intact cells. *J. Virol.* **66**, 1702–1708.

Lips, D.L., Majerus, P.W., Gorga, F.R., Young, A.T. & Benjamin, T.L. 1989 Phosphatidylinositol 3-phosphate is present in normal and transformed fibroblasts and is resisitant to hydrolysis by bovine brain phospholipase C II. *J. biol. Chem.* **264**, 8759–8763.

Matsuda, M., Mayer, B.J., Fukui, Y. & Hanafusa, H. 1990 Binding of transforming protein, P47$^{gag\text{-}crk}$, to a broad range of phosphotyrosine-containing proteins. *Science, Wash.* **248**, 1537–1539.

McGlade, C.J., Ellis, C., Reedjik, M. *et al.* 1992 SH2 domains of the p85α subunit of the phosphatidylinositol 3-kinase regulate binding to growth factor receptors. *Molec. cell. Biol.* **12**, 991–997.

Morgan, S.J., Smith, A.D. & Parker, P.J. 1990 Purification and characterization of bovine brain type 1 phosphatidylinositol 3-kinase. *Eur. J. Biochem.* **191**, 761–767.

Musacchio, A., Gibson, T., Lehto, V.-P. & Saraste, M. 1992*a* SH3 - an abundant protein domain in search of function. *FEBS Lett.* **307**, 55–61.

Musacchio, A., Noble, M., Pauptit, R., Wierenga, R. & Saraste, M. 1992*b* Crystal structure of a *src*-homology 3 (SH3) domain. *Nature, Lond.* **359**, 851–855.

Otsu, M., Hiles, I., Gout, I. *et al.* 1991 Characterization of two 85 kd proteins that associate with receptor tyrosine kinases, middle-T/pp60$^{c\text{-}src}$ complexes, and PI3-kinase. *Cell* **65**, 91–104.

Overduin, M., Rios, C.B., Mayer, B.J., Baltimore, D. & Cowburn, D. 1992 Three-dimensional solution structure of the src homology 2 domain of c-abl. *Cell* **70**, 697–704.

Panayotou, G., Bax, B., Gout, I. *et al.* 1992 Interaction of the p85 subunit of PI 3-kinase and its N-terminal SH2 domain with a PDGF receptor phosphorylation site: structural features and analysis of conformational changes. *EMBO J.* **11**, 4261–4272.

Pawson, T. & Gish, G.D. 1992 SH2 and SH3 domains: from structure to function. *Cell* **71**, 359–362.

Schu, P.V., Takegawa, K., Fry, M.J., Stack, J.H., Waterfield, M.D. & Emr, S.D. 1992 A yeast gene essential for protein sorting in the secretory pathway encodes a phosphatidylinositol 3-kinase. *Science, Wash.* **260**, 88–91.

Serunian, L.A., Haber, M.T., Fukui, T. *et al.* 1989 Polyphosphoinositides produced by phosphatidylinositol 3-kinase are poor substrates for phospholipases from rat liver and bovine brain. *J. biol. Chem.* **264**, 17809–17815.

Shibasaki, F., Homma, Y. & Takenawa, T. 1991 Two types of phosphatidylinositol 3-kinase from bovine thymus. *J. biol. Chem.* **266**, 8108–8114.

Skolnik, E.Y., Margolis, B., Mohammadi, M. *et al.* 1991 Cloning of PI3-kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. *Cell* **65**, 83–90.

Waksman, G., Kominos, D., Robertson, S.C. *et al.* 1992 Crystal structure of the phosphotyrosine recognition domain SH2 of v-src complexed with tyrosine-phosphorylated peptides. *Nature, Lond.* **358**, 646–653.

Whitman, M., Kaplan, D.R., Schaffhausen, B., Cantley, L. & Roberts, T.M. 1985 Association of phosphatidylinositol kinase activity with polyoma middle-T component for transformation. *Nature, Lond.* **315**, 239–242.

Whitman, M., Downes, C.P., Keeler, M., Keller, T. & Cantley, L. 1988 Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol 3-phosphate. *Nature, Lond.* **332**, 644–646.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES