

# SYNTHESIS OF AND SIGNALING THROUGH D-3 PHOSPHOINOSITIDES

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## SUBSTRATES FOR THE SYNTHESIS OF D-3 PHOSPHOINOSITIDES 495

### THE PI 3-KINASE FAMILY 496

Class I PI 3-Kinases 496  
Class II PI 3-Kinases 499  
Class III PI 3-Kinases 499

### PI 3-KINASE INHIBITORS 500

## ALTERNATE PATHWAYS FOR GENERATING D-3 POLYPHOSPHOINOSITIDES 500

Structure of PIP Kinase Family 501  
Activity and Regulation of PIP Kinases 501  
Phosphatases 501

## LIPID PRODUCTS AS MEDIATORS OF PI 3-KINASE DOWNSTREAM SIGNALING 502

FYVE Domains 502  
Pleckstrin Homology Domains 503  
Other Targets for D-3 Phosphoinositides 504

Inositol-containing lipids were recognized as early as 1930 as components of biological membranes in mycobacteria, plants, and mammalian cells (reviewed in ref. 1). Over the next 20 years, the structure of these lipids as phosphorylated derivatives of phosphatidylinositol (PI) was elucidated (2). PI is the simplest component of this family and has an inositol ring attached by its D-1-OH group to phosphatidic acid. In cells, the free hydroxyl groups of inositol ring, except those at the D-2 and D-6 positions, can be phosphorylated in different combinations, which gives each carbon atom its unique identity and creates the diversity among the inositol-containing phospholipids. Eight different PI species have been identified in eukaryotic cells to date. The function of these lipids began to be clarified in the early 1950s. However, their participation in intracellular signal transduction was not recognized until the early 1980s, and the nature of the protein–phospholipid interactions has only been appreciated in the last few years. In this chapter, we focus on the enzymes that generate polyphosphoinositides phosphorylated in the D-3 position and their role in signal transduction.

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Regarding the liver, PI 3-kinase has been increasingly demonstrated to play a critical role in apoptosis (Chapter 34), differentiation (Chapter 2), regeneration (Chapter 42 and website chapter [□ W-3](#)), malignant transformation (Chapter 68), intracellular trafficking (Chapter 24), and membrane transport (Chapter 10). PI 3-kinase participates in these and other functions in hepatocytes (Chapter 36), endothelial cells (Chapter 30), Kupffer cells (website chapter [□ W-26](#)), stellate cells (Chapter 31), and biliary epithelial cells (Chapter 29). These rapid developments are remarkable because, until recent years, PI 3-kinase had been associated only with activity of tyrosine kinase growth factors and oncogenes. In addition, the mechanisms whereby the 3' phosphoinositide products of the enzyme affect these processes were unknown.

## SUBSTRATES FOR THE SYNTHESIS OF D-3 PHOSPHOINOSITIDES

Phospholipids constitute approximately 3% of the total cell weight of mammalian cells (3). The inositol-containing phospholipids represent less than 20% of total cellular phospholipids (reviewed in ref. 4). The majority (up to 80%) of the inositol-containing lipids are present as PI (132). PI 4-phosphate (PI 4-P) and PI 4,5-bisphosphate (PI 4,5-P<sub>2</sub>) share the remaining fraction of the inositol-containing lipids in nonstimulated mammalian cells. The 3' phosphorylated polyphosphoinositide, PI 3-P, constitutes a

small fraction (less than 0.25%) of total inositol-containing phospholipids, and even smaller fractions are detected as PI 3,4-P<sub>2</sub> and PI 3,4,5-P<sub>3</sub> in transformed or growth factor-stimulated cells. These polyphosphoinositides are not substrates for the PI-specific phospholipases C (PLC) (6,7), enzymes that cleave inositol phospholipids into membrane-bound diacylglycerol and soluble inositol phosphates. Thus, this separates PI 3-kinase signaling from the PLC/PI 4,5-P<sub>2</sub> pathway, a pathway that leads to Ca<sup>2+</sup> release and activation of protein kinase C (PKC). Instead, phosphoinositides phosphorylated in the D-3 position are substrates for lipid kinases and phosphatases that act on the inositol ring.

## THE PI 3-KINASE FAMILY

PI 3-kinase was initially recognized as a novel enzymatic activity associated with the virally encoded protein-tyrosine kinases, *v-src*, and *v-ros* (8,9). Subsequently, this activity was found in association with other receptor and nonreceptor protein-tyrosine kinases, such as platelet-derived growth factor receptor (PDGFR) and the polyoma middle T/pp60<sup>c-src</sup> complex (10–15). This PI kinase activity was shown to be different from the previously characterized PI 4-kinase based on its sensitivity to detergents and adenosine (16,17). Further studies identified a distinct activity that phospho-

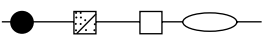
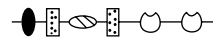
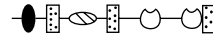
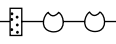
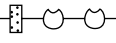
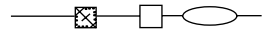

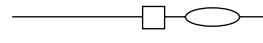
rylated the D-3 position of the inositol ring of purified PI, PI 4-P and PI 4,5-P<sub>2</sub> (18). This discovery led to the purification of PI 3-kinase from rat liver and brain (19,20). Nine mammalian genes have been cloned to date, and their protein products are part of the PI 3-kinase family that has been grouped into three classes (I–III) (21) based on their structure, *in vitro* substrate specificity and probable mechanism of regulation (Table 35.1).

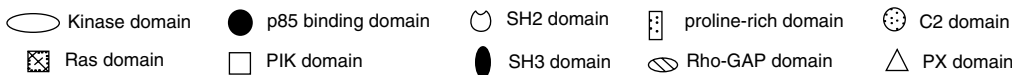
## Class I PI 3-Kinases

### Structure of Class I PI 3-Kinases

Class I PI 3-kinases are heterodimeric complexes that consist of a catalytic subunit and an adaptor/regulatory subunit. Class I enzymes are further subdivided into Class I<sub>A</sub> PI 3-kinases and Class I<sub>B</sub> PI 3-kinases, based on association with different regulatory subunits (Table 35.1). The mammalian Class I<sub>A</sub> catalytic subunits include three isoforms: p110 $\alpha$ , p110 $\beta$ , and p110 $\delta$ . The catalytic subunits, p110 $\alpha$  and p110 $\beta$  are found in most mammalian tissues, whereas p110 $\delta$  is expressed in leukocytes (22). There are five adaptor/regulatory subunits for mammalian Class I<sub>A</sub> PI 3-kinases. These arise from three different genes: p85 $\alpha$ , p85 $\beta$ , and p55PIK/p55 $\gamma$  with two additional protein products (p55 $\alpha$  and p50 $\alpha$ ) that result from the alternate splicing of p85 $\alpha$  (reviewed in ref. 23). p85 $\alpha$  is ubiquitously distrib-

**TABLE 35.1. CLASSIFICATION OF THE MAMMALIAN MEMBERS OF THE PI 3-KINASE FAMILY: STRUCTURAL AND DOMAIN ORGANIZATION OF THE SUBUNITS, LIPID SUBSTRATES OF THE ENZYMES, AND MECHANISMS OF REGULATION**

