to be determined. Does palmitoylation increase the effective concentration of Hh or increase its affinity for Ptc? Does cholesterol mediate the interaction of Hh-Np with HSPGs? And when and where does Disp act to regulate the release of Hh-Np from secreting cells? It also seems likely that the lipid environment plays a critical role in regulating the activity of the Ptc and Smo proteins, although, again, the details of these processes remain obscure. The studies reviewed here have given some tantalizing glimpses into the roles of lipids in these processes, but a great deal more analysis at the cellular and biochemical levels will be required before the picture can be completed.

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REVIEW

Location, Location, Location: Membrane Targeting Directed by PX Domains

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Phosphoinositide (PI)–binding domains play critical roles in the intracellular localization of a variety of cell-signaling proteins. The 120–amino acid Phox homology (PX) domain targets proteins to organelle membranes through interactions between two conserved basic motifs within the PX domain and specific PIs. The combination of protein-lipid and protein-protein interactions ensures the proper localization and regulation of PX domain–containing proteins. Upon proper localization, PX domain–containing proteins can then bind to additional proteins and execute their functions in a diverse set of biological pathways, including intracellular protein transport, cell growth and survival, cytoskeletal organization, and neutrophil defense.

With 30,000 to 40,000 genes potentially expressed in the human genome, cells face the difficult task of assembling these gene products into functional complexes and localizing them to appropriate sites. Of course, cells have developed a number of different strategies to deal with this problem, one of which is to spatially restrict proteins to their site of function and thus improve the probability that they will interact with their proper partners. In particular, the targeting of proteins to specific membrane-bound organelles has proven to be an effective cellular mechanism in maintaining the fidelity and efficiency of protein activities. Research within the past decade has identified protein domains that specifically bind the phosphatidylinositol (PtdIns) phospholipids, collectively called phosphoinositides (PIs), as major determinants in localizing proteins to their site of function (*1, 2*). These PI-binding motifs, which include the C2 (PKC conserved region 2), PH (Pleckstrin homology), FYVE (Fab1p/YOTP/Vac1p/EEA1), ENTH (Epsin NH_2-terminal homology) and tubby domains, are found in proteins implicated in a diverse array of cellular processes, such as protein transport, exocytosis, endocytosis, actin cytoskeletal organization, cell growth regulation, and control of gene expression. Through the regulated synthesis of distinct PIs on specific organelles, proteins containing these lipid-binding domains can be targeted and activated at the appropriate site of function. The importance of membrane targeting by PIs is exemplified by a number of human diseases linked to defects in PI signaling (*3–5*), including cancer, immunodeficiency disorders (X-linked agammaglobulinemina and chronic granulomatous disease), myotubular myopathy, kidney and neurological diseases (oculocerebro-renal syndrome of Lowe), and faciogenital dysplasia (Aarskog-Scott syndrome).

Even with the large number of PI-binding proteins previously identified, genetic and biochemical studies suggest the existence of additional effector molecules. For example, it has long been known that PI synthesis is necessary for the generation of superoxides by the human NADPH oxidase complex, though the connection between these processes had been elusive. Recently, it was determined that Phox Homology (PX) domains, including those in two NADPH oxidase subunits, bind to PIs, identifying another family of effector proteins [(*6–11*); reviewed in (*12*)]. Many members of this effector family contain additional motifs that mediate protein-protein interactions and other biochemical activities, such as protein phosphorylation and lipid modification (*13*). As with other lipid-binding motifs, PX domains play important roles in ensuring that proteins reach their appropriate intracellular location through the binding of membrane-restricted PIs.

PI Lipids and PI Kinases

In contrast to the headgroups of other phospholipids, the biological versatility of PtdIns is derived from its unique ability to be reversibly phosphorylated at three distinct positions of the inositol headgroup (Fig. 1). Single or combinatorial phosphorylation of the D-3, D-4, and D-5 positions on the inositol ring of PtdIns can generate at least seven unique PI derivatives. Furthermore, linkage between the inositol ring to diacylglycerol anchors PIs within lipid membranes. Thus, simple changes in the phosphorylation of PtdIns can trigger a number of distinct, membrane-restricted signals.

