REVIEW ARTICLE

The Phox homology (PX) domain, a new player in phosphoinositide signalling

Yue XU, Li-Fong SEET, Brendon HANSON and Wanjin HONG[1]

Membrane Biology Laboratory, Institute of Molecular and Cell Biology, 30 Medical Drive, Singapore 117609, Singapore

Phosphoinositides are key regulators of diverse cellular processes. The pleckstrin homology (PH) domain mediates the action of PtdIns(3,4)P_2, PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, while the FYVE domain relays the pulse of PtdIns3*P*. The recent establishment that the Phox homology (PX) domain interacts with PtdIns3*P* and other phosphoinositides suggests another mechanism by which phosphoinositides can regulate/integrate multiple cellular events via a spectrum of PX domain-containing proteins. Together with the recent discovery that the epsin N-terminal homologue (ENTH) domain interacts with PtdIns(4,5)P_2, it is becoming clear that phosphoinositides regulate diverse cellular events through interactions with several distinct structural motifs present in many different proteins.

Key words: endosomes, membranes, phosphoinositide-binding domains, protein traffic, signal transduction.

INTRODUCTION

Phosphoinositide (PI) metabolism

Cellular membranes, composed primarily of lipids and proteins, not only serve as structural manifolds that demarcate the cell from its extracellular milieu and compartmentalize its interior into discrete functional organelles but also participate in the regulation of many cellular processes. While the functions of a myriad of membrane proteins, such as signalling receptors and ion channels, have been well studied, it is increasingly recognized that the lipid constituents of the membrane are also involved in many regulatory events [1]. PI is a collective term for PtdIns and its phosphorylated derivatives (Figure 1) [2–6]. PtdIns contains a 1D-*myo*-inositol phosphate group linked to diacylglycerol. The D-*myo*-inositol head group of PtdIns contains five hydroxy groups (at positions 2, 3, 4, 5 and 6), three of which (at positions 3, 4 and 5) are known to be targets of phosphorylation. These positions may be reversibly phosphorylated singly, doubly (in various combinations) or triply, yielding a myriad of PtdIns derivatives: PtdIns3*P*, PtdIns4*P*, PtdIns5*P*, PtdIns(3,4)P_2, PtdIns(3,5)P_2, PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3. Among the eight PI species known in eukaryotic cells, PtdIns is most abundant and its concentrations can be 10–20-fold higher than those of PtdIns4*P* and PtdIns(4,5)P_2, which are present at comparable levels. Among the singly phosphorylated PIs, PtdIns4*P* is the most abundant (90–96 %), while PtdIns3*P* and PtdIns5*P* each make up approx. 2–5 %. PtdIns(4,5)P_2 is the most abundant (representing $>$ 99 %) of the doubly phosphorylated PIs, while PtdIns(3,4)P_2 and PtdIns(3,5)P_2 exist at levels that are approx. 500-fold lower than that of PtdIns(4,5)P_2. The cellular levels of triply phosphorylated PtdIns(3,4,5)P_3 vary dramatically in response to both external and internal regulation and can be up-regulated to levels comparable with those of PtdIns(3,4)P_2 and PtdIns(3,5)P_2. Generally, PtdIns(3,4)P_2, PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 are enriched in the plasma membrane [7–11], whereas PtdIns3*P* is enriched in the endosomal compartments [12].

PIs are now known to regulate many biological processes, including cell proliferation, cell survival, differentiation, signal transduction, cytoskeleton organization and membrane trafficking [2,4–6,13,14]. The regulation of their activities and the mechanisms of their actions have therefore been the subject of much investigation. The activities of specific PIs are regulated by controlling their levels in the cell, which is achieved by intricate networks of proteins that control their synthesis, transport and degradation.

Lipid kinases

PI synthesis is regulated by PI kinases and phosphatidylinositol phosphate (PIP) kinases (PIPKs), and PI degradation is governed by lipid phosphatases, while their transport is mediated by PtdIns transfer proteins (PITPs). The hydroxy groups at positions 3, 4 and 5 on the inositol ring can be phophorylated by the lipid kinases: PtdIns 3-kinases (PI3Ks), PtdIns 4-kinases (PI4Ks),

