Erratum

The PX domain: a new phosphoinositide-binding module

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In the printed version of this Commentary, Table 1 was omitted. The Table is shown below and is included in the on-line versions.

Table 1. PX domain phosphoinositide-binding specificities

Accession Number	Protein	Species	Res	Specificity	Reference
Q15080	p40^{phox}	*Hs*	R	3P	Ellson et al. (2001a); Kanai et al. (2001)
P14598	p47^{phox}	*Hs*	R	3,4	Ago et al. (2001); Kanai et al. (2001)
O60493	SNX3	*Hs*	R	3P	Xu et al. (2001a)
Q9UNH6	SNX7	*Hs*	R	3P	Xu et al. (2001a)
P57768	SNX16	*Hs*	R	3P	Xu et al. (2001a)
Q9BSD1	RGS-PX1	*Hs*	R	3P	Zheng et al. (2001)
P32912	Vam7	*Sc*	R	3P	Cheever et al. (2001); Song et al. (2001); Yu et al. (2001)
Q08826	SNX3	*Sc*	R	3P	Yu et al. (2001)
Q01846	Mdm1	*Sc*	R	3P	Yu et al. (2001)
P38815	Yhr105w	*Sc*	R	3P	Yu et al. (2001)
P29366	Bem1	*Sc*	Y	4P	Ago et al. (2001)
Q9ERE3	CISK	*Mm*	R	3P/3,4,5 ?	Virbasius et al. (2001); Xu et al. (2001b)
Q9VTN5	CPK PI3K	*Mm*	S	4,5	Song et al. (2001)

Species abbreviations are the same as used in Fig. 1. Phosphoinositide species are abbreviated as standard. That is, 3P=PtdIns(3)*P*, 3,4,5=PtdIns(3,4,5)P_3 etc. Residues (Res) at the position equivalent to Arg58 of p40^{phox} are indicated as standard single letter amino acid code. A basic residue (R or K) at this position predicts 3-phosphoinositide binding. Only the high-affinity PX domains from Yu and Lemmon (2001) are included.

The PX domain: a new phosphoinositide-binding module

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Summary

The PX domain, which until recently was an orphan domain, has emerged as the latest member of the phosphoinositide-binding module superfamily. Structural studies have revealed that it has a novel fold and identified key residues that interact with the bound phosphoinositide, enabling some prediction of phosphoinositide-binding specificity. Specificity for PtdIns(3)*P* appears to be the most common, and several proteins containing PX domains localise to PtdIns(3)*P*-rich endosomal and vacuolar structures through their PX domains: these include the yeast t-SNARE Vam7p, mammalian sorting nexins (involved in membrane trafficking events) and the Ser/Thr kinase CISK, which is implicated in cell survival. Additionally, phosphoinositide binding to the PX domains of p40*phox* and p47*phox* appears to play a critical role in the active assembly of the neutrophil oxidase complex.

Key words: Phox, PI3-kinase, PtdIns(3)*P*, Trafficking, NADPH oxidase, SNX

Introduction

Phosphoinositide levels are exquisitely regulated within cells and constitute important spatial and temporal signals coordinating a wide range of cellular processes. The effects of phosphoinositides are mediated primarily by direct interaction with a large number of downstream effector proteins, and these protein-lipid interactions involve numerous, specific lipid-binding modules, including the pleckstrin homology (PH), FYVE and ENTH domains (Itoh et al., 2001; Ford et al., 2001) (reviewed in Hurley and Meyer, 2001). Recently, the PX domain has extended this list.

First identified in two cytosolic components of the NADPH oxidase (Ponting, 1996), PX domains are found in >100 known and hypothetical eukaryotic proteins, which have divergent functions and include the p40*phox* and p47*phox* subunits of the NADPH oxidase, class II phosphoinositide 3-kinases (PI3Ks), cytokine-independent survival kinase (CISK), members of the phospholipase D (PLD) family, sorting nexins (SNX), bud emergence (Bem) proteins and the t-SNARE Vam7p (Fig. 1).

Recent simultaneous studies from several laboratories have now shown that several PX domains act as specific phosphoinositide-binding modules (Cheever et al., 2001; Ellson et al., 2001a; Kanai et al., 2001; Song et al., 2001; Xu et al., 2001a) that have varying lipid-binding specificities (Table 1). The task of understanding their structure and ultimately their context-specific function is now well underway.