Enzyme	Subunits		Structural Features	Lipid Substrates			Regulation	
	Catalytic	Adaptor		<i>in vitro</i>	<i>in vivo</i>			
Class I A	p110 $\alpha$ p110 $\beta$ p110 $\delta$			p110 $\alpha, \beta, \delta$	PI PI 4-P PI 4,5-P <sub>2</sub>	PI 4,5-P <sub>2</sub>	p85 Ras Heterotrimeric G-proteins	
		p85 $\alpha$		p85 $\alpha$				
		p85 $\beta$		p85 $\beta$				Tyrosine Kinases SH3 domains PIP <sub>3</sub>
		p55 $\alpha, \gamma$		p55 $\alpha, \gamma$				
		p50 $\alpha$		p50 $\alpha$				
Class I B	p110 $\gamma$	p101		p110 $\gamma$			Ras Heterotrimeric G-proteins	
					p101			
Class II	PI3K-C2 $\alpha$ PI3K-C2 $\beta$ PI3K-C2 $\gamma$			PI3K-C2	PI PI 4-P PI 4,5-P <sub>2</sub>	?	Unknown ? Tyrosine Kinases or Heterotrimeric G-proteins. Not Ras	
Class III	Vp534p homologue (PI 3-K III)	Vp515p homologue (p150)		PI 3-K III Vp534p				
					p150 Vp515p	PI	PI	Constitutive



uted, whereas p85 $\beta$  is expressed primarily in bovine brain and lymphoid tissues (24). Although there are no reports of preferential interactions between the regulatory and catalytic subunits, differential tissue distribution may be responsible for some differences in function.

The p110 subunits contain a C-terminal catalytic domain that is also found in related protein and lipid kinases, a PIK domain that is found in all lipid kinases to which no known function has been assigned, and a region for association with p21<sup>ras</sup> (25). The Class I<sub>A</sub> PI 3-kinases also have an N-terminal region that is required for interaction with the regulatory subunits. In addition to lipid kinase activity, the catalytic subunit has an intrinsic serine/threonine (Ser/Thr) protein kinase activity and phosphorylates the inter-SH2 domain of p85 (19,26), and autophosphorylates p110 $\delta$  (27), which results in downregulation of the lipid kinase activity. The p110 $\delta$  PI 3-kinase has one proline-rich region and a leucine-zipper-like domain whose functions have not been fully characterized (27).

The p85 $\alpha$  and  $\beta$  regulatory subunits contain two *src* homology-2 (SH2) domains and one N-terminal *src* homology-3 (SH3) domain. The region between the two SH2 domains (inter-SH2 domain) interacts with the Class I<sub>A</sub> catalytic subunits (26,28). Two proline rich regions (P1 and P2) of p85 associate with SH3 domains of *c-abl*, p56<sup>lck</sup>, p59<sup>fyn</sup>, and p60<sup>v-src</sup>. The SH3 domain of the p85 subunit can also self-associate through the proline-rich regions and bind to proline-rich regions in other proteins (29). p85 also has a domain which is homologous to the C-terminal region of the breakpoint cluster region gene product (BH domain). The BH domain of p85 contains a GTPase-activating protein domain (GAP domain) which provides binding sites for the Rho family proteins, Cdc42 and Rac1, although p85 itself does not have GTPase activity (30).

Alternative splicing of p85 $\alpha$  mRNA gives rise to proteins that migrate at 50 and 55 kd in sodium dodecyl sulfate polyacrylamide gels (31,32). These proteins contain only the second proline-rich region and lack the SH3 and GAP domains. The N-terminal region of p55 $\alpha$  contains a 34-amino-acid (aa) fragment which is not found in p85. The other splice form, p50 $\alpha$ , contains a 6-aa extension (32). The third gene, p55PIK/p55 $\gamma$ , encodes for a protein with similar overall structure to p55 $\alpha$  (33). Thus, there is a multitude of mechanisms by which the regulatory subunits could modulate the constitutive and signal-mediated interactions of PI 3-kinase with other intracellular proteins.

The only Class I<sub>B</sub> PI 3-kinase identified to date is p110 $\gamma$ , which is found only in mammals and is expressed highly in white blood cells (34). The structure of p110 $\gamma$  is similar to other Class I enzymes except for the lack of the N-terminal p85-binding sequence. The p110 $\gamma$  isoform binds to a 101-kd regulatory protein which has no sequence homology to any other known protein (35). Although the binding sites have not been mapped, p101 is required for activation of p110 $\gamma$  by heterotrimeric G proteins (35–37).

### Activity and Regulation of Class I<sub>A</sub> PI 3-Kinases

The *in vitro* substrates for all Class I PI 3-kinases include PI, PI 4-P, and PI 4,5-P<sub>2</sub>, whereas their preferred substrate *in vivo* is PI 4,5-P<sub>2</sub> (38). The delayed generation of PI 3,4-P<sub>2</sub> in response to some growth factors is likely due to dephosphorylation of PI 3,4,5-P<sub>3</sub> by a 5'-inositol phosphatase rather than a direct effect of the Class I PI 3-kinases (39,40). PI 3,4-P<sub>2</sub> or PI 3,4,5-P<sub>3</sub> are not detectable in quiescent cells, and transient accumulation of these products is only observed in response to growth factors (18,41). Class I<sub>A</sub> PI 3-kinases can also phosphorylate PI 5-P *in vitro* (38), although there is no evidence for accumulation of PI 3,5-P<sub>2</sub> in response to Class I<sub>A</sub> PI 3-kinase agonists (reviewed in ref. 42).

Activation of Class I<sub>A</sub> PI 3-kinases is tightly controlled by growth factor receptors that have intrinsic protein tyrosine kinase activity, as well as by receptors coupled to *src*-like protein tyrosine kinases, p21<sup>ras</sup> or heterotrimeric G proteins. Receptor-mediated PI 3-kinase activation, which have been well characterized, include: platelet-derived growth factor (PDGF), insulin, insulin-like growth factor 1 (IGF-1) (see Chapter 36), nerve growth factor (NGF), stem cell growth factor (SCF), epidermal growth factor (EGF), and hepatocyte growth factor (HGF) (see Chapter 43) (reviewed in refs. 23,42). Translocation of PI 3-kinase to the plasma membrane brings the catalytic subunit in proximity to its substrates, which is required for accumulation of PI 3-kinase lipid products in adherent cells (43,44), but not in hematopoietic cells (44). Activation of PI 3-kinase based on translocation to the plasma membrane has been verified by myristoylated or C-terminal isoprenylated PI 3-kinase mutants (45). The adaptor subunits are recruited to the membrane by an interaction of their SH2 domains with specific tyrosine-phosphorylated motifs, pTyr-X1-X2-Met (YXXM) on the intracellular domain of the receptor or other adaptor proteins (46). The mechanism whereby p85 increases the lipid kinase activity in the catalytic subunit is still unclear. However, association of phosphotyrosine-containing synthetic peptides with the SH2 domain of p85 increases the catalytic activity of the p110 subunit *in vitro* (47,48). The downregulation of PI 3-kinase activity could be mediated by the highly phosphorylated product, PI 3,4,5-P<sub>3</sub>, which competes with pYXXM motifs for p85 SH2 docking sites and could result in dissociation of PI 3-kinase from the plasma membrane (49). The versatility of the regulatory subunits to interact with multiple signaling molecules and the ability to form direct complexes with the receptors allows tight regulation of intracellular PI 3-kinase.