The biological activity of PIs can be

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spatially controlled by regulating the enzymes that produce (kinases) and degrade (phosphatases and lipases) these phosphorylated lipids (*3, 14*). Compartment-specific localization of the PI kinases and their regulatory machinery restricts PI synthesis to specific organelle membranes. The in vivo visualization of hybrid proteins in which green fluorescent protein (GFP) is fused to specific PI-binding domains has elucidated the membrane sites where specific PIs are produced. For example, using GFP fusion proteins with the PtdIns(4,5)P_2-specific PH domain of phospholipase C-δ (*15*) and the PtdIns(3,4,5)P_3-specific PH domain of Brunton's tyrosine kinase (*16*), both PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 were determined to be generated on the plasma membrane. PtdIns(4,5)P_2 in mammalian cells mediates a number of biological functions at the plasma membrane, such as actin cytoskeletal organization, phagocytosis, exocytosis, and clathrin-mediated endocytosis (*17*). In yeast, the plasma membrane-localized PtdIns(4)P 5-kinase, Mss4, does not function in membrane trafficking, although Mss4 is necessary for actin cytoskeletal organization (*18*). The mammalian class I PI 3-kinases (PI3Ks) are recruited to the plasma membrane upon the activation of several cell surface receptor tyrosine kinases, where they phosphorylate PtdIns(4,5)P_2 to generate PtdIns(3,4,5)P_3 on the inner leaflet of the plasma membrane (*3*). Newly synthesized PtdIns(3,4,5)P_3 mediates the regulation of actin cytoskeletal organization, cell growth, DNA synthesis, and apoptosis in response to growth factors. The intracellular localization of the class III PtdIns 3-kinase, Vps34, and its product, PtdIns(3)P, to *trans*-Golgi and endosomal compartments (*19*) correlates with their functional activities in Golgi-to-lysosome protein sorting in yeast (*20*) and mammalian systems (*21*). Maintenance of Golgi structure and the formation of secretory vesicles that carry cargo from the Golgi to the plasma membrane requires synthesis of PtdIns(4)P by the Golgi-localized PtdIns 4-kinase Pik1 in yeast (*22*) and PI4Kβ in mammalian cells (*23*). Thus, by restricting PI synthesis to specific membrane compartments, PI signaling is also restricted to discrete locations within the cell.

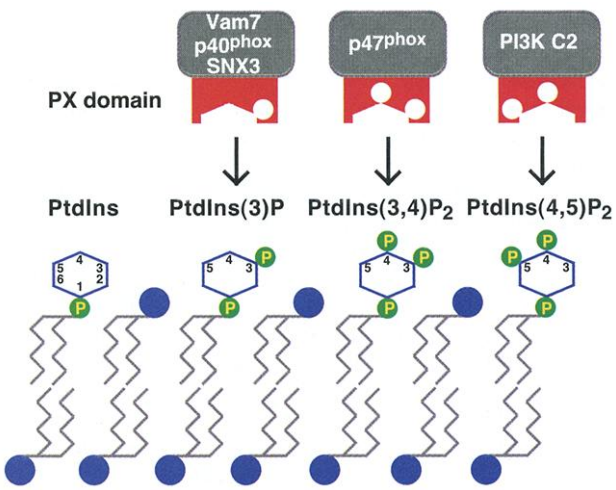

Fig. 1. Schematic diagram of phosphatidylinositol (PtdIns) and three example PIs that bind PX domains. Each of the carbon atoms in the inositol ring are numbered as shown.

PX Domains as PI-Binding Motifs

PX domains were initially identified in 1996 through a database search for proteins homologous to a COOH-terminal region of the class II PI3K C2-γ (*24*). From this search, 36 proteins contained this domain of 120 amino acids. Since then, more than 100 PX domain–containing proteins have been identified in eukaryotes from yeast to humans (*13*). In general, conservation between PX domains is relatively low, although they do contain two highly conserved sequence motifs: (R/K)(R/K)(Y/F)xxFxxLxxxL and R(R/K)xxLxx(Y/F) (where x is any amino acid residue) (*25*). Many PX domain proteins function in a variety of distinct biological processes (Table 1) that involve specific membrane compartments. These include the $p40^{phox}$ and $p47^{phox}$ subunits of the human NADPH oxidase complex that assemble on phagosomal membranes together with the membrane-bound flavocytochrome b_{558} to regulate the generation of bacteriocidal superoxides in neutrophils (*4*). The t-SNARE (target-soluble NSF attachment protein receptor) Vam7 also contains a PX domain and functions in the docking and fusion of transport vesicles to the lysosome-like vacuole in yeast (*26*). Lastly, the human sorting nexin SNX3, whose PX domain composes almost three-quarters of the protein, is required for endosomal function (*10*). Through protein-lipid binding assays and membrane localization studies, it was determined that the PX domains from Vam7 (*6, 9*), $p40^{phox}$ (*7, 8*), and SNX3 (*10*) specifically bind PtdIns(3)P and targets them to their sites of function (Fig. 1 and Table 1). Although the sorting nexins SNX7 and SNX16 also bind to PtdIns(3)P (*10*), their functions are

Table 1. Domains found within PX domain–containing proteins. In addition to binding to PIs, many PX domain–containing proteins have other domains that interact with protein targets. Together, these interactions likely play important roles in defining protein function.