Abbreviations used: AP180, adaptor protein 180; ARF, ADP-ribosylation factor; ALK, activin-like receptor; ARNO, ARF nucleotide binding site opener; BTK, Bruton's tyrosine kinase; CALM, clathrin assembly lymphoid myeloid leukaemia protein; CISK, cytokine-independent survival kinase; CPY, carboxypeptidase Y; DPAP A, dipeptidyl aminopeptidase A; EEA1, early endosome autoantigen 1; EGF, epidermal growth factor; ENTH, epsin N-terminal homologue; FANCA, Fanconi anaemia protein A; GAP, GTPase-activating protein; GEF, guanine nucleotide exhange factor; GFP, green fluorescent protein; GRP1, general receptor for phosphoinositides-1; HIP, huntingtin interacting protein; Hrs, hepatocyte growth factor-regulated tyrosine kinase substrate; HS1, haemopoietic-specific protein 1; HS1BP3, HS1 SH3 domain binding protein; IL, interleukin; I-1 RCP, imidazoline-1 receptor candidate protein; MAP, mitogen-activated protein; MDC protein, metalloprotease, disintegrin, cysteine-rich protein; MTM1, myotubularin; MTMR, myotubularin-related protein; PDGF, platelet-derived growth factor; PDK1, phoshoinositide-dependent kinase 1; PH, pleckstrin homology; PI, phosphoinositide; PI3Ks, PtdIns 3-kinases; PI4Ks, PtdIns 4-kinases; PI5Ks, PtdIns 5-kinases; PIP, phosphatidylinositol phosphate; PIPK, PIP kinase; PITPs, PtdIns transfer proteins; PKB, protein kinase B; PLC, phospholipase C; PLD, phospholipase D; PX, Phox homology; PXA, PX domain-associated; PXK, PX domain-containing protein kinase; SARA, Smad anchor for receptor activation; SM domain, SNX1–Mvp1p domain; SDPs, SM domain proteins; SH, Src homology; SHIP, SH2 domain-containing inositol 5-phosphatase; SKIP, skeletal muscle and kidney-enriched 5-inositol phosphatase; SNARE, soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor; SNX, sorting nexin; TGF, transforming growth factor; VHS, Vps27p/Hrs/Stam.

[1] To whom correspondence should be addressed (e-mail mcbhwj@imcb.nus.edu.sg).

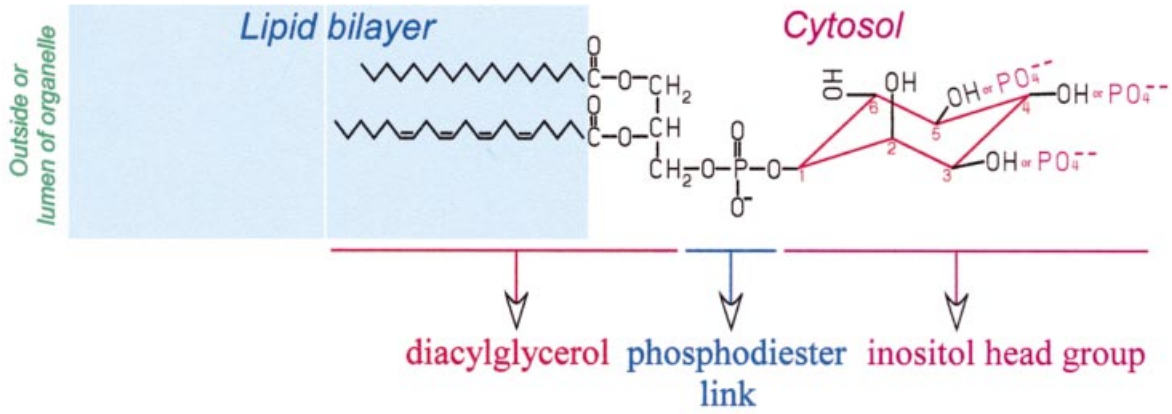

Figure 1 Chemical structure of PIs

As indicated, the hydroxy groups at positions 3, 4 and 5 of the inositol ring can be reversibly phosphorylated singly, doubly (in various combinations) or triply to give rise to various species of PIs.