PI 3-kinase may also be recruited by interaction with the p85 SH3 domain. Two proline-rich consensus motifs with higher affinity for the p85 SH3 domain have been identified, RXLPPRPXX and XXXPPXPXX, where X could be any amino acid except cysteine (50). A constitutive interaction of the p85 SH3 domain with widely expressed adaptor proteins, such as *c-cbl*, *in vitro* and *in vivo* has been identi-

fied (51,52). The SH3 domain of p85, expressed as a GST-fusion protein, binds to the microtubule-associated protein, dynamin (53) (see Chapter 10), and this interaction occurs *in vivo* (231).

The p85 $\alpha$  SH3 domain also binds to each of the proline-rich regions, P1 and P2, within p85 itself (29). The P1 and P2 domains of p85 can also associate with SH3 domains of Src family kinase members, *src*, *lck*, *lyn*, *fyn*, and *abl* (29,54–57). The binding of p85 to *lyn* and *fyn* increases the lipid kinase activity (58). The N-terminal proline-rich region, preferentially binds to the SH3 domain of *c-src* (58). The p50 $\alpha$ , p55 $\alpha$ , and p55 $\gamma$  subunits have only one proline-rich motif, and lack the N-terminal proline-rich region that preferentially binds *c-src*. The p85 $\beta$  possesses a distinct C-terminal proline-rich motif. Thus, a different subset of SH3-containing proteins could associate *in vivo* with each of the regulatory subunits.

The catalytic subunits also have intrinsic protein Ser/Thr kinase activity (26,27,59). The substrates of this activity are serine residues within the catalytic and the regulatory subunits. The p85 $\alpha$  regulatory subunit has been shown to be phosphorylated by p110 $\alpha$  on Ser 608, which reduces the lipid kinase activity several-fold (26,59). p110 $\delta$  autophosphorylation also downregulates the lipid kinase activity (27). In contrast, p110 $\gamma$  autophosphorylation does not seem to affect its enzymatic activity (60). Several investigators have reported that p85 subunits become tyrosine-phosphorylated in response to growth factors (11,61–65). No change in lipid kinase activity has been attributed to this modification (66,67).

Class I<sub>A</sub> and I<sub>B</sub> enzymes are activated by the interaction of p110 subunits with GTP-loaded p21<sup>ras</sup>, which signals through a number of downstream effectors. When GTP-loaded Ras is bound to p110 $\alpha$ , the lipid kinase activity increases two- to three-fold *in vitro* and *in vivo* (25,68). Immunoprecipitation with anti-Ras antibodies provided evidence for the physical association of p21<sup>ras</sup> and the p85/p110 $\alpha$  heterodimer (69). Amino acid residues 133–314 within p110 $\alpha$  are necessary for binding to GTP-Ras (68,70). Transient transfection of constitutively active Ras (V12Ras) leads to an accumulation of PI 3,4-P<sub>2</sub> and PI 3,4,5-P<sub>3</sub> lipid products, while dominant negative Ras (N17Ras) abrogates growth factor-mediated activation of PI 3-kinase (25). These data provided evidence for Ras as an upstream regulator of PI 3-kinase, whereas other studies using a PDGFR mutant that lacks the PI 3-kinase docking site fail to activate Ras (71), which suggests that Ras could be a downstream target of PI 3-kinase.

In addition,  $\beta\gamma$  subunits of G-protein coupled receptors stimulate p85 immunoprecipitable PI 3-kinase activity (72,73), and insulin-activated PI 3-kinase is synergistically activated by a pertussis toxin-sensitive G-protein (72). The  $\beta\gamma$  subunits specifically activate p85-associated p110 $\beta$  but not p110 $\alpha$  isoforms *in vitro*, and G $\beta\gamma$ -sensitive PI 3-kinase partially purified from rat liver is activated synergistically by phosphotyrosyl peptides (72–75). Thus, the p110 $\beta$  isoform

can function as a cross-talk between protein-tyrosine kinases and G-protein-mediated signaling.

Other proposed models for activation of Class I<sub>A</sub> PI 3-kinase have been reported, but their significance in intracellular signaling is controversial. The phosphatidylinositol transfer protein (PITP) (76), profilin and gelsolin have also been shown to increase PI 3-kinase activity in an Src-dependent manner (reviewed in ref. 77). A membrane-permeable peptide generated from the PI 4,5-P<sub>2</sub>-binding site of gelsolin is a potent activator of PI 3-kinase in intact cells (78,79,230). It has been proposed that calmodulin could activate PI 3-kinase by binding to p85 SH2 domains (80). The binding of the p85 BH domain to the Rho family GTPases, Rac and Cdc42, also increases PI 3-kinase activity (30,81), although other studies failed to detect this interaction *in vivo* (25).

The use of knockout and transgenic mice has been useful in establishing the function of Class I<sub>A</sub> PI 3-kinases. Deletion or expression of catalytically inactive PI 3-kinase subunits p110 $\alpha$  or p110 $\beta$  result in embryonic lethality between E9.5 and E10.5 (82). The results for the p110 $\alpha$  knockout were not surprising due to the broad tissue distribution, whereas the similar phenotype observed for the p110 $\beta$  mutant was less expected. The study of Class I PI 3-kinases in the nematode *C. elegans* has also revealed interesting results. Deletion of Class I<sub>A</sub> PI 3-kinase results in the entry into the arrested dauer stage and prolongs the lifespan of the nematode (83), which suggests a role for PI 3-kinase as a regulator of longevity.

Most mice which lack p85 $\alpha$  and its splice variants, p50 $\alpha$  and p55 $\alpha$ , do not survive to birth, and those that do have impaired B cell development with normal T cell lineages (84). Gene targeting of the first exon of p85 $\alpha$ , which leads to deletion of p85 $\alpha$  without affecting the expression of p50 $\alpha$  and p55 $\alpha$  isoforms, results in mice that are viable but develop B cell immunodeficiency that is reminiscent of Btk<sup>-/-</sup> mice. These mice also have increased sensitivity to insulin by producing elevated PI 3,4,5-P<sub>3</sub> levels, which suggests that p50 $\alpha$  participates in insulin-mediated signaling (85,86). Mice which lack p85 $\alpha$  invariably manifest extensive hepatic necrosis of unknown pathogenesis. The p85 $\beta$  knockout mice are also viable, which is consistent with the restricted expression of this subunit (87). The p85 $\alpha$  is overexpressed in p110 $\beta$ <sup>-/-</sup> animals, whereas expression of catalytic subunits is reduced in p85 $\alpha$ <sup>-/-</sup> mice (84), which indicates that the expression of PI 3-kinase subunits is interdependent.