Protein	Function	Domains	Target	Ref.
Vam7	Golgi-to-vacuole transport	PX	PtdIns(3)P	(*6, 9*)
		α-helical SNARE	Vam3	(*26*)
SNX3	Endosomal protein trafficking	PX	PtdIns(3)P	(*10*)
SNX7	Unknown	PX	PtdIns(3)P	(*10*)
SNX16	Unknown	PX	PtdIns(3)P	(*10*)
		Coiled-coil	Unknown	(*13*)
$p40^{phox}$	NADPH oxidase regulation	PX SH3	PtdIns(3)P	(*7, 8*)
		SH3	$p47^{phox}$	(*4*)
$p47^{phox}$	NADPH oxidase regulation	PX	PtdIns(3,4)P_2	(*8*)
		SH3a	$p47^{phox}$ COOH-terminal, cytochrome b_{558}	(*4*)
		SH3b	$p67^{phox}$, $p47^{phox}$ PX domain	(*4, 27*)
P13K C2-γ	EGF receptor signaling	PX	PtdIns(4,5)P_2	(*9*)
		Ras-binding	Unknown	(*13*)
		PI3-kinase	PtdIns, PtdIns(4)P_2	(*41*)
		C2	Unknown	(*42*)
CISK	Cell survival	PX	PtdIns(3,5)P_2, PtdIns(3,4,5)P_3	(*11*)
		Ser-Thr kinase	Transcription factors?	(*43*)

not yet known. However, not all PX domains bind PtdIns(3)P. The PX domain of p47^{phox} preferentially binds to PtdIns(3,4)P_2 (*8*), whereas the human class II PI3K C2-γ PX domain binds PtdIns(4,5)P_2 (*9*). The PX domain of CISK (cytokine-independent survival kinase) preferentially binds to PtdIns(3,5)P_2 and PtdIns(3,4,5)P_3 (*11*). So, like PH domains, different PX domains possess preferences for different PIs, likely reflecting the variability within the PX domain sequence. This is in contrast to FYVE domains, whose primary sequence is highly conserved among family members, accounting in part for its exclusive specificity for PtdIns(3)P. This diversity in PI-binding specificity of PX domains may have played a critical role in their evolution within proteins with distinct biological functions.

Structural Basis of PX Domain Interactions with PIs

The solution structure of the p47^{phox} PX domain revealed PI binding and regulatory sites. The p47^{phox} PX domain structure is composed of two lobes whose β sheet and helical bundle (*27*) (Fig. 2) closely match the Vam7 PX topology (*6*). Between the lobes, there is a binding cleft with a pair of conserved binding motifs pinpointed by changes in chemical shifts induced by PtdIns(3)P binding to the Vam7 PX domain (*6*). Specificity for a particular PI may be determined in part by the orientation and context of two motifs: RR(Y/F) (basic motif I) and R(R/K) (basic motif II). The PI interaction alters the conformation of the domain as indicated by the extent of chemical shift changes throughout the structure (*6*). Because PtdIns(3)P is anchored in the membrane bilayer, additional interactions between the PX domain and the membrane surface could be envisaged. Indeed, a membrane-binding loop was identified by mapping chemical shift changes induced in the domain by interactions with lipid micelles. Although variable in length, this exposed loop contains conserved hydrophobic groups that could insert into the bilayer, and is positioned next to basic motif II. Thus, penetration of the PX domain into the membrane may orient basic motifs I and II toward the inositol phosphates at the D3, D4, and D1 positions, respectively, by analogy to the orientation of the FYVE domain in PtdIns(3)P-containing micelles (*28*). The PX domains that bind polyphosphorylated PIs, such as p47^{phox} and the class II PI3K C2-γ, may have additional basic elements in their binding sites to coordinate additional phosphates. Like the PH domain, specificity for a particular PI may be achieved in several ways, including variable loops near the binding pocket. Thus, a predictive basis for PI specificity by PX domains must await the elucidation of the structures of several lipid complexes.