Table 1 Summary of mammalian lipid kinases

Lipid kinase	Isoforms	Number of residues	Regulatory subunits	Major substrates	Yeast homologues	GenBank® accession number
PI3K						
Class IA	p110α	1068	p85α, p55α	PtdIns(4,5)P_2		P42336
	p110β	1070	p50α, p85α/β	PtdIns(4,5)P_2		P42338
	p110δ	1044	p55γ	PtdIns(4,5)P_2		O00329
Class IB	p110γ	1101	p85α, p55α	PtdIns(4,5)P_2		P48736
Class II	PI3K-C2α	1686		PtdIns4P, PtdIns?		NP_002636
	PI3K-C2β	1634		PtdIns4P, PtdIns?		O00750
	PI3K-C2γ	1448		PtdIns4P, PtdIns?		O75747
Class III	Vps34	887	p150/Vps15p	PtdIns	Vps34p	NP_002638
PI4K						
Class II	PI4KIIα	479		PtdIns	YJL100w	AJ303098
	PI4KIIβ	481		PtdIns		AF411320
Class III	PI4KIIα	2041		PtdIns	Stt4p	D83538
	PI4KIIβ	816	Frequenin?/Frq1p	PtdIns	Pik1p	D84667
PI5K	PIKfyve	2052		PtdIns3P, PtdIns	Fab1p	NP_035216
PIPK						
Class I	PIP5Kα	549		PtdIns4P, PtdIns3P?	Mss4p	NP_003548
	PIP5Kβ	546		PtdIns4P, PtdIns3P?		NP_032873
	PIP5Kγ	661		PtdIns4P, PtdIns3P?		AB006916
Class II	PIP4Kα	406		PtdIns5P, PtdIns3P		NP_005019
	PIP4Kβ	416		PtdIns5P, PtdIns3P		NP_003550
	PIP4Kγ	420		PtdIns5P, PtdIns3P		AF030558

PtdIns 5-kinases (PI5Ks) and PIPKs (Table 1) [2,5,6]. Three classes of PI3Ks have been identified based on their primary structure, regulation and substrate specificity. Class I PI3Ks can produce PtdIns3P, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 and are regulated by receptor tyrosine kinases and G-protein-coupled receptors; class II PI3Ks can generate PtdIns3P and PtdIns(3,4)P_2 and contain Phox homology (PX) and C2 domains at their C-terminal regions; and class III PI3Ks phosphorylate mainly PtdIns to produce PtdIns3P. Class III PI3Ks are generally thought to be responsible for the generation of the majority of PtdIns3P. The yeast Vps34p is a class III PI3K and is essential for the trafficking of hydrolytic enzymes from the late Golgi to the vacuole [15]. In mammalian cells, class III PI3K (Vps34p homologue) is likely to be involved in spatial regulation of target molecules, since it is specifically recruited on to or activated in the endosomes [16], and its activation depends on a 150 kDa (p150) adaptor that is homologous to yeast Vps15p [17]. Two classes (type II and type III) of PI4Ks have been molecularly described that use PtdIns as substrate to produce PtdIns4P. The recently cloned 55 kDa PI4KIIα and PI4KIIβ (which are 56% identical) represent type II PI4Ks [18,19]. Type III PI4Ks are represented by the 230 kDa PI4Kα, which is homologous to yeast Stt4p, and the 92 kDa PI4Kβ, which is homologous to yeast Pik1p [2]. PIKfyve/p235, the mammalian homologue of yeast Fab1p, is a known PI5K that may use PtdIns and PtdIns3P to produce PtdIns5P and PtdIns(3,5)P_2 respectively [5,20–23].

Table 2 Summary of mammalian lipid phosphatases

Among the MTMRs, the amino acid sequences in the catalytic domains of MTMR5/SBF1 (GenBank® accession number = AAC39675) and MTMR8 [203] are altered, suggesting that they do not have catalytic activities and are therefore not included. Although Inp51p/Sjl1p also contains a Sac1p-homologous domain, it is not functional, while those in Inp52p/Sjl52p and Inp53p/Sjl53p are functional. MTMR3 is also known as FYVE-DSP1, and MTMR4 is also known as FYVE-DSP2. PIP_3, PtdIns(3,4,5)P_3; PIPP, proline-rich inositol polyphosphates-phosphatase.