PX domain structure

Overall topology

The PX domain is approximately 120 residues long, and sequence comparisons have shown that it contains several well conserved regions, including a number of basic residues and a proline-rich stretch (Ponting, 1996). NMR spectroscopy studies of two different PX domains (from p47*phox* and Vam7p) indicated that the overall structure of the domain is a three-stranded β-sheet followed by three α-helices (Cheever et al., 2001; Hiroaki et al., 2001). When the Vam7p PX domain was studied in the PtdIns(3)*P*-bound state and compared with the unligated form, a collection of basic residues that undergo significant chemical shift upon ligation of PtdIns(3)*P* were identified. This suggested that several of the conserved basic residues identified in the primary sequence are indeed those most likely to participate in interactions with the phosphoinositide.

Very recently, Bravo et al. (Bravo et al., 2001) reported a crystal structure of the PX domain of p40*phox* bound to di-butanoyl-PtdIns(3)*P* (a soluble form of PtdIns(3)*P*) at 1.7Å resolution. This study is the most detailed and accurate to date, allowing assignment of roles for individual residues in interactions with the bound phosphoinositide. The crystal structure confirmed that the overall topology of the PX domain is similar to that reported for the p47*phox* and Vam7p PX domains: the domain forms an overall wedge shape, one face forming the phosphoinositide-binding pocket (Fig. 2A,B).

The interactions between all PX domains and their bound phosphoinositides most likely to be shared, regardless of specificity, involve the 1-phosphate and the inositol ring, because they are present in all phosphoinositide species. A hydrogen bond between a well conserved basic residue, Lys92*, and the 1-phosphate is the most important interaction with this group. Tyr59 forms the floor of the lipid-binding pocket in the PX domain, allowing the inositol ring of PtdIns(3)*P* to stack tightly against it (Fig. 2C). This carbohydrate-aromatic stacking is likely to be a common

*All residue assignments refer to p40*phox*.

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feature of binding, because all PX domains have either Tyr or Phe at the analogous position.

Lipid-binding specificity

Two of the most well conserved basic residues in the PX domain are at positions 57 and 105 in p40^{phox}. Arg105 forms hydrogen bonds with the 4- and 5-hydroxyl groups of PtdIns(3)*P*; analogous residues in other PX domains are probably well positioned to bind to phosphate groups at these positions, perhaps allowing varied lipid-binding specificities. Arg57, however, fulfils an entirely different role that was not expected on the basis of previous work. Studies of the analogous residue in other proteins had indicated that it might have a critical role in phosphate ligation of the bound phosphoinositide. NMR spectra had revealed a large chemical shift upon ligation of PtdIns(3)*P* in Vam7p (Cheever et al., 2001). In addition, a naturally occurring Arg→Gln mutation in p47^{phox} results in a non-functional NADPH oxidase (Noack et al., 2001), and mutation of this residue in p47^{phox} abrogates lipid binding (Kanai et al., 2001). However, the crystal structure shows that this residue does not project into the binding pocket but faces away from it (Fig. 2C). This suggests that Arg57 actually performs a vital structural role, forming hydrogen bonds within the hydrophobic core to stabilise the unique fold of the PX domain (Fig. 2C).

The residue that emerged as crucial for ligation of the 3-phosphate was Arg58, which is sandwiched between the fundamentally important Arg57 and Tyr59 residues. Mutation of this residue completely abolishes PtdIns(3)*P* binding in vitro and in vivo, despite the fact that structural integrity of the domain remains intact (Bravo et al., 2001).