PX Domains in Protein Trafficking

The role of PX domains as membrane-targeting motifs is exemplified by their presence in proteins required for protein transport between membrane-bound organelles (Fig. 3A). Genetic studies have implicated a number of PX domain family members, such as Vam7, in yeast protein transport. The yeast Mvp1 is suggested to function in anterograde protein transport from the Golgi to the endosome (*29*), while Vps5, Vps17 (*30*), and the SNX3 ortholog, Grd19 (*31*), are required for retrograde transport from endosomal compartments. The requirement for PX domains in endosomal protein transport was not unexpected, as both PtdIns(3)P and the FYVE domain–containing proteins Vac1 (related to human EEA1 and rabenosyn-5) and Vps27 (related to human HRS) have also been implicated in endosomal trafficking (*2, 5*). However, the finding that the Vam7 PX domain

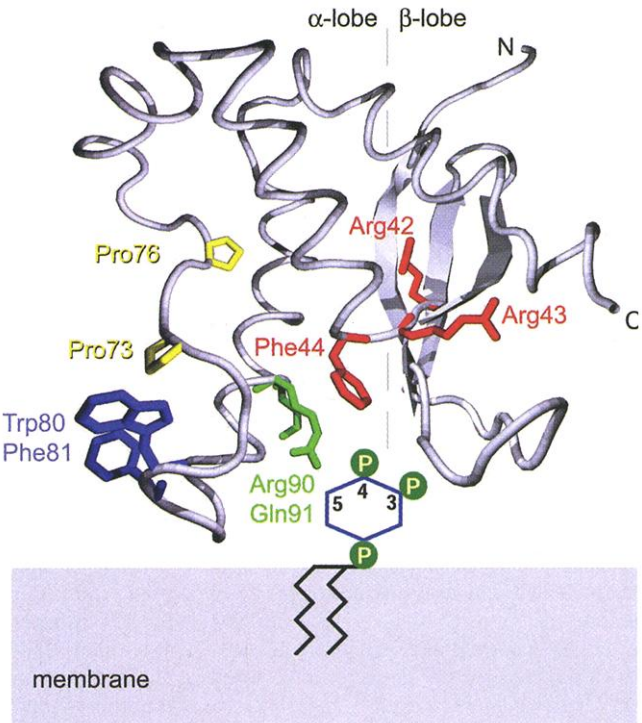

Fig. 2. Structure of the PX domain of p47^{phox}. The ribbon depicts three β strands followed by a bundle of helices. A pair of proline side chains are drawn in yellow to indicate the motif recognized by SH3 domains. The residues that correspond to the two predicted PI binding motifs; basic motif I (residues 42 to 44) and basic motif II (residues 90 and 91) are shown in red and green, respectively, and form an accessible basic pocket. The membrane interaction loop includes exposed hydrophobic residues such as Trp^{80}-Phe^{81} which may penetrate into the bilayer. The β sheet and helical lobes of the PX domain are separated by a dashed line. The NH_2- and COOH-termini are indicated by "N" and "C," respectively.