### Activity and Regulation of Class I<sub>B</sub> PI 3-Kinase

The p110 $\gamma$  catalytic subunit is the only member of this group and differs from the Class I<sub>A</sub> PI 3-kinases in that it lacks an N-terminal p85-binding domain. Class I<sub>B</sub> PI 3-kinase is activated directly by the  $\beta\gamma$  subunits of heterotrimeric G proteins (30,90–92). Some groups have also reported that p110 $\gamma$  can be activated by G-protein associated  $\alpha$  subunits (90,93). In addition, a modest activation of

p110 $\gamma$  is mediated by p21<sup>ras</sup> (94). The interaction of Ras with p110 $\gamma$  occurs through the N-terminal region homologous to the Ras-binding site of Class I<sub>A</sub> isoforms (94). Interestingly, the Ras/p110 $\gamma$  interaction prevents Ras from GTPase activity, suggesting a reciprocal modulation (95).

Receptors coupled to heterotrimeric G proteins (GPCR) are one of the largest known families of integral membrane receptors. Binding of ligands to growth factor receptors or activation of odor and taste receptors coupled to heptahelical GPCR triggers the exchange of GDP for GTP in the  $\alpha$  subunit. This leads to dissociation of  $\beta\gamma$  subunits. This process is amplified in a way that activation of one GPCR leads to activation of multiple G proteins, leading GTP-bound  $\alpha$  and free  $\beta\gamma$  subunits to participate, independently, in regulation of other pathways. G-protein-mediated activation of PI 3-kinase *in vivo* was reported prior to cloning and characterization of the catalytic subunits (96). The precise mechanism of regulation of p110 $\gamma$  is not well understood. However, recent identification of an adaptor protein, p101 (35), which is required for activation of p110 $\gamma$  by G $\beta\gamma$  (36), provides a plausible mechanism.

Ligands that activate heterotrimeric G-proteins have also been reported to stimulate Class I<sub>A</sub> PI 3-kinase activity as discussed in the section that describes the activity and regulation of Class I<sub>A</sub> PI 3-kinases. This activity is most likely mediated by p110 $\beta$ . The activation of p85-associated PI 3-kinase isoforms and p110 $\gamma$  are probably interdependent and may explain the synergy between responses initiated by protein-tyrosine kinase receptors and heterotrimeric G-protein receptors.

The functional role of p110 $\gamma$  has been difficult to study because of the crossover effects between the Class I PI 3-kinase isoforms. Recent studies involving mice which lack p110 $\gamma$  identified a link to a chemotactic response provided by G-type receptors, interleukin-8 (IL-8), C5a and the formylated peptide, fMLP (97,98). Neutrophils from these mice have defective homing with increased accumulation in the peripheral blood, impaired chemoattractant-induced migration and activation of Akt but no apparent changes in responses generated by receptor protein-tyrosine kinases. These results complement studies which suggest that activation of chemotactic GPCRs is required for migration of neutrophils in response to chemoattractants (99,100).

Mice which lack p110 $\gamma$  have impaired thymocyte development (101), in contrast to p85 knockout mice that showed a defect in B-cell development (84). Thus, type I PI 3-kinase-mediated activation of Akt is required for survival of lymphoid cells in both lymphoid lineages at specific stages of differentiation. In addition, p110 $\gamma$ <sup>-/-</sup> mice are defective in T cell-mediated secretion of IL-2 (101) despite normal phosphotyrosine-mediated binding of PI 3-kinase to the T cell receptor. Therefore, T cell receptor-mediated secretion of IL-2 requires functional p110 $\gamma$ , which suggests that p110 $\gamma$  participates in vesicular trafficking and secretion.

## Class II PI 3-Kinases

### Structure of Class II PI 3-Kinases

Class II PI 3-kinase catalytic subunits are larger proteins (170 to 220 kd) that contain a C2 domain, a PX domain, a PIK domain and a catalytic domain which shares 40% to 50% sequence homology with Class I enzymes. Three different isoforms of Class II PI 3-kinases are encoded by separate genes: PI 3-K-C2 $\alpha$ ,  $\beta$ , and  $\gamma$ . PI 3-K-C2 $\alpha$  and  $\beta$  have a PX domain identified in other signaling molecules, phox-40 and phox-70, whose function is unclear (reviewed in ref. 42). PI 3-K-C2 $\alpha$  and  $\beta$  are found ubiquitously in mammals, whereas PI 3-kinase-C2 $\gamma$  is found primarily in the liver (102,103).

The defining feature of this class of PI 3-kinases is the C-terminal C2 domain (104–106). C2 domains have been implicated in the Ca<sup>2+</sup>-dependent binding of proteins to lipid vesicles. However, Class II PI 3-kinases lack a critical Asp residue that is required for Ca<sup>2+</sup>-sensitive phospholipid binding. In contrast to the cytosolic distribution of Class I enzymes, Class II enzymes are associated with the membrane fraction (104).

### Activity and Regulation of Class II PI 3-Kinases

*In vitro*, Class II PI 3-kinases phosphorylate only PI and PI 4-P, although PI 3-K-C2 $\alpha$  also phosphorylates PI 4,5-P<sub>2</sub> in the presence of phosphatidylserine (106). Regulation may be provided by weak acidic phospholipid binding to the C2 domain in a Ca<sup>2+</sup>-independent fashion (105). Class II PI 3-kinases are activated by insulin, EGF, PDGF, integrins, and a chemokine, MCP-1, and are sensitive to nanomolar concentrations of wortmannin (107–109). No adaptor proteins have been identified, and the mechanisms of control for these PI 3-kinases is still unknown. The *Drosophila* Class II PI 3-kinase, PI3K-68D/cpk, has been found in association with 90 kd and 190 kd tyrosine-phosphorylated proteins, implying that they could participate in the activation of Class II PI 3-kinases (110).

## Class III PI 3-Kinases

### Structure of Class III PI 3-Kinases

The first Class III PI 3-kinase was identified in yeast (Vps34p). This enzyme exists as a complex with Vps15p, whereas in mammalian cells the homologue of Vps34p, PI 3-K III, associates with a p150 regulatory subunit (111–114). The catalytic subunit of PI 3-K III has a kinase domain, a PIK domain, and a Vps15p/p150 binding motif (114). The mammalian regulatory subunit, p150, has 30% identity with its yeast counterpart (115). Vps15p and p150 are N-terminally myristoylated Ser/Thr kinases. Myristoylation of these proteins recruits them to the plasma membrane, thus providing a constitutive interaction of PI 3-kinase with its substrates. In addition to having a Ser/Thr

domain, the p150 adaptor subunit has a series of HEAT repeats (116) and C-terminal WD motifs (117) which may regulate interactions with other proteins.

### Activity and Regulation of Class III PI 3-Kinases

Vps34p and its homologues in *Dictyostelium*, *Drosophila* and humans use only PI as their substrate and will not phosphorylate PI 4-P or PI 4,5-P<sub>2</sub> *in vitro* (114). PI 3-P is the only 3' phosphorylated phosphoinositide detected in yeast and is constitutively present in all mammalian cells (114,118). Although PI 3-P levels do not change in response to activation of receptor-tyrosine kinases, large increases in PI 3-P are observed only in platelets in response to agonists (119). Mutational analysis of the yeast Vps34p demonstrated that this lipid kinase is essential for accurate vesicle-mediated transport, osmoregulation, and endocytosis (120).