Since Arg58 is so crucial for binding to the 3-phosphate, is it the residue that confers selectivity upon the PX domain? Indeed, all PX domains possessing a basic residue in this position that have been studied are specific for 3-phosphoinositides (Cheever et al., 2001; Ellson et al., 2001a; Kanai et al., 2001; Song et al., 2001; Virbasius et al., 2001; Xu et al., 2001a; Xu et al., 2001b; Yu and Lemmon, 2001). Conversely, the PX domains that exhibit alternative phosphoinositide-binding specificity, that is, the mouse class II PI3K [which binds to PtdIns(4,5)P_2 (Song et al., 2001)] and the yeast Bem protein Bem1p [which binds to PtdIns(4)*P* (Ago et al., 2001)] both lack the analogous basic residue (Fig. 1; Table 1). Such a theory, however, does not explain, for example, why the p40^{phox} PX domain does not bind to PtdIns(3,4)P_2, whereas the p47^{phox} PX domain does. One possibility may be that the binding pocket is too cramped to allow access to the 4-phosphate. This means that Arg105, which would be well placed to interact with the 4- and 5-phosphates of bound phosphoinositides, instead forms hydrogen bonds with the hydroxyl groups in these positions of PtdIns(3)*P*. In contrast, residues in this region of the p47^{phox} PX domain presumably do not present such stearic hindrances, allowing p47^{phox} to show a preference for PtdIns(3,4)P_2. Therefore, in addition to Arg58, the specific sequences in the variable loop and around the residue analogous to Arg105 of PX domains seem to be instrumental in determining lipid-binding specificity. A combination of molecular modelling and sequence comparisons of PX domains with known specificity may allow lipid-binding profiles to be predicted for those PX domains without known ligands. However, only comprehensive lipid-binding experiments will demonstrate actual specificities.

The NMR studies of the PtdIns(3)*P*-bound Vam7p PX domain (Cheever et al., 2001) also defined a membrane-interaction loop that immediately follows the proline-rich region and spans approximately eight hydrophobic and polar residues. This feature of the PX domain might facilitate interactions with both the surface and interior of the lipid bilayer (Fig. 2A,B). Interestingly, mutation of one residue in this region of p40^{phox} (Tyr94 to Ala) has little effect on binding to soluble di-C4-PtdIns(3)*P*, demonstrating that a lipid bilayer is necessary for detection of this interaction (Bravo et al., 2001).

PX domains as a ligand of SH3 domains

The existence of a conserved polyproline motif (PxxP) in many PX domains suggested that it may act as a target for SH3 domains (Fig. 2A,B). Numerous PX-domain-containing proteins also contain SH3 domains and form multiple contacts with other proteins. In fact, an NMR study of the isolated domains revealed that the PX domain of p47^{phox} binds to its own C-terminal SH3 domain through this PxxP motif (Hiroaki et al., 2001). It is tempting to speculate that this is an inhibitory

intramolecular association, but studies on the full-length protein are required to test this idea. The crystal structure shows that both proline residues in the PxxP motif of the PtdIns(3)P-bound p40*phox* PX domain are internal and therefore unavailable for interaction with an SH3 domain (Bravo et al., 2001). This implies either that the PX domain of p40*phox* has no SH3 ligand or that a conformational change (perhaps linked to lipid dissociation) allows the interaction to occur. Given that only one SH3-PX domain interaction has been found, the issue of whether SH3 domain binding is a general feature of PX domains is yet to be resolved.