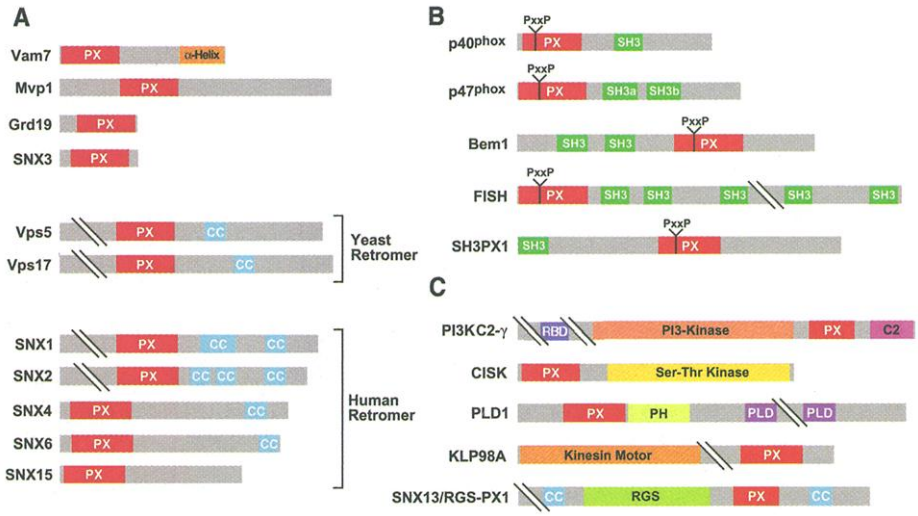

Fig. 3. Schematic representation of protein motifs found within PX domain proteins. (**A**) PX domain proteins implicated in protein trafficking. Known components of the yeast and human retromers are indicated. (**B**) Proteins containing SH3 domains and PxxP motifs within their PX domains. (**C**) PX domain proteins containing motifs with enzymatic activities implicated in cell signaling. Abbreviations: α-Helix, α-helical domain found in SNARE proteins; CC, coiled-coil domain; SH3, Src homology 3 domain; RBD, Ras-binding domain; PI 3-kinase, PI 3-kinase catalytic domain; C2, PKC conserved region 2; PH, Pleckstrin homology domain; PLD, phospholipase D catalytic motif; Kinesin Motor, Kinesin-like motor domain; RGS, regulator of G-protein signaling domain.

is a PtdIns(3)P-binding motif was more surprising, because Vam7 functions on vacuolar membranes (*26*). When separated from the rest of the protein, the Vam7 PX domain localizes to endosomes and vacuoles, indicating that it does recognize PtdIns(3)P on both compartments (*6*). However, its ability to selectively target to and function on vacuolar membranes is likely due to its additional interaction with the vacuolar-resident integral membrane t-SNARE protein, Vam3 (*26*). At its COOH-terminus, Vam7 contains an α-helical SNARE motif that binds Vam3. This α-helical domain alone cannot mediate its membrane association, because deletion of the PX domain results in complete redistribution of Vam7 into the cytoplasm (*6*). This suggests that the PX domain may function by initially targeting Vam7 to PtdIns(3)P-containing endosomes and vacuoles, while the α-helical domain mediates interactions with Vam3 that stabilize Vam7 onto the vacuolar membrane. Thus, through a combination of protein-lipid and protein-protein interactions, Vam7 is restricted to vacuolar membranes, where it functions together with Vam3 in SNARE-mediated membrane docking and fusion reactions.

PX Domain Function in Sorting Nexins

The identification of SNX3 as a PtdIns(3)P-binding protein provides new insight into the regulation of the human sorting nexins. Both human SNX3 and its yeast ortholog Grd19 mediate the retrieval of membrane proteins within the endocytic and secretory pathways. SNX3 is required to deliver endocytosed transferrin receptors from early endosomes to recycling endosomes where they are subsequently recycled back to the plasma membrane (*10*). Yeast Grd19 mediates the retrieval of resident late-Golgi transmembrane proteins from endosomal compartments by interacting with the cytoplasmic tails of the recycled cargo (*31*). Therefore, it is likely that the PX domain plays a key role in defining SNX3 and Grd19 functions in recycling by restricting their interactions with the cytoplasmic domains of cargo on PtdIns(3)P-enriched endosomal compartments, rather than on the plasma membrane and late-Golgi, respectively.

Observations that the PX domains of SNX3, SNX7, and SNX16 bind to PIs suggest that other sorting nexins may also be regulated by membrane targeting. The SNX family represents the largest subgroup of the PX domain–containing proteins. Twenty-one human SNX proteins have been identified on the basis of their homology to the PX domain of SNX1 (*13*). SNX1 forms a protein complex with the PX domain family members SNX2, SNX4, SNX6, and SNX15 (and excludes SNX3), most likely through coiled-coil interactions (*32, 33*). The SNX complex then binds to the cytoplasmic domain of numerous cell-surface receptors, including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin, and leptin receptors. In contrast to SNX3, the interactions between other SNX proteins and receptor tails mediate receptor trafficking from the plasma membrane to the lysosome where they are degraded. Studies of the yeast SNX1 homolog, Vps5, further support a role for SNX proteins in receptor trafficking (*30*). Like SNX1, Vps5 assembles into a large protein complex, called the retromer (*34*). This heterooligomeric complex contains several other proteins, including another PX domain–containing protein, Vps17. The retromer binds to the cytoplasmic tails of protein sorting receptors and processing enzymes, mediating their recycling from endosomal compartments back to the late-Golgi (*35*).