### PI 3-KINASE INHIBITORS

There are two extensively studied cell-permeable inhibitors of PI 3-kinases, wortmannin and LY294002 (121–123). They are not structurally related and block the enzymatic activity by different mechanisms. Wortmannin binds covalently to the PI 3-kinase catalytic subunits (124), whereas LY294002 is a competitive inhibitor of ATP binding (123). Formation of a Schiff-base between p110 $\alpha$  and wortmannin involves a conserved Lys<sup>802</sup> residue which is also required for ATP hydrolysis (124). *In vitro*, Class I PI 3-kinases are blocked by 1 nM wortmannin or by 1  $\mu$ M LY294002. Treatment of cells with 25 to 50 nM wortman-

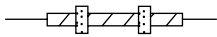
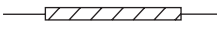
nin or by 10 to 20  $\mu$ M LY294002 blocks PI 3-kinase-dependent biological responses. At higher concentrations, these compounds lose specificity. Class II PI 3-kinases are less sensitive to these agents, and the ubiquitously expressed PI 3-K-C2 $\alpha$  isoform is ten-fold more resistant to wortmannin (106,125). The mammalian Class III PI 3-kinase are also sensitive to wortmannin (114), whereas their *S. cerevisiae* and *S. pombe* counterpart, Vps34, is resistant (126,127).

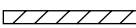

### ALTERNATE PATHWAYS FOR GENERATING D-3 POLYPHOSPHOINOSITIDES

Until recently, PI 4-P was the only PIP product detected in cells. Although PI 4-P is the most abundant monophosphoinositide, PI 3-P and PI 5-P also occur physiologically. A number of kinases and phosphatases that generate PI 3,4-P<sub>2</sub> or PI 3,5-P<sub>2</sub> from PIPs have recently been identified.

In the early 1990s, two types of PIP kinases were isolated from human erythrocytes based on their ability to phosphorylate PI 4-P *in vitro* (128). These were subsequently designated as type I (68 kd) and type II (53 kd) PIP kinases based on their biochemical properties (Table 35.2). In addition, a 90 kd PIP kinase was isolated from rat brain which was immunologically similar to type I PIP kinase and was, therefore, designated type Ib (129). Biochemical characterization of these enzymes indicated that both type Ia and type Ib, but not the type II isoform, were stimulated *in vitro* by phosphatidic acid (PA) (129).

**TABLE 35.2. CLASSIFICATION OF THE PIP 4-KINASES AND PIP 5-KINASES: STRUCTURAL FEATURES OF THE ENZYMES, THEIR LIPID SUBSTRATES, AND MECHANISMS OF REGULATION**

Enzyme	Subunits		Structural Features	Lipid Substrates		Regulation		
	Catalytic	Adaptor		<i>in vitro</i>	<i>in vivo</i>			
PIP 4-K	<i>Human</i>			PIP 4-KII ( $\alpha$ , $\beta$ , $\gamma$ )	PI 5-P PI 3-P	PI 5-P PI 3-P	Integrin-mediated pathway TNF $\alpha$ stimulates PIP 4-KII $\beta$ Inhibited by: PI 4,5-P <sub>2</sub> Heparin	
	PIP 4-K	II $\alpha$ (47 kd) II $\beta$ (47 kd)						TNFR
	<i>Rat</i>	PIP 4-K						II $\gamma$ (47 kd)
PIP 5-K	<i>Human</i>			PIP 5-KI ( $\alpha$ , $\beta$ )	PI 4-P PI 3-P PI 3,4-P <sub>2</sub> PI 3,5-P <sub>2</sub>	PI 4-P PI 3-P PI 3,4-P <sub>2</sub> PI 3,5-P <sub>2</sub>	Regulated by: Heterotrimeric G proteins Small g proteins Rac I and Rho A Activated by: Phosphatidic acid Heparin Spermine Inhibited by: PI 4,5-P <sub>2</sub>	
	PIP 5-K	I $\alpha$ (61 kd) I $\beta$ (68 kd) (90 kd) (110 kd)						
	<i>Mouse</i>							
	PIP 5-K	I $\alpha$ (68 kd) I $\beta$ (68 kd) I $\gamma$ (90 kd)						

 catalytic domain  proline-rich domain

## Structure of PIP Kinase Family

The type II PIP kinase (type II $\alpha$ ) was the first enzyme in this family to be cloned (130,131). Since then, two other type II PIP kinases ( $\beta$  and  $\gamma$ ) have been cloned and characterized (reviewed in ref. 132). The structure and distribution of the type II PIP kinases are similar to those of the type I PIP kinases and are discussed in the next section.

Three distinct type I PIP 5-kinases have been cloned in the mouse. The type I $\alpha$  and  $\beta$  isoforms migrate at 68 kd, whereas the third, type I $\gamma$ , migrates at 87 to 90 kd (133). There are several different isoforms for each of the type I PIP 5-kinases which result from alternate splicing. Human homologues of the murine type I $\alpha$  and  $\beta$  isoforms have also been cloned (reviewed in ref. 132). The human type I $\alpha$  is closer to the mouse type I $\beta$  (134). Two PIP kinases have also been identified in *S. cerevisiae*; Mss4, which is biochemically similar to mammalian type I PIP 5-kinase (135,136), and FAB1. FAB1 is unique in that it converts PI 3-P to PI 3,5-P<sub>2</sub> and does not utilize other types of phosphoinositides (137). Therefore, this enzyme could be considered as a type III PIP kinase.

PIP 4-kinases and PIP 5-kinases are about 35% identical in their putative catalytic core domain, but are different in their N- and C-terminal regions, and neither share homology with other lipid or protein kinases (reviewed in refs. 42,132). The PIP 4- but not 5-kinases contain two proline-rich domains within their catalytic core which could provide binding sites for SH3 domain-containing proteins.

Although at least one of the isoforms of types I and II PIP kinases is found in mammalian tissues, the expression level varies considerably for each isoform (reviewed in ref. 132). With the exception of the type II $\alpha$  PIP kinase, both types I and II PIP kinases are found at very low levels in the liver (132).

## Activity and Regulation of PIP Kinases

Despite homology in the DNA sequence, PIP kinases phosphorylate the inositol ring at different positions. Type II PIP kinase phosphorylates the D-4- position of PI 3-P and PI 5-P *in vitro* (138,139) and *in vivo* (140,141), and could be considered a 4-OH kinase. Type I PIP kinase phosphorylates PI 4-P on the D-5-position of the inositol ring and thus can be considered a 5-OH kinase as suggested previously (138,139) and could provide an alternative pathway for the synthesis of PI 3,4-P<sub>2</sub>. PI is not a substrate for PIP 4-kinases (139). The activity of PIP 4-kinases is inhibited by its product, PI 4,5-P<sub>2</sub>, and by heparin. Indirect evidence suggests that PIP 4-kinases associate with the p55 subunit of the tumor necrosis factor (TNF) receptor. Binding of TNF $\alpha$  to its receptor leads to an increase in PIP 4-kinase II $\beta$  activity (142). In platelets, the generation of PI 3,4-P<sub>2</sub> from PI 3-P in integrin-dependent signaling involves PIP 4-kinase (143).