A recent report also claims that the PX domains of p40*phox*

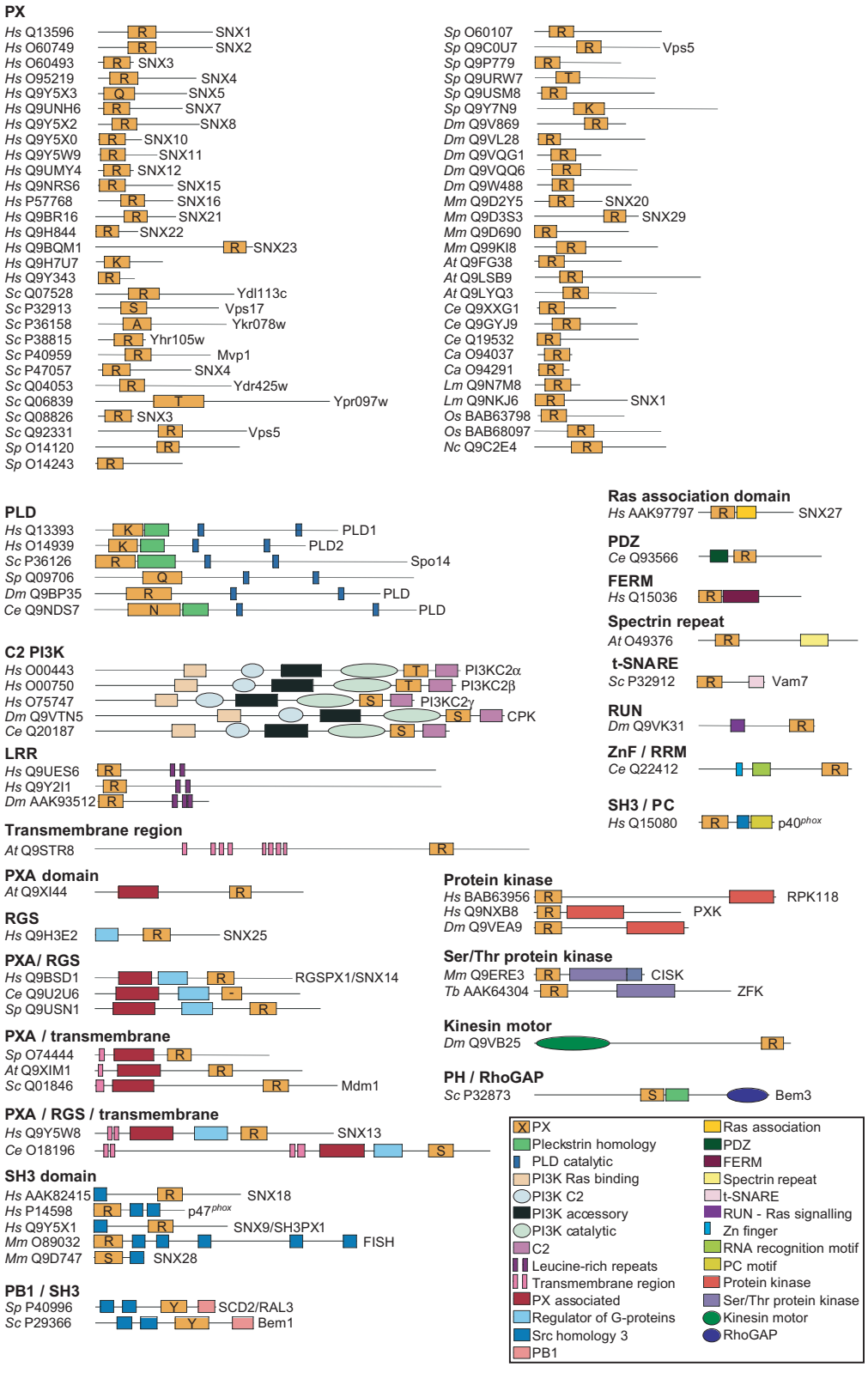

Fig. 1. Domain profiles of all PX-domain-containing proteins. Proteins were selected from the SMART database (Schultz et al., 2000), aligned, and unique proteins are shown (proteins with >80% homology were excluded as redundant or orthologous). Where appropriate, the human version is shown. Species abbreviations precede the accession numbers; *At*, *A. thaliana*; *Ca*, *C. albicans*; *Ce*, *C.elegans*; *Dm*, *D. melanogaster*; *Hs*, *H.sapiens*; *Mm*, *M.musculus*; *Nc*, *N.crassa*; *Os*. *O.satvia*; *Sc*, *S.cerevisiae*; *Sp*, *S.pombe*; *Tb*, *T.brucei brucei*; *Um*, *U.maydis*. The residue at the at the equivalent position to Arg58 of p40*phox* is shown as the standard single letter amino acid code in each PX domain. Domains with a basic residue (R or K) at this position are suggested to bind 3-phosphoinositides, whereas domains lacking a basic residue are predicted to bind other phosphoinositide species (see text).