Observations suggest that retromer function may be regulated by PIs. Components of both the yeast and mammalian retromers localize to endosomal compartments that contain PIs (*30, 34, 36*). Activated PDGF and EGF receptors recruit and stimulate PI3Ks to mediate their postendocytic trafficking and degradation (*37*). The localized synthesis of PIs by activated PI3Ks may be necessary for the PX domain–mediated recruitment of the retromer to the membrane where these receptors reside. This is consistent with observations that the PX domain is essential for SNX15 partitioning to membrane fractions and for SNX15 interactions with PDGF receptors and other SNX proteins (*33*). Thus, PX domain–mediated membrane targeting and retromer assembly may be prerequisites for SNX complex associations with receptors. The presence of PX domains in SNX1, SNX2, SNX4, SNX6, and SNX15 further suggests that these retromer components may have independent capacities to associate with membranes. However, it remains possible that these complexes may be recruited from the cytoplasm upon stimulation of PI synthesis or that the oligomerization of PX domains is necessary to achieve the binding affinity and/or specificity for the correct target membrane. Like Vam7, the combination of both protein-lipid and protein-protein interactions may ensure proper targeting of the retromer. Formal proof of this PI- and protein-mediated recruitment of the retromer to the membrane awaits further experiments.

PX Domain Regulation by SH3 Domains

Many PX domain–containing proteins have one or more Src homology 3 (SH3) domains, which interact with poly-proline PxxP motifs (Fig. 3B). For example, membrane targeting by PX domains is important in coordinating the SH3 domain–dependent assembly of the human NADPH oxidase complex (*4*). In quiescent neutrophils, the $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$ oxidase subunits are present in the cytoplasm and not associated with the membrane-bound cytochrome b_{558} subunit. The phagocytosis of an invading bacterial or fungal microorganism stimulates PI synthesis on the phagocytic membrane and the resulting PIs are thought to recruit $p40^{phox}$ and $p47^{phox}$ through interactions with PX domains (*7, 8*). These oxidase subunits can then assemble with cytochrome b_{558} to form the active complex on the phagocytic membrane. Assembly is mediated in part through interactions between two SH3 domains in $p47^{phox}$ with PxxP motifs in $p67^{phox}$ and cytochrome b_{558}. The coordination of PI synthesis and these specific protein-lipid and protein-protein interactions ensures proper complex assembly on the neutrophil phagosome. This is necessary for the proper generation of highly reactive superoxides, as a mutation in the PX domain basic motif I of $p47^{phox}$ that blocks the interaction with PtdIns(3,4)P_2 is found in immunodeficient patients with chronic granulomatous disease (*8*).

In addition to binding PxxP motifs from other proteins, SH3 domains may play an important role in mediating intramolecular interactions. Analysis of the $p47^{phox}$ PX domain solution structure revealed an SH3-binding regulatory element that appears to be present in many other PX domain–containing proteins (*27*). This SH3 domain–docking site is formed by a partly exposed PxxP motif within the helical lobe of the $p47^{phox}$ PX domain (Fig. 2). Interestingly, the COOH-terminal SH3 domain of $p47^{phox}$ binds to this PxxP motif and causes a conformational change in the PX domain (*27*). Because the PxxP motif, the membrane interaction loop, and the proposed PI-binding motifs are structurally adjacent (Fig. 2), the SH3 domain interaction may lock $p47^{phox}$ in an autoinhibited state that is sterically hindered from binding membranes. Upon binding to PtdIns(3,4)P_2, the $p47^{phox}$ SH3 domain would be released from the PX domain and freed to assemble with the PxxP motifs of other oxidase subunits. Alternatively, PI binding could render the PxxP motif more solvent-accessible, allowing the PX domain to simultaneously bind to the membrane and to a SH3 domain. Indeed, the PxxP motif from the Vam7 PX domain registers dramatic chemical shift changes upon binding to PtdIns(3)P (*6*), indicating a change in the immediate environment of this element. Thus, the interaction of PX domain–containing proteins with PIs may be modulated by SH3 domain docking, although the biological role and structural basis of this regulatory mechanism remains to be elucidated.