In addition, I $\alpha$  and  $\beta$  isoforms of PIP 5-kinase generate PI 3,5-P<sub>2</sub> from PI 3-P and PI 3,4,5-P<sub>3</sub> from PI 3,4-P<sub>2</sub> *in vitro*, although at a lower rate than PI 3-kinases (139,144,145). Recombinant purified PIP 5-kinase generates significant amounts of PI 3,4,5-P<sub>3</sub> from PI 3,4-P<sub>2</sub> (139). It is unclear whether PI 3-P is preferentially phosphorylated to PI 3,4-P<sub>2</sub> or PI 3,5-P<sub>2</sub> (139) and whether these intermediates can be further phosphorylated to PI 3,4,5-P<sub>3</sub> (reviewed in ref. 42).

The activity of PIP 5-kinases is stimulated by heparin and spermine (138) and, as much as 50-fold, by phosphatidic acid (129,146). PIP 5-kinase activity is inhibited by its product, PI 4,5-P<sub>2</sub>. *In vivo*, PIP 5-kinase activity may be influenced by small G proteins. The nonhydrolyzable GTP analog GTP $\gamma$ S increases PIP 5-kinase activity in several cell types (147). PIP 5-kinase has also been found to associate with Rac1 (30) and RhoA (148) and, in intact cells, the addition of rac-GTP or RhoA results in the accumulation of PI 4,5-P<sub>2</sub> (79,148,149). Furthermore, PIP 5-kinase activity is regulated by a cholera toxin-sensitive heterotrimeric G protein in rat liver membranes (150). It is clear that the number of enzymes belonging to these two families will continue to grow.

## Phosphatases

Phosphatases can be divided into three groups based on the specificity for the phosphate on the inositol ring to use PI 3-P, PI 3,4-P<sub>2</sub> or PI 3,5-P<sub>2</sub>. It has been proposed that a phosphatase that hydrolyzes the phosphate on the D-3 position could play a role in tumor suppression. A tumor suppressor gene located on human chromosome 10q23, PTEN (phosphatase and tensin homologue deleted on chromosome ten) or MMAC1 (mutated in multiple advanced cancers), was identified by several groups (151,152). PTEN is deleted or mutated in a wide variety of tumors (reviewed in ref. 153). The DNA sequence of PTEN suggested that it is a dual-specificity phosphatase, and PTEN can dephosphorylate some Ser/Thr phosphorylated proteins, although it prefers highly acidic substrates, such as poly(Glu-pTyr) (154). In contrast to a weak protein phosphatase activity, PTEN is a potent phosphatase for PI 3,4,5-P<sub>3</sub> *in vivo* and *in vitro* (155). Subsequent studies established that PTEN hydrolyzes PI 3,4-P<sub>2</sub>, PI 3,4,5-P<sub>3</sub> and, to a lesser extent, PI 3-P (154). In addition to its catalytic domain, PTEN has a C-terminal potential binding site for PDZ domain-containing proteins and a region which overlaps the catalytic domain that is similar to the cytoskeletal proteins tensin and auxilin. The function of these domains is still unclear, but other PDZ proteins have been shown to direct the assembly of multiprotein complexes, often at the membrane/cytoskeletal interface, whereas the "tensin domain" may constitute a binding site for the phosphoinositides. PTEN affects several signal transduction pathways including PI 3-kinase-dependent activation of Akt (156) and dephosphorylation of focal adhesion kinase (FAK) (157). The lipid phosphatase activity of PTEN is required for

its tumor suppressor activity and for its role in normal development (reviewed in ref. 153).

A group of magnesium-independent phosphatases that remove the phosphate from the D-4 position of the inositol ring also participate in the turnover of PI 3-kinase lipid products. The D-4-phosphatases are encoded for by two genes (types I and II) which are alternately spliced and contain a phosphatase consensus sequence (CKSAKDRT) within the catalytic domain (158). Both types catalyze the hydrolysis of PI 3,4-P<sub>2</sub> and, to a lesser extent, inositol 3,4-bisphosphate (158,159). The alternately spliced isoforms contain a C-terminal putative transmembrane domain (158). Type I phosphatase can be inactivated by proteolytic cleavage from the transmembrane domain by calcium-dependent protease, calpain, which may be responsible for the calcium-dependent accumulation of PI 3,4-P<sub>2</sub> in platelets (158).

Several 5'-phosphatases have been cloned and characterized, and there are others for which the sequence has not been identified (40,160). All identified 5'-phosphatases are magnesium-dependent phosphomonoesterases (160). Members of this family share a conserved domain of about 300 amino acids in the central portion of the molecule and are defined by two signature motifs: (F/I)WXGDXN(F/Y)R and (R/N)XP(S/A)(W/Y)(C/T)DR(I/V)(L/I) and are subdivided based on their substrate specificity (reviewed in ref. 160). Type I 5'-phosphatases hydrolyze only inositol polyphosphates. Type II enzymes specifically remove D-5-phosphate from soluble inositols and phosphatidylinositol polyphosphates. Type II enzymes are further subdivided based on their sequences and subcellular localization (40). Human and mouse platelet 5'-phosphatase (INPP5P) have a C-terminal prenylation site that targets this enzyme to mitochondria and plasma membranes, in addition to an N-terminal domain which is also thought to be important for association with the cellular membranes (75). Another member of this group, OCRL-1, is not modified by a lipid moiety but is found on the surface of lysosomes (161). INPP5P and OCRL-1 may have overlapping functions in mice, although this is probably not the case in humans (162). Cell lines derived from the proximal tubules of the kidney from a patient with Lowe syndrome were shown to lack OCRL-1 and have misrouting of lysosomal enzymes and accumulation of PI 4,5-P<sub>2</sub> and PI 3,4,5-P<sub>3</sub> (162). Two other members of this family isolated from nerve terminals are synaptojanin 1 and 2. These proteins are related to synaptic vesicle trafficking proteins and have PI 4,5-P<sub>2</sub> 5'-phosphatase activity (163). Synaptojanins play a role in osmoregulation and vacuole morphology in yeast (164) and form complexes with dynamin and amphiphysin and thus may participate in vesicular trafficking.

The SH2 domain-containing 5'-inositol phosphatases (SHIP1 and SHIP2) constitute the type III enzymes and hydrolyze specifically the 5'-phosphate from PI 3-kinase lipid products (reviewed in ref. 160). In addition to the N-

terminal SH2 domain, both enzymes contain a C-terminal proline-rich region which interacts with SH3-containing proteins (165,166). SHIP1 contains two phosphotyrosine-binding (PTB) domains within the proline-rich region, whereas SHIP2 contains only one. The SH3 and PTB domains of SHIP1 and SHIP2 are probably responsible for their association with Shc and Grb2-Sos1 complexes, which link these phosphatases to signal transduction initiated by protein-tyrosine kinases (167,168). SHIP1 is expressed almost exclusively in cells of hematopoietic origin, but it is also found in lung and testis (168), whereas SHIP2 is expressed ubiquitously. SHIP1 and SHIP2 have been implicated in the downregulation of cellular responses to cytokines and growth factors, specifically insulin (169).