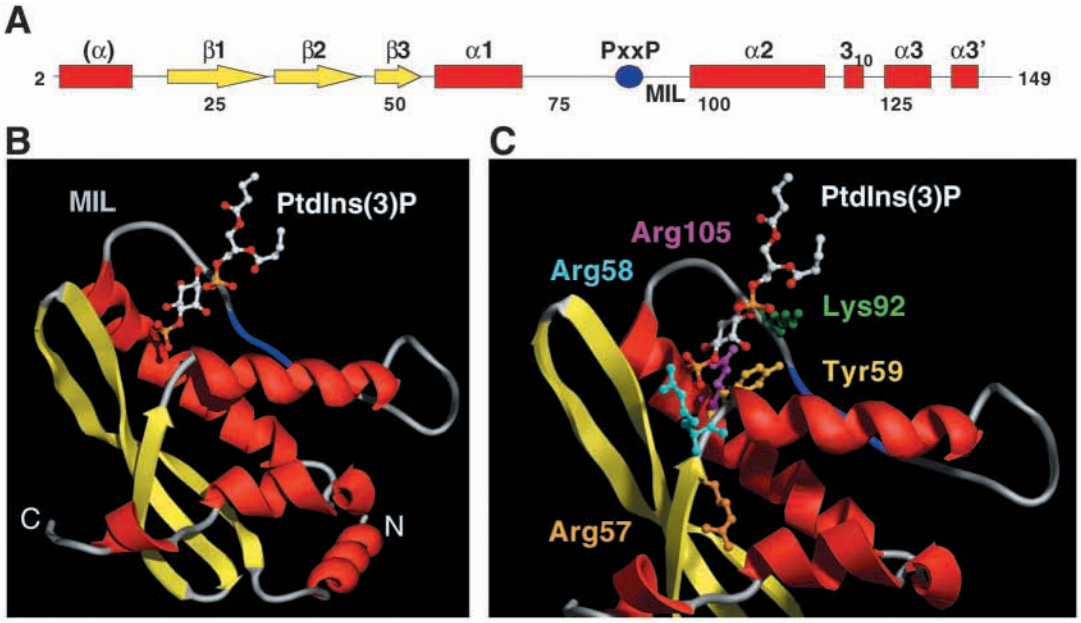

Fig. 2. (A) Secondary structure of the p40*phox* PX domain. The first α-helix is not part of the core PX domain but was included in the crystallised p40*phox* PX domain construct. MIL, membrane interaction loop; PxxP, polyproline region. (B) Tertiary structure of the overall topology of the p40*phox* PX domain bound to PtdIns(3)*P*. The membrane interaction loop of the PX domain and the acyl chains of the PtdIns(3)*P* would insert into a membrane located towards the top of the diagram. (C) Details of the lipid-binding pocket shown in (B). Key residues are illustrated to demonstrate how each one is positioned to fulfil specific roles; Lys92 hydrogen bonds with the 1-phosphate, Tyr59 stacks against the inositol ring, Arg105 interacts with the 4- and 5-hydroyl groups, Arg58 contacts the 3-phosphate and Arg57 faces away from the lipid binding pocket, fulfilling a structural role in the domain.

and p47*phox* can interact with the ERM protein moesin, although the molecular mechanism for this interaction remains to be defined (Wientjes et al., 2001).

Regulation of proteins by the PX domain binding to phosphoinositides

Several paradigms of protein regulation by lipid binding have been formulated from the extensive work on FYVE domains and PH domains. The main modes of regulation conferred by lipid binding to these domains are spatiotemporal and allosteric.

PtdIns(3)*P* in endosome and vacuole targeting

The majority of PX domains studied so far show binding selectivity for PtdIns(3)*P*, and the importance of this lipid in vesicle trafficking in yeast and mammalian cells is well described (reviewed in Stenmark and Aasland, 1999). The only yeast PI3K is Vps34p, which exclusively generates PtdIns(3)*P*. Mutation of the *vps34* gene results in aberrant sorting of proteins to the vacuole, defective endosomal processing and abnormal vacuole morphology (Herman and Emr, 1990). Levels of PtdIns(3)*P* in yeast appear to be constant, presumably maintained by an equilibrium of synthesis and degradation. By extrapolation, the human homologue of the yeast PI3K, hVps34, is thought to be responsible for maintaining the constitutively high levels of PtdIns(3)*P* found in mammalian cells (Siddhanta et al., 1998). Previously, the only known targets for PtdIns(3)*P* were proteins containing FYVE domains. FYVE domains are present in many proteins involved in vesicle trafficking, including EEA-1 and Hrs (Burd and Emr, 1998; Gaullier et al., 1998). PtdIns(3)*P* binding is required for the correct localisation and hence function of several of these proteins (Gaullier et al., 2000). Two examples of PX-domain-containing proteins thought to be involved in this type of trafficking pathway have been described: the yeast t-SNARE Vam7pp (Cheever et al., 2001) and the human sorting nexins SNX3 (Xu et al., 2001a) and RGS-PX1 (Zheng et al., 2001).