Lipid phosphatase	Isoforms	Number of residues	Major substrates	Yeast homologues	GenBank® accession number
3-Phosphatases	PTEN	403	PtdIns3*P*, PtdIns(3,4)P_2, PtdIns(3,5)P_2, PIP_3	Tep1p	O00633
	MTM1	603	PtdIns3*P*	YJR110w	Q13496
	MTMR1	662	PtdIns3*P*		Q13613
	MTMR2	643	PtdIns3*P*		Q13614
	MTMR3	1198	PtdIns3*P*		NP_066576
	MTMR4	1195	PtdIns3*P*		NP_004678
	MTMR6	661	PtdIns3*P*		AF406619
	MTMR7	Partial	PtdIns3*P*		AF073482
	Sac1	587	PtdIns3*P*, PtdIns4*P*, PtdIns(3,5)P_2	Sac1p, Fig4p	AF251186
4-Phosphatases	Type I	938	PtdIns(3,4)P_2, PIP_3?		U26398
	Type II	924	PtdIns(3,4)P_2, PIP_3?		U96922
5-Phosphatases	SKIP	448	PtdIns(4,5)P_2, PIP_3	Inp51p/Sjl1p, Inp54p	NP_057616
	OCRL protein	901	PtdIns(4,5)P_2, PIP_3		Q01968
	5-Phosphatase II	1017	PtdIns(4,5)P_2, PIP_3		T42384
	SHIP1	1190	PtdIns(4,5)P_2, PIP_3		U39203
	SHIP2	1258	PtdIns(4,5)P_2, PIP_3		Y14385
	Synaptojanin 1	1575	PtdIns3*P*, PtdIns4*P*, PtdIns5*P*, PtdIns(3,5)P_2, PtdIns(4,5)P_2, PIP_3	Inp52p/Sjl2p	O43426
	Synaptojanin 2	1248	PtdIns3*P*, PtdIns4*P*, PtdIns5*P*, PtdIns(3,5)P_2, PtdIns(4,5)P_2, PIP_3	Inp53p/Sjl3p	O55207
	Pharbin	647	PtdIns(3,5)P_2, PIP_3		AF226683
	PIPP	1001	PtdIns(4,5)P_2, PIP_3		BAA90553

Finally, two classes of PIPKs are known: the 68 kDa PIP5K(α, β and γ) that uses PtdIns4*P* to generate PtdIns(4,5)P_2 and the 53 kDa PIP4K(α, β and γ) that phosphorylates PtdIns3*P* and PtdIns5*P* to produce PtdIns(3,4)P_2 and PtdIns(4,5)P_2. It is noted that some of these lipid kinases are regulated by small GTPases. Both PI4Kβ and PIP5Kα have been shown to be recruited on to the Golgi and activated by ADP-ribosylation factor (ARF)1 [24,25], while the recruitment of PIP5Kα on to the plasma membrane is mediated by ARF6 [26]. Rab5 has been shown to regulate recruitment of class I (p110β-p85α) and class III (Vps34-p150) PI3Ks on to clathrin-coated vesicles and early endosomes respectively [16].

Lipid phosphatases

Lipid phosphatases that remove phosphate groups at specific positions on the phosphorylated inositol head group are divided into three major categories based on their ability to hydrolyse 3-, 4-, or 5-phosphorylated PIs (Table 2) [6,27–29]. PtdIns 3- and 4-phosphatases are characterized by the CXXXXXR consensus motif in the catalytic domain, whereas type II PI 5-phosphatases contain two conserved motifs [WXGDXNXR and PXW(C/T)DRXLW; where single-letter amino-acid notation has been used] that are located approx. 60 amino acids apart in the catalytic core region of approx. 300 residues. PI 3-phosphatases include the tumour suppressor PTEN, myotubularin (MTM1), myotubularin-related proteins (MTMR1, MTMR2, MTMR3, MTMR4, MTMR6, and MTMR7) and Sac1. Two types of PtdIns 4-phosphatases are known to down-regulate PtdIns(3,4)P_2. Type II PI 5-phosphatases that can catalyse the hydrolysis of PtdIns(3,4,5)P_3 and possibly other 5′-PIs form a family of five subgroups: the skeletal muscle and kidney-enriched 5-inositol phosphatase (SKIP); GTPase-activating protein (GAP)-containing inositol 5-phosphatases ('GIPs') which have two members (the OCRL protein and 5-phosphatase II); Src homology (SH)2 domain-containing inositol 5-phosphatases (SHIPs) which have two members (SHIP1 and SHIP2); Sac1p-containing inositol 5-phosphatases ('SCIPs'), which include synaptojanin 1 and synaptojanin 2; and proline-rich domain-containing inositol 5-phosphatases that include pharbin and PIPP (proline-rich inositol polyphosphates-phosphatase). In addition, multiple isoforms of each of the phosphatases exist to refine the regulatory process. For example, synaptojanin 2, a Sac1 homology domain-containing 5-phosphatase, has at least eight isoforms [30,31].