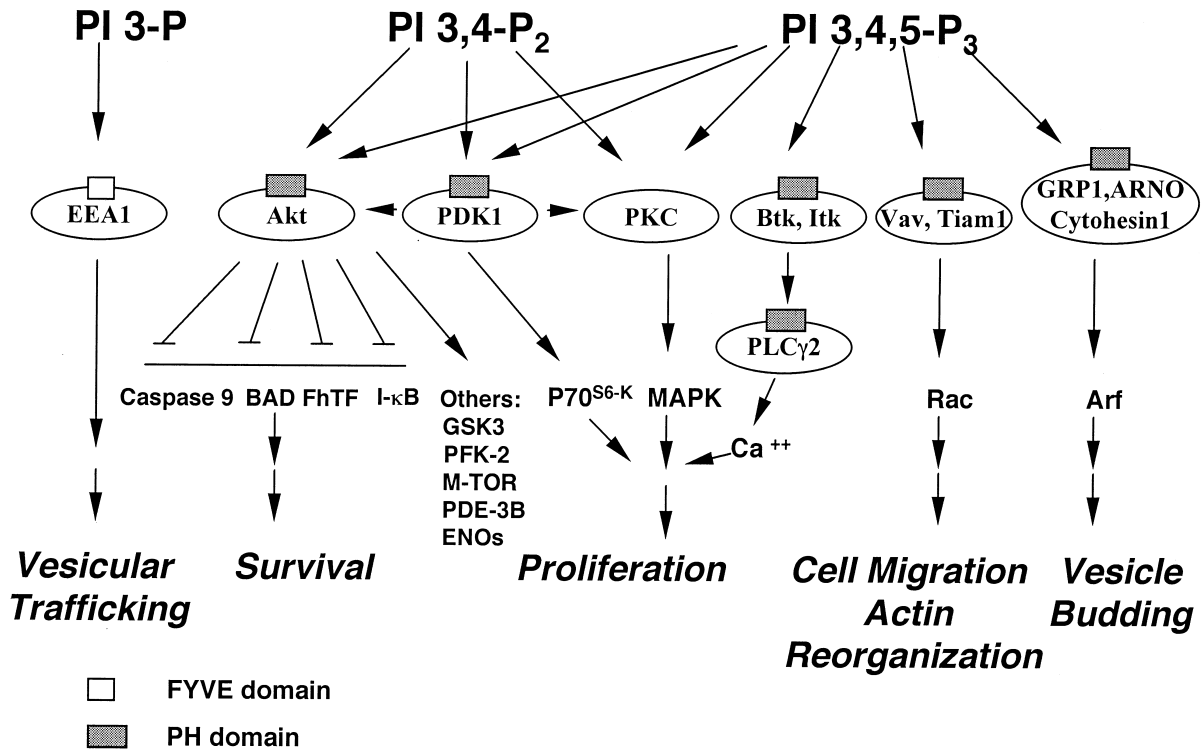
A different enzymatic activity has recently been isolated from platelets and placed in a separate group (type IV). This enzyme is found in a complex with PI 3-kinase and specifically hydrolyzes PI 3,4,5-P<sub>3</sub> (170).

## LIPID PRODUCTS AS MEDIATORS OF PI 3-KINASE DOWNSTREAM SIGNALING

The ability of PI 3-kinase to initiate intracellular signals is derived from the direct interaction of the catalytic and regulatory subunits with other proteins and from protein-lipid interactions provided by lipid-binding domains in target proteins (Fig. 35.1). Two lipid-binding domains have been identified that specifically recognize inositol-containing phospholipids: the FYVE and PH domains. A novel method for the identification of protein-lipid interactions has recently been described (171). This technology is based on screening peptide libraries with biotinylated analogs of the lipids and may uncover novel mechanisms for interaction with lipid moieties.

### FYVE Domains

The FYVE domain was named after the first four proteins known to contain the conserved protein sequence: *F*ab1p, *Y*OTB, *V*ac1p, and *E*EA1. It selectively binds to PI 3-P (172–174). To date, over 40 FYVE domain-containing proteins have been described in mammals, and most of them have been implicated in intracellular trafficking. The discovery of the FYVE domain as a target of PI 3-P opened a new level of understanding for the role of PI 3-P in vesicular trafficking (reviewed in ref. 175). The 60–80 amino acid residue FYVE domain is a special RING zinc finger structure with eight conserved cysteines forming two separate zinc coordination centers (173,176). Many FYVE domain-containing proteins are involved in secretion, vacuolar targeting, receptor recycling and multivesicular endosome formation (reviewed in ref. 175). The FYVE domain of EEA1, a protein that regulates fusion of endocytic membranes (177), is necessary for Rab-5-directed vesicular trafficking (178,179). The crystal structure of



**FIGURE 35.1.** Downstream signaling through PI 3-kinase lipid products. The targets of lipid products are indicated, as are their roles in cellular responses. The PH and FYVE protein domains that bind phospholipids are highlighted.

VPS27p protein suggests that PI 3-P but not other lipids, including PI 5-P, can be accommodated in the binding site, and mutation of the zinc coordinating cysteines or removal of zinc reduces the affinity for PI 3-P (180). The crystal structure of a homodimer of FYVE domains derived from *Drosophila* Hrs (HGF-regulated tyrosine kinase substrate) has a parallel rather than perpendicular orientation to the membrane (181). Hrs is involved in targeting the endosome to the lysosome and is required for normal mouse development. Dimerization of the Hrs FYVE domain creates two identical pockets, resulting in higher affinity for PI 3-P.

### Pleckstrin Homology Domains

The pleckstrin homology (PH) domain contains 90–110 amino acids and was initially found in pleckstrin, the major protein kinase C substrate in platelets. PH domains are found in more than 100 proteins with a wide range of cellular functions, including kinases, phospholipases, nucleotide-exchange factors, and adaptor proteins and proteins which mediate protein–phospholipid and protein–protein interactions (182). The inositol head group binding site in the PH domain is formed by two  $\beta$ -sheets capped by an  $\alpha$ -helix. Divergent basic residues in the PH domain core structure determine the binding affinity (reviewed in ref. 175). Most

PH domains bind inositol-containing phospholipids but only some bind with high affinity. There are no PH domains that bind PI 3-P, a role that seems to be assigned to FYVE domains. Some PH domains preferentially bind to PI 3,4-P<sub>2</sub>, whereas others specifically bind PI 3,4,5-P<sub>3</sub> (138,183). In addition, PH domains have been used as probes for the visualization of subcellular compartments that transiently accumulate polyphosphoinositides in response to specific stimuli (184), and led to the identification of novel signaling cascades in membrane ruffling, adhesion, and chemotaxis (reviewed in ref. 175).