Vam7pp is a yeast t-SNARE localised to the vacuole, comprising an N-terminal PX domain and a C-terminal coiled-coil region. It has a role in the docking and subsequent fusion processes of vesicles with the vacuole, acting as a SNAP-25 homologue. Vam7p-null (*Vam7pΔ*) mutant cells accumulate aberrant membranous compartments (Sato et al., 1998). A mutant protein lacking the PX domain (Vam7pΔPX) is cytosolic, demonstrating that the PtdIns(3)*P*-specific PX domain of Vam7p is required for appropriate vacuolar localisation (Cheever et al., 2001). Studies using the *Vam7pΔ* mutant cells show that high levels of exogenous Vam7pΔPX can complement the *Vam7pΔ* phenotype, demonstrating that the PX domain is not essential for Vam7pp function. Taken together, these data suggest that the role of the PX domain is to concentrate Vam7pp on the vacuole, where it interacts with other components that regulate docking and fusion (Fig. 3A).

Mammalian sorting nexins are a family of related proteins implicated in the endocytic pathway that also contain PX domains (Teasdale et al., 2001). Several also bind to cell-surface receptors, but their exact function remains undefined

(Haft et al., 1998). Xu et al. have studied SNX3 (Xu et al., 2001a), an interesting protein in that it is little more than a PX domain (130 residues of 162 make up the PX domain). Selective binding of the SNX3 PX domain to PtdIns(3)*P* primarily localises this protein to early endosomes. Overexpression leads to swelling of the endosomal compartment reminiscent of that seen after overexpression of PtdIns(3)*P*-binding FYVE domain constructs (Gillooly et al., 2000). These SNX3-induced expanded membranous structures contain markers of sorting, recycling and late endosomes, perhaps indicating mixing of these compartments. In addition, overexpression of SNX3 leads to internalised EGF being retained in the swollen structures rather than being targeted to the lysosome for degradation. Microinjection of anti-SNX3 antibodies inhibited transport of internalised transferrin receptors from early to recycling endosomes, implicating SNX3 in the regulation of this trafficking event. Exactly how SNX3 performs its function is unclear, but, if it acts within a multiprotein complex, the only evident protein-protein-interaction module is the polyproline region in the PX domain. In this view, the PX domain of SNX3 could then act as a regulator of a secondary protein, which raises the possibility that PX domains of this protein family differ by binding to distinct protein partners or phosphoinositide species.

RGS-PX1 (also called SNX14) is another mammalian sorting nexin that is localised to endosomes through a PtdIns(3)*P*-specific PX domain (Zheng et al., 2001). In contrast to SNX3, RGS-PX1 has a defined catalytic activity. It is a GTPase-activating protein (GAP) that specifically binds to $G\alpha_s$ and accelerates the catalytic rate of GTP hydrolysis. Overexpression of RGS-PX1 significantly attenuates the effects of $G\alpha_s$-mediated signalling, such as increases in cAMP levels, through its GAP activity. Additionally, overexpression also causes a delay in EGF receptor degradation following ligand-dependent internalisation, as well as influencing EGF-dependent MAP kinase activation. RGS-PX1 may therefore act as a bridge between G protein signalling and regulation of vesicular traffic.

Two independent groups have also described PX-domain-dependent endosomal localisation of a serum- and glucocorticoid-regulated kinase (SGK) family member (Virbasius et al., 2001; Xu et al., 2001b). Cytokine-independent survival kinase (CISK) is a Ser/Thr kinase that can protect cells from apoptosis following serum withdrawal. It shares significant homology with protein kinase B (PKB) in its kinase domain and is also thought to share at least a subset of the same substrates, such as Bad and the forkhead transcription factor FKHRL1 (which is inhibited by phosphorylation) (Liu et al., 2000). Work on PKB has shown that it has a PH domain specific for PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 N-terminal to the kinase domain. Production of PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 at the plasma membrane recruits PKB, colocalising it with its upstream activating kinase PDK1 (which also contains a PtdIns(3,4,5)P_3-specific PH domain) (Alessi et al., 1997; Stephens et al., 1998). Binding of lipids to PKB is thought to induce a conformational change in the protein that relieves an inhibitory constraint, allowing activating phosphorylation by PDK1 (Stokoe et al., 1997).

