The Role of Phosphoinositide 3-Kinase Lipid Products in Cell Function*

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Recent advances in the field have been achieved by the development of new techniques to probe for the direct targets of PI 3-K lipid products. The chemical synthesis of short chain fatty acid versions of these lipids (3–5) has been a crucial step in determining the specificity of lipid-binding proteins. Additionally, new cloning strategies have been developed to isolate new lipid-binding proteins (6). Here we will review the most recent advances in our understanding of the role of PI 3-K in cell function by dissecting the contribution of each of its lipid products.

PtdIns-3-P

Regulation—PtdIns-3-P is constitutively present in both mammalian and yeast cells (7, 8). It can be produced in vitro via phosphorylation of PtdIns by Class I, II, or III PI 3-Ks (Fig. 1). However, the majority of PtdIns-3-P in mammalian cells is probably produced by Class III PI 3-K (9). The mammalian Class III enzyme is highly related to the yeast Vps34 gene product (10), and, like the yeast enzyme, is specific for PtdIns and will not phosphorylate PtdIns-4-P or PtdIns-4,5-P2 (11).

Targets—PtdIns-3-P was recently shown to specifically interact with a 70-residue protein module called the FYVE finger domain. This domain is a special type of RING zinc finger that is characterized by two zinc-binding sites and a highly conserved stretch of basic residues surrounding the third zinc-coordinating cysteine. Liposomes containing PtdIns-3-P were shown to associate with several FYVE domains (15–17). Other phosphoinositides bound poorly to the FYVE domains investigated, showing that this interaction is specific for PtdIns-3-P. Proper folding of the domain is important for its function because mutation in one of the zinc-coordinating cysteines or removal of zinc with EDTA or TPEN reduced PtdIns-3-P binding (16, 17). Moreover, mutations in the basic motif also eliminated binding (16). The interaction between FYVE domains and PtdIns-3-P is presumed to occur in vivo, because localization of the FYVE-containing protein EEA1 on early endosomes depends on an intact FYVE domain (17, 18) and on PI 3-K activity (based on Wortmannin effects in mammalian cells) (19). When overexpressed in cells, the FYVE domain of EEA1 is sufficient to determine subcellular localization (16). Simonson and colleagues (20) have shown that, in addition to PtdIns-3-P, the EEA1 protein associates with GTP-bound Rab5 through separate domains, and interactions with both PtdIns-3-P and GTP-Rab5 are necessary for the stable association of EEA1 with membranes in vivo.

Cellular Functions—Mutations in the yeast Class III PI 3-K, VPS34, cause mis-sorting of vacular proteins, changes in vacuole morphology, and defects in the endocytic pathway (reviewed in Ref. 10). In mammalian cells, inhibition of PI 3-K by the drug Wortmannin blocks transport of proteins from the Golgi to the lysosome, inhibits early endosome trafficking, and causes the accumulation of prelysosomal vesicles (22, 23). Mutations in the PDGF receptor that disrupt its association with Class I PI 3-Ks interfere with trafficking of this receptor to the lysosome and its subsequent degradation (12).

Regulation—PtdIns-3,4-P2 levels can be regulated by extracellular signals. PDGF stimulation of quiescent fibroblasts as well as fMLP peptide stimulation of neutrophils result in rapid PtdIns-3,4-P2 synthesis (7, 13, 25). Stephens and collaborators (25) have proposed that the elevation in PtdIns-3,4-P2 levels in these cells is caused by the dephosphorylation of PtdIns-3,4,5-P3, as opposed to the phosphorylation of PtdIns-4-P or PtdIns-3-P. Recent studies have indicated that, in platelets, PtdIns-3,4-P2 can also be synthesized by phosphorylation of the 4-position of PtdIns-3-P by an unidentified PtdIns-3-P 4-kinase (26, 27). Although the PtdIns-5-P 4-kinase α (also called Type II PtdIns-P kinase) can catalyze this reaction in vitro, it is unlikely that this enzyme is responsible for the elevation of PtdIns-3,4-P2 levels in vivo because PtdIns-3-P is a poor substrate for this enzyme when compared with PtdIns-5-P (28).

The class II PI 3-Ks can phosphorylate PtdIns-4-P to generate PtdIns-3,4-P2, independent of PtdIns-3,4,5-P3 synthesis (Fig. 1). The contribution of this pathway to the intracellular levels of PtdIns-3,4-P2 is unknown.