Other PX domain–containing proteins that contain SH3 domains may mediate both intermolecular and intramolecular interactions through PxxP motifs. The yeast Bem1 protein contains the characteristic PxxP motif within its

PX domain in addition to two SH3 domains, one of which interacts with other proteins required for actin-mediated polarized growth (*38*). The requirement for PtdIns(4,5)P_2 synthesis in proper actin cytoskeletal organization strongly suggests that Bem1 functions as an adaptor molecule to connect PtdIns(4,5)P_2 with actin assembly. The mammalian FISH protein, which contains five SH3 domains and the PX domain PxxP motif, is phosphorylated by the Src tyrosine kinase (*39*). It is possible that the PX and SH3 domains function in targeting FISH to specific membrane compartments for phosphorylation by Src. Alternatively, phosphorylation may trigger the targeting of FISH to specific membranes, possibly by destabilizing an intramolecular SH3-PX domain interaction, and thus allow the SH3 domains to recruit other proteins to the membrane. Lastly, the SH3PX1 protein contains one SH3 domain that binds to the cytoplasmic domains of the integral membrane metalloprotease disintegrins, MDC9 and MDC15 (*40*). This SH3 domain may also adopt a closed, inactive conformation by binding to the PxxP motif of its PX domain. Thus, interactions with PIs may release the SH3 domain for binding to the PxxP motifs of disintegrins, or vice versa.

PX Domains in Signaling Molecules

PI-binding by PX domains is likely to play an important role in the spatial regulation of cell signaling pathways (Fig. 3C). For example, in addition to a PX domain, the class II PI3K C2 enzymes contain a C2 domain and PtdIns kinase domain that phosphorylates PtdIns and PtdIns(4)P to generate PtdIns(3)P and PtdIns(3,4)P_2, respectively (*41*). The observation that the PI3K C2 PX domain specifically binds PtdIns(4,5)P_2 suggests that the PX domain targets this lipid kinase to the plasma membrane. However, deletion of both the PX and C2 domains does not affect its association with membranes in general (*42*). Thus, the PI3K C2 PX domain is not absolutely required for membrane association as seen with other PX domain proteins, but may instead either target the lipid kinase specifically to PtdIns(4,5)P_2-containing membranes or regulate kinase activity by PtdIns(4,5)P_2 binding. Interactions between the PX domain and PIs localizes CISK to early endosomal compartments (*11*), where this serine-threonine protein kinase functions in the inhibition of apoptosis through the phosphorylation of downstream signaling targets (*43*). Finally, phospholipase D (PLD), which plays an important role in exocytosis (*44*), contains NH_2-terminal PX and PH domains. PLD hydrolyzes phosphatidylcholine (PC) to generate phosphatidic acid and choline, and its activity is stimulated by PtdIns(4,5)P_2. The presence of two different lipid-binding domains with PLD may serve a number of functions. The PX and PH domains may bind to distinct PIs for coordinating PLD activity at different sites. PLD also functions on the Golgi network for secretory protein transport in yeast (*45*), suggesting that one of these domains may bind to PtdIns(4)P. Alternatively, PLD recruitment and activation may require two distinct lipid signals recognized by PX and PH domains. Other PX domain proteins with associated enzymatic activities include *Drosophila* KLP98A and the human SNX13/RGS-PX1 (*13, 46*). KLP98A contains a kinesin motor domain that may transport PI-enriched cargo vesicles and organelles along microtubules. SNX13/RGS-PX1 contains both an RGS (regulator of G protein signaling) domain and a PX domain. RGS-PX1 is reported to bind to endosomal membranes and to interact with PtdIns(3)P and PtdIns(5)P (*46*). This localization may be important for its specific roles in accelerating GTP hydrolysis on $G_{\alpha s}$ and in the regulation of EGF receptor transport to and degradation in the lysosome.

Conclusions

The identification of the PX domain as a PI-binding motif increases the number of candidate effector proteins in PI signaling and helps to explain many of the requirements for PIs in a number of biological processes. Through a combination of protein-lipid and protein-protein interactions, PX domain proteins are targeted to their sites of function on specific intracellular membrane compartments. Once in the proper location, these proteins can then carry out additional biochemical activities, such as binding other proteins through SH3 or coiled-coil domains, regulating the assembly and activity of protein complexes, protein phosphorylation, lipid modification, and cytoskeletal organization.

Note added in proof: The crystal structure of the PX domain of the $p40^{phox}$ subunit of the NADPH oxidase complexed with PtdIns(3)P was recently published (*47*). The structure reveals how specificity for 3-phosphoinositides is achieved in an extensive basic pocket, and rules out an intramolecular effect of the SH3 domain on PI binding by this PX domain.

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25. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. x indicates any residue.
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