CISK has an N-terminal PX domain, rather than the PH domain of PKB, and is activated through stimulation of cell surface receptors coupled to IGF-1 and EGF. Non-lipid-binding mutants prevent both endosomal localisation of the protein and downstream inhibition of FKHRL1. Interestingly, deletion of the PX domain enhanced inhibition of FKHRL1 activity, suggesting that the unliganded PX domain autoinhibits CISK in a manner analogous to that predicted for the PH domain of PKB (Xu et al., 2001b). These analogies may extend further in that the key activating phosphorylation sites in PKB (Thr308 in the kinase domain and Ser473 in the C-terminal tail) also appear to be conserved in CISK, which suggests that PDK1 or a PDK-like kinase is responsible for the phosphoinositide-dependent phosphorylation and activation of CISK (Fig. 3B). The lipid-binding properties of the CISK PX domain are somewhat disputed: Xu et al. report specificity for PtdIns(3,4,5)P_3 and PtdIns(3,5)P_2, whereas Virbasius et al. demonstrate exclusive binding to PtdIns(3)*P*. The reason for this discrepancy is not clear because both groups use

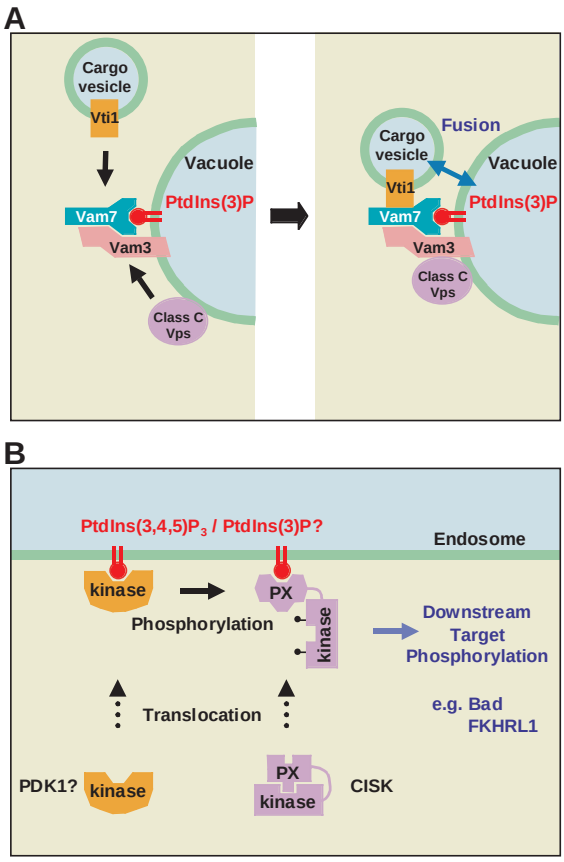

Fig. 3. (A) A model for the role of Vam7p in vesicle-vacuole docking. Vam7p is localised to the vacuole by interaction of its PX domain with PtdIns(3)*P* and through interaction with Vam3 (explaining how overexpression of Vam7pΔPX can still facilitate docking, as it can be localised through Vam3, albeit to a lower extent than the wild type). The cargo vesicle docks to Vam7p through Vti1, and the Class C Vps complex also joins. Assembly of this complex facilitates vesicle-vacuole fusion (Sato et al., 2000). (B) A model of regulation of CISK by interaction with phosphoinositides. CISK is localised to membranes by PtdIns(3)*P* or PtdIns(3,4,5)P_3. This may allow it to be activated through phosphorylation by an upstream kinase, perhaps PDK or a PDK-like kinase. Once active, CISK can phosphorylate downstream targets to exert effects on cell survival.

essentially the same 'protein-lipid overlay' binding assay, although Virbasius et al. also present convincing liposome binding data. Certainly, PtdIns(3)*P* binding more easily explains the endosomal localisation of CISK; however, PtdIns(3,4,5)P_3 binding would more easily explain how CISK couples to agonist-stimulated events originating at the plasma membrane. Whatever the precise lipid-binding specificity of CISK, there seem to be very strong parallels, both in the way CISK and PKB are regulated by phosphoinositides and in the roles these two kinases play in the regulation of cell survival.