Other regulators

In addition to these enzymes that regulate PI metabolism, PIs are also substrates for other enzymes, such as phospholipase C (PLC) and PtdIns-directed phospholipase D (PLD) [32,33]. These proteins not only alter the levels of membrane PIs, but at the same time convert the PIs into soluble inositol polyphosphates and diacylglycerol that can act as second messengers. In addition, regulation of membrane trafficking and inositol lipid signalling also involves two closely related PtdIns/phosphatidylcholine transfer proteins (PITPα and PITPβ) [34,35]. In yeast, the major PITP is represented by Sec14p which is important for trafficking from Golgi to the plasma membrane [36,37]. These proteins, as the name implies, have the ability to transfer specific phospholipids such as PtdIns and phosphatidylcholine between membrane compartments. PITPs may also facilitate the phosphorylation of PtdIns by presenting these lipids to appropriate lipid kinases. The synthesis of PtdIns3*P*, PtdIns4*P*, PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 has been reported to be stimulated by the action

of PITPs. In addition, three other proteins (retinal degeneration Bβ, Nir2 and Nir3) also contain the PITP domain, but their physiological roles in PI metabolism remain to be established [38,39].

Physiological relevance of PI metabolism

Maintenance of the delicate balance of different types of PIs in the cell is central towards facilitating their roles as critical signals for diverse processes. The importance of the proper regulation of PI levels is highlighted by the presence of several abnormalities that are associated with mutations in the enzymes responsible for their metabolism. For example, human Lowe oculocerebrorenal syndrome is associated with mutations in the OCRL-1 gene [40]. Mutations in PTEN have been discovered in many tumours [28,41], while mutations in the MTM1 gene have been shown to cause the muscle disease X-linked myotubular myopathy [27]. Charcot-Marie-Tooth type 4B, an autosomal recessive demyelinating neuropathy with myelin outfoldings, is caused by mutations in the gene encoding MTMR2 [42]. Another example is the lethal neurodegenerative disease in the *vibrator* mice which results from reduced levels of PITPα [43,44]. The importance of PI turnover is also exemplified by synaptojanin 1 knockout mice which accumulated clathrin-coated vesicles [45].

KNOWN MECHANISMS OF PI-MEDIATED ACTIONS

Action of PIs

In addition to their roles as precursors for secondary messengers generated by PLC and PI-specific PLD, as well as local modulators of lipid compositions of membranes, the major underlying mechanism by which PIs regulate cellular processes is via their ability to serve as site-specific membrane signals to affect the intracellular localizations and/or biological properties of effector proteins (or protein complexes). An increasing number of PI-binding motifs with specific lipid binding specificities and affinities have been identified (Figure 2) [3,5,6,13,14,46,47], including the FYVE domain [48], the pleckstrin homology (PH) domain [49,50], the epsin N-terminal homologue (ENTH) domain [51,52] and the PX domain [53]. An interesting observation is that the PH domain can coexist with either the FYVE or the PX domain in the same protein, while the coexistence of the PX and the FYVE domains has not been encountered. The ENTH domain has not been found to coexist with any of the other three domains and is always present at the extreme N-terminus. It will be of interest to investigate the relationship between PH and FYVE (or PX) domains in terms of spatial regulation and functional activities in proteins containing both domains. Other domains, such as C2 domains and some SH2 domains, have also been shown to interact with PIs [54,55].

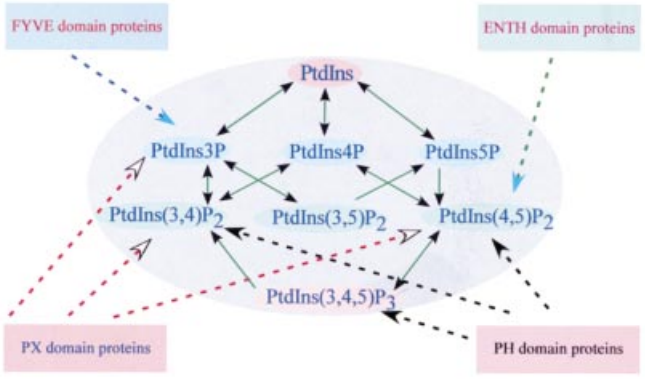

Figure 2 Summary of PI-interacting domains

The PI species that are targeted by the various PI-binding domains are indicated. The relationship between these PIs is indicated. Conversions between different PIs are catalysed by various lipid kinases (see Table 1) and phosphatases (see Table 2).