In summary, it is clear that mammalian cells have evolved a variety of mechanisms for independently controlling the levels of PtdIns-3,4-P2 and PtdIns-3,4,5-P3.

Targets—The serine/threonine protein kinase B (PKB), also known as Akt, is the most well characterized target of PtdIns-3,4-P2. The PH domain of Akt has been shown to bind phosphoinositides in vitro with the order of preference being PtdIns-3,4-P2 >> PtdIns-3,4,5-P3 >> PtdIns-4,5-P2 (30, 31). PtdIns-3,4,5-P2 binding to Akt causes a 3–5-fold stimulation of its activity in vitro (30–32). In thrombin-stimulated platelets, Akt activation correlates with PtdIns-3,4-P2 production rather than PtdIns-3,4,5-P3 production (30).
PI-3,4,5-P₃

Regulation—The majority of the PI-3,4,5-P₃ synthesized in response to extracellular signals is most likely generated by phosphorylation of PtdIns-4,5-P₂ at the 3-position of the inositol ring (25). The Class I PI 3-Ks are the only enzymes that can use PtdIns-4,5-P₂ as a substrate to synthesize PtdIns-3,4,5-P₃ (Fig. 1). Activation of class IA PI 3-K by growth factor stimulation of cells is mediated in part by interaction of their SH2 domain with tyrosine-phosphorylated proteins and results in a rapid elevation of PtdIns-3,4,5-P₃ levels (reviewed by Ref. 2). Class IB PI 3-Ks can also be regulated by the GDP-bound form of the small G protein Ras (59). The Class IB PI 3-K can be directly activated by the βγ subunits of heterotrimeric G proteins (reviewed by Ref. 58). In addition, one of the Class IA enzymes (p110α) can be activated synergistically by phosphotyrosine peptides plus βγ subunits (60).

Recently, a PtdIns-4,5-P₂-independent pathway for PtdIns-3,4,5-P₃ synthesis was described. PtdIns-5-kinases α and β have been shown to utilize PtdIns-3-P as a substrate to produce PtdIns-3,4,5-P₃ by phosphorylating the α- and γ-positions of the inositol ring in a concerted reaction (54, 61). The contribution of this new pathway to intracellular levels of PtdIns-3,4,5-P₃ is still unknown. Several PtdIns-3,4,5-P₃ phosphatases have now been isolated. Of special interest is the PtdIns-3,4,5-P₃ tumor suppressor protein (see below), which can dephosphorylate PtdIns-3,4,5-P₃ at the 3-position (62), and SHIP (discussed above), which can dephosphorylate the 5-position (63). Little is known about the in vivo metabolism of this lipid. However, it is resistant to hydrolysis by phospholipase C, types β, γ, and δ (64).

Targets—In addition to Akt, PDK1, and PKCe (discussed above), many targets for PtdIns-3,4,5-P₃ have now been described. Several of these proteins have PH domains that mediate binding (for a review, see Ref. 65). Many PtdIns-3,4,5-P₃-binding PH domains can also bind to PtdIns-4,5-P₃, and only those that have at least a 10-fold higher affinity for PtdIns-3,4,5-P₃ than for PtdIns-4,5-P₃ will be considered here.

The PH domain of the Bruton’s tyrosine kinase (Btk) was shown to interact with PtdIns-3,4,5-P₃ and its head group, inositol 1,3,4,5-P₄, with high affinity (66–68). Substituting cysteine for arginine 28 (R28C) in the PH domain of Btk, a natural mutation that causes X-linked immunodeficiency in mice, significantly affects the binding of PtdIns-3,4,5-P₃ and inositol 1,3,4,5-P₄. In vitro, overexpression of the Class I PI 3-K enzyme p110α (a constitutively active form of PI 3-K) or Class IA PI 3-K by a 5-K (presumably the Class III enzyme) and of PtdIns-3-P by a PtdIns-3-P 5-kinase (53). In vitro, this second reaction can be catalyzed by the PtdIns-5-kinase α and β (also known as type I PtdIns-5-kinases) (54). Likewise, PtdIns-3,5-P₃ synthesis in yeast involves phosphorylation of PtdIns-3-P by a 5-kinase and requires Vps34p PI 3-K (55). Fab1, a gene that is highly homologous to the mammalian PtdIns-5-kinase, was recently identified as the yeast PtdIns-3-P 5-kinase (56). Dramatic increases in PtdIns-3,5-P₃ levels were observed in response to hyperosmotic shock of yeast cells. In mammalian cells, the levels of PtdIns-3,5-P₃ decrease moderately with hyperosmotic shock and increase with hypo-osmotic shock. In vitro, PtdIns-3,5-P₃ can also be generated through phosphorylation of the novel lipid PtdIns-5-P by the Class IA PI 3-K (28).