PtdIns(3)*P* as a target on phagosomes

Two reports have recently demonstrated a new cellular site of PtdIns(3)*P* generation (Vieira et al., 2001; Ellson et al., 2001b). These studies focused on professional phagocytic cells that engulf pathogenic material and cell debris and breakdown the contents of the ingested phagosome by the co-ordinated delivery of vesicle-bound digestive enzymes and reactive oxygen species (reviewed in Berón et al., 1995; Desjardins et al., 1994; Desjardins et al., 1997; Babior, 1999). Both groups observed that PtdIns(3)*P* is rapidly and transiently generated on phagosomal membranes following closure of the phagosome. The kinetics of this rise and fall in PtdIns(3)*P* levels strongly suggest a role for this lipid in temporal localisation of proteins involved in maturation of the phagosome as a prerequisite for entry into the lysosomal pathway. Using inhibitory antibodies, Vieira et al. also demonstrated that hVps34 activity is required for this rise in PtdIns(3)*P* to occur and that the PtdIns(3)*P* is necessary for efficient maturation of the phagosome.

These are the first reports to show rapid upregulation of PtdIns(3)*P* synthesis in response to extracellular stimuli, which contrasts with the maintenance of steady-state levels of PtdIns(3)*P* in endosomes. What are the targets of PtdIns(3)*P* in this pathway? Clearly, PX-domain-containing proteins involved in vesicle trafficking, such as members of the sorting nexin (SNX) family, are obvious candidates. FYVE-domain-containing proteins (such as EEA1) could also interact with this PtdIns(3)*P*, allowing docking and fusion of digestive-enzyme-containing vesicles.

In addition to these reports, previous studies have shown that the entire process of phagocytosis correlates with spatially and temporally regulated synthesis of three other phosphoinositide species: PtdIns(3,4,5)P_3, PtdIns(3,4)P_2 and PtdIns(4,5)P_2 (Botelho et al., 2000; Marshall et al., 2001). This again provides potential localisation signals for lipid-binding-domain-containing proteins, including those that have PX domains.

In conjunction with content delivery from intracellular vesicle populations, production of microbicidal reactive oxygen species into the phagosomal vacuole is also essential for efficient killing of ingested material. The enzyme complex responsible for the generation of these superoxide anions is the NADPH oxidase. It contains a membrane-bound cytochrome and four cytosolic components – Rac, p67^{phox}, p47^{phox} and p40^{phox} (reviewed in Babior, 1999). Many regulatory inputs are known to impinge on the assembly and activity of the oxidase, and now lipid regulation through PX-domain-containing proteins must be integrated. p40^{phox} and p47^{phox} contain PX domains showing specificity for PtdIns(3)*P* and PtdIns(3,4)P_2, respectively (although the p47^{phox} PX domain is much less selective, exhibiting significant binding to several other phosphoinositides) (Ellson et al., 2001a, Kanai et al., 2001). The cytosolic phox proteins are generally thought to exist in a basal complex, translocating to the membrane-bound cytochrome upon activation by stimulation. The phagovacuole-localised rise in PtdIns(3)*P* levels is clearly a potential target for the translocation of PtdIns(3)*P*-binding p40^{phox}. This translocation may help recruit other members of the cytosolic complex, facilitating interaction with the cytochrome and, ultimately, activation of the oxidase. Similarly, PtdIns(3,4)P_2, which was shown to accumulate in the phagocytic cup (Marshall et al., 2001), could target the cytosolic complex by interacting with the p47^{phox} PX domain. This potential mode of activation illustrates the importance of establishing the credentials of the p47^{phox} PX-SH3 domain interaction, that is, what is the relationship between phosphoinositide binding and SH3 domain binding? Phosphorylation of p47^{phox} is thought to disrupt intramolecular associations (perhaps including the PX-SH3 domain interaction) to expose previously masked domains, subsequently allowing direct interaction of p47^{phox} with the cytochrome (p40^{phox} has not been shown to bind to the cytochrome) (Ago et al., 1999). This suggests that the PX domains in this pathway act as both spatiotemporal and allosteric regulators. Note, however, that, to date, there is no direct evidence for these PX-domain-mediated mechanisms in oxidase activation.

Conclusion

The PX domain has emerged as a major phosphoinositide-binding module. PtdIns(3)*P* produced constitutively in endosomes or following stimulation in phagocytes is a clear target for proteins that possess PtdIns(3)*P*-binding PX domains. In this respect, PX domains resemble FYVE domains (although the two are structurally distinct), and it is not surprising that proteins containing either PX or FYVE domains are implicated in similar trafficking pathways. In addition, PX domains that have alternative specificities could be targeted to other phosphoinositides and participate in pathways in which proteins with other lipid-binding modules (such as PH or ENTH domains) participate. With this knowledge, we can now rapidly study the function and regulation of PX-domain-containing proteins.

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