The PH domain of Akt, also referred to as PKB, preferentially binds to PI 3,4-P<sub>2</sub> and PI 3,4,5-P<sub>3</sub> over other phosphoinositides. The mechanism of activation of Akt is not fully understood, but the interaction of PH domain with phosphoinositides leads to a three- to five-fold stimulation of its activity *in vitro* (185–187). *In vivo*, Akt activation requires PI 3-kinase and phosphorylation of Thr<sup>308</sup> and Ser<sup>473</sup> (188). Thr<sup>308</sup> is phosphorylated by PDK1, a constitutively active Ser/Thr kinase that also contains a PH domain with high affinity for PI 3,4,5-P<sub>3</sub> and, to a lesser degree, PI 3,4-P<sub>2</sub> (189–192). Phosphorylation of Akt on Thr<sup>308</sup> is enhanced over one thousand-fold in the presence of lipid micelles containing PI 3,4-P<sub>2</sub> or PI 3,4,5-P<sub>3</sub>. An enzymatic activity named PDK2 phosphorylates Ser<sup>473</sup>. Embryonic stem cells, which lack PDK1, fail to phosphorylate Thr<sup>308</sup>

in response to IGF-1 but have phosphorylation on Ser<sup>473</sup>, which suggests that PDK2 is functional in these cells (193).

PI 3-kinase-dependent activation of Akt is required for its role in cell survival. Akt participates in counteracting the apoptotic pathway on several levels, and its effect on cell survival may depend on the specific apoptotic pathway affected in different cell types. Akt phosphorylates BAD, a protein that in a dephosphorylated state promotes apoptosis by binding to Bcl-x<sub>L</sub>, a cell survival factor. Phosphorylation of BAD disrupts the BAD/Bcl-x<sub>L</sub> complex and allows Bcl-x<sub>L</sub> to homodimerize and thereby protect mitochondria from apoptosis. Caspase 9, a protease necessary for the initiation of the apoptotic cascade, is also phosphorylated and inhibited by Akt (194,195). Two additional targets that interfere with the apoptotic pathway have recently been identified. Akt phosphorylates forkhead transcription factor, which leads to its accumulation in the cytosol, thus preventing expression of proapoptotic molecules such as Fas (196–199). Akt also associates with and activates I-κB kinases (200–202). This leads to degradation of I-κB, which allows NF-κB-dependent transcription of antiapoptotic proteins (203). In the liver, PI 3-kinase-dependent activation of Akt also protects hepatocytes from apoptosis (204,205).

In addition to cell survival, activation of Akt affects other cellular responses, including regulation of enzymes involved in glycogen turnover in response to insulin. These include glycogen synthase kinase 3 (GSK3) (206), the cardiac isoform of 6-phosphofructo-2-kinase (207), phosphodiesterase-3B (208) which is involved in the regulation of intracellular levels of cAMP, and mTOR (209), which regulates protein translation. Akt also participates in the generation of nitric oxide (NO) in endothelial cells by activation of endothelial NO synthase (210–212).

PDK1 is a constitutively active protein Ser/Thr kinase that is recruited to the plasma membrane by the interaction of its PH domain with PI 3,4-P<sub>2</sub> and PI 3,4,5-P<sub>3</sub>. PDK1 has targets other than Akt. PDK1 phosphorylates and activates p70 S6 kinase (213,214) and several members of the PKC family, PKCε, and PKCζ (215). These PKC isoforms also contain PH domains and can be independently activated by PI 3,4-P<sub>2</sub> or PI 3,4,5-P<sub>3</sub> (216,217). Additional targets for PDK1 may be activated independently of PI 3-kinase signaling.

Another large group of proteins which contain PH domains are GTP/GDP exchange factors (GEFs) and GTPase-activating proteins (GAPs), proteins which regulate the GTPase activity of small G proteins. The presence of PH domains in Rho family GTPases and ARF-GTPases supports the experimental evidence that these small G proteins are regulated by 3'-polyphosphoinositides (218). Two GEFs for Rac, Vav and Tiam1, specifically bind to PI 3,4,5-P<sub>3</sub>, which results in activation of Rac (182,219). PI 3-kinase-dependent activation of Rac may be responsible for growth factor-mediated membrane ruffling, actin reorganization and chemotaxis (220,221). Other examples include

GAP1<sup>m</sup> and GAP1<sup>IP4BP</sup>, which are Ras-GAPs; their PH domains also bind PI 3,4,5-P<sub>3</sub>. Although the role of this binding is unclear because it does not lead to increased Ras-GAP activity, it may facilitate the recruitment of PI 3-kinase to the plasma membrane and Ras-dependent activation of PI 3-kinase, as discussed in the earlier section entitled Activity and Regulation of Class I<sub>A</sub> PI 3-kinase.

Three ARF-GEFs which participate in vesicular membrane trafficking are known to be regulated by PI 3-kinase lipid products: GRP1, ARNO, and cytohesin-1 (reviewed in refs. 222,223). Their PH domains preferentially bind to PI 3,4,5-P<sub>3</sub> and have relatively low affinity for PI-3-P and PI 3,4-P<sub>2</sub>. This leads to translocation of these proteins from the cytosol to the plasma membrane, where they regulate vesicle coating and budding. In addition, centaurins, which are ARF-GAPs, specifically bind to PI 3,4,5-P<sub>3</sub> or the soluble Ins(1,3,4,5)-P<sub>4</sub> *in vitro*. The role of PI 3-kinase in vesicular trafficking is further supported by studies of PDGF-dependent trafficking of the PDGF receptor to lysosomes and for the insulin-dependent trafficking of the glucose transporter, GLUT4, to the plasma membrane (224,225).

Other proteins that contain PH domains are Tec nonreceptor tyrosine kinases. Tec family members include Bruton's tyrosine kinase (Btk) and the inducible T-cell kinase (Itk). Btk is critical for B cell development and function. Mutation in the Btk PH domain that affects binding to PI 3,4,5-P<sub>3</sub> results in X-linked immunodeficiency in mice (226). The mechanism of Btk activation resembles the activation of Akt by PDK1. The PH domain of Btk mediates its translocation to the plasma membrane where it can be further activated by phosphorylation on Tyr and Ser by Src family kinases (reviewed in ref. 23). Btk regulates tyrosine phosphorylation of PLCγ2, which also has a PI 3,4,5-P<sub>3</sub>-specific PH domain (227). Thus, the recruitment to the plasma membrane and activation of PLCγ2 provides a role for PI 3-kinase lipid products in hematopoietic cells.

## Other Targets for D-3 Phosphoinositides

As described in the earlier section entitled Activity and Regulation of Class I<sub>A</sub> PI 3-kinases, PI 3,4,5-P<sub>3</sub> binds to p85 SH2 domains and may compete for its interaction with phosphotyrosine-containing sequences. This binding could terminate the PI 3-kinase cascade by inducing dissociation of PI 3-kinase from the lipid substrates. In contrast, binding of PI 3,4,5-P<sub>3</sub> to the SH2 domain of PLCγ enhances its phospholipase activity (49,228,229). Other targets for PI 3-kinase lipid products are continuously identified.

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