Targets—PtdIns-3,5-P₃ is a newly identified molecule, and no direct target for this lipid has been found. PH domain-containing proteins are likely candidates for PtdIns-3,5-P₃ downstream effectors. Previous studies of lipid binding specificity of PH domains did not investigate this lipid.

Cellular Functions—Because mutations in the yeast Fab1 cause enlargement of the vacuole (57), PtdIns-3,5-P₃ may be involved in vesicle trafficking (reviewed by Emr and colleagues (94)).
Grp1 exchange activity toward myristoylated Arf1 can be enhanced 
in vitro by PtdIns-3,4,5-P3-containing micelles, suggesting that 
PtdIns-3,4,5-P3 regulates Grp1 by recruiting it to membranes 
where Arf is localized (73).

The presence of PH domains in a wide range of guanine nucleotide 
exchange factors for small G proteins (74) suggests that phosphoi-
notid side regulation of these proteins may be widespread. PDGF-
诱导 binding of GTP to the small G protein Rac depends on PI 3-K 
activation (75). This result suggests that PI 3-K lipids may directly 
affection the exchange factors for Rac. Indeed, PtdIns-3,4,5-P3 and Ptd-
Ins-4,5-P2 were bound to the nucleotide exchange factor Vav 
and stimulate its exchange activity toward Rac, Cdc42, and RhoA 
(76). Interestingly, PtdIns-4,5-P2 was also able to bind to Vav, but 
in this case, the exchange activity of Vav was inhibited by this lipid. 
Because water-soluble (short chain fatty acid) lipids were used in 
these experiments, it was suggested that PtdIns-3,4,5-P3 binds to the 
PH domain of Vav and allosterically activates it.

The SH2 domains of Src and p85 (the PI 3-K Class Iα regulatory 
subunit) can bind PtdIns-3,4,5-P3 in competition with phosphoty-
rosine-containing proteins (72). More recently, PtdIns-3,4,5-P3 was 
shown to bind to the SH2 domains of PLCγ and to enhance its 
phospholipase activity toward PtdIns-4,5-P2 and Ptd-
Ins-3-P to activate PLCγ (77, 78). Inhibition of PI 3-K activity (by wortmannin treatment, mutation of PI 
3-K-binding sites in the PDGF receptor, or overexpression of domi-
nant-negative enzyme) partially inhibits PDGF-dependent inositol-
1,4,5-P3 production in intact cells, implicating PtdIns-3,4,5-P3 as a 
positive regulator of PLCγ in vivo. Another report showed that the 
PLCγ PH domain also binds PtdIns-3,4,5-P3 and mediates PLCγ 
translocation to the cell membrane in response to growth factors (79).

Cellular Functions—With the identification of several PtdIns-
3,4,5-P3 targets, many of the cellular functions attributed to PI 3-Ks 
can now be understood at the molecular level. Elevation in cytosolic 
calcium in response to B cell stimulation appears to be modulated by 
PI 3-K, based on studies with PI 3-K inhibitors. This result can be 
explained by PtdIns-3,4,5-P3-dependent activation of Btk and per-
haps also by direct effects of PtdIns-3,4,5-P3 in recruitment of PLCγ 
to the membrane (69, 70, 77, 79).

A role for PI 3-K in vesicle recruitment to the plasma membrane 
has been proposed based on the observation that wortmannin and 
dominant-negative PI 3-K block GLUT 4 translocation to the plasma 
membrane in response to insulin (80, 81). The observation that Ptd-
Ins-3,4,5-P3 mediates recruitment of Grp1 to membranes and en-
forces Grp1 nucleotide exchange activity toward Arf1 provides an 
explanation for how PtdIns-3,4,5-P3 may regulate coating and bud-
ing of intracellular vesicles (6, 73). As discussed above, a role for 
PtdIns-3-P and FYVE domain proteins in vesicle fusion is also likely.

PI 3-K recruitment and activation is also necessary for PDGF-
duced chemotaxis and membrane ruffling (82, 83). PtdIns- 
3,4,5-P3 activation of Vav2 (or other Rac exchange factors) (76) and 
consequently binding of Rac to GTP may explain the mechanism 
by which PI 3-K is involved in growth factor and Ras-stimulated 
cytoskeleton rearrangements that lead to cell migration.

Several studies support the idea that PI 3-K is necessary for 
growth factor and oncogene-induced cell proliferation. Recently, a 
natural oncogenic form of PI 3-K, v-p3k, was isolated from a 
chicken retrovirus that causes hemangiosarcomas, ASV16 (84). 
Expression of v-p3k protein as well as its cellular counterpart, 
the chicken p110α PI 3-K, causes elevation in PtdIns-3,4-P2 and Ptd-
Ins-3,4,5-P3 levels, activation of Akt, and transformation of chicken 
embryo fibroblasts. Another oncogenic form of PI 3-K that consists 
of a truncated version of p85 (p85e) associated with the p110 cata-
ytic subunit has been isolated from transformed lymphoid cells 
(85). In cells expressing this constitutively active PI 3-K, Akt is also 
up-regulated. Strong evidence indicating that PtdIns-3,4,5-P3 is 
inolved in cell proliferation came with the recent finding that the 
tumor suppressor protein, PTEN, is a 3-phosphatase that dephospho-
phytlates PtdIns-3,4,5-P3 (62). The PTEN gene is deleted or mu-
tated in a wide variety of human cancers, and it is capable of 
suppressing the growth of glioma cells (86–88).

The gene encoding the mouse PI 3-K adapter subunit, p85α, has 
now been disrupted by two independent groups (89, 90). Defects in 
B cell development and proliferation were observed in both studies. 
This phenotype resembles the phenotype of Btk-deficient mice. 
These data support the hypothesis that PtdIns-3,4,5-P3 activation of 
Btk is likely to mediate B cell functions in animals (89, 90).

PI 3-K as a Protein Kinase

PI 3-K is a dual specificity kinase that can phosphorylate serine 
and threonine residues in addition to phosphoinositide lipids (91, 
92). p110α can phosphorylate itself, the associated p85 regulatory 
subunit, and the insulin receptor substrate (IRS1) (reviewed in Ref. 
58). Because phosphorylation of p85 by PI 3-K decreases the lipase 
kinase activity of the complex, it was proposed that the protein 
kinase intrinsic to PI 3-K has a regulatory function. The possibility 
that the protein kinase activity of PI 3-K plays a role in signaling 
is suggested by a recent study. Bondeva et al. (88) have now 
demonstrated MAPK activation by a PI 3-K hybrid protein that 
has protein kinase activity but lacks lipid kinase activity. More-
over, membrane-bound PI 3-K was unable to stimulate MAPK 
activation, indicating that the substrate for PI 3-K protein kinase 
is not at the cell membrane. On the other hand, this enzyme failed 
to stimulate Akt consistent with Akt activation being dependent on 
PtdIns-3,4-P2 and/or PtdIns-3,4,5-P3 synthesis (93). These results 
show that PI 3-K-mediated signaling involves independent path-
ways that lead to MAPK activation or Akt activation. Activation of 
the MAPK pathway may be an additional mechanism by which PI 
3-K mediates the transduction of proliferation signals.

Concluding Remarks

Given that PI 3-K is involved in so many different cellular 
responses to a variety of different signals and that several different 
proteins are direct targets for 3-phosphorylated phosphoinositides 
(Fig. 2), an important question is: how is specificity in downstream 
signaling maintained?

One level of specificity may be obtained by synthesis of different 
lipid products by different PI 3-K isoforms. A second level of spec-
ificity can be obtained by recruitment of PI 3-K to specific subcel-
ular compartments and a consequent increase in local production 
of lipids where a specific target may be available (for example, 
EEA1 recruitment by PtdIns-3-P and Rab5). Finally, it is possible 
that the specificity of the response is determined by convergence of 
two parallel pathways triggered by a specific signal (for example, 
Btk activation by PtdIns-3,4-P2 and Lyn).

As other targets for PI 3-K lipids are unveiled, the job of untan-
gling the intricate network of PI 3-K signaling will continue.

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