The FYVE domain

The term FYVE domain is derived from the first letters of the four proteins first identified to contain it: Fab1p, YOTB, Vac1p and EEA1 [48]. The FYVE domain of human early endosome autoantigen 1 (EEA1) was first defined as a motif responsible for binding exclusively to PtdIns3*P* [56–59]. As a subclass of double zinc-finger domains, the FYVE domain is composed of 60–80 amino acids, including eight conserved cysteine residues (characteristic of the zinc-finger) and a cluster of basic residues surrounding the third and fourth cysteine residues: R(R/K)HHCRXCG (characteristic of the FYVE domain). These eight conserved cysteine residues co-ordinate binding of the FYVE domain with two zinc atoms. The FYVE domain is conserved throughout evolution, being present in yeast, worm, fly and mammals. More than 30 mammalian proteins containing the FYVE domain have been identified [13,60]. The interaction of some of these proteins, including mammalian EEA1, hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs), Rabenosyn-5, endofin, Rabip4, and the yeast Vac1p/Pep7p, Vps27p, Fab1p and Pib1p, with PtdIns3*P* via the FYVE domain has been verified by *in vitro* binding studies [5,13,56,58,61–63]. Only the FYVE domain of endofin and yeast Pib1p is by itself sufficient for endosomal association [63,64], EEA1 and Hrs are targeted to the endosomes via the concerted action of the FYVE domain and/or Rab5-binding domain and coiled-coil domain respectively [65–67].

The structural basis for the FYVE domain to interact with PtdIns3*P* has been studied [68]. The crystal structure of the yeast Vps27p FYVE domain has been determined at 1.15 Å resolution (where 1 Å = 0.1 nm) [69]. Vps27p is the proposed yeast homologue of the mammalian Hrs. The Vps27p FYVE domain consists of a pair of double-stranded antiparallel β-sheets (β1–β2 and β3–β4) that are stabilized by the two tetrahedrally co-ordinated zinc ions followed by a C-terminal α-helix (see Figure 5). The characteristic cluster of basic residues is located on the β1-strand and the following β-turn and, together with a conserved arginine residue in the β4-strand, forms a defined basic pocket for interaction with the negatively charged 1,3-bisphospho-*myo*-inositol head group of PtdIns3*P*. The shallow groove that forms the PtdIns3*P* binding site is too small for PtdIns(4,5)P_2 or PtdIns(3,4,5)P_3 and is incompatible with other PIs, such as PtdIns4*P*, thereby accounting for the binding specificity of the motif. In addition, hydrophobic residues preceding the first β-strand are predicted to form a hydrophobic loop for interaction with the membrane. The crystal structure of the *Drosophila* Hrs FYVE domain in tandem with the Vps27p/Hrs/Stam (VHS) domain at 2 Å resolution has also been defined and the FYVE domain has a structure similar to that of Vps27p. In addition, it suggests a homodimer model for PtdIns3*P* binding [70]. The solution structure of the PtdIns3*P*-bound FYVE domain of EEA1 has been defined by heteronuclear NMR analysis, which suggests a multistep binding mechanism [71,72]. The first event involves non-specific insertion of the hydrophobic loop (preceding the β1-strand) into the lipid bilayer. This weak interaction with the membrane opens the binding site for PtdIns3*P* in the

basic pocket, allowing specific interaction with PtdIns3*P*. Ligation of PtdIns3*P* then induces a global structural change, resulting in stable interaction with the PtdIns3*P*-enriched membrane.

Many FYVE domain proteins also contain other domains, such as protein kinase, lipid kinase, phosphatase, Smad-binding, C_2H_2 finger or VHS, PH, RUN (RPIP8, UNC-14 and NESCA) or Rho guanine nucleotide exhange factor (GEF) domains, suggesting that they may regulate diverse cellular processes in response to interaction with PtdIns3*P* [13,60]. The FYVE domain in this diverse array of proteins is thought to function as a membrane-targeting motif that specifically recognizes PtdIns3*P* so that proteins containing them are selectively recruited to the correct cellular compartment for activity and/or as a regulatory domain to control their functional activities. Many of the characterized FYVE domain proteins have been localized to early endosomes, suggesting that they may be involved in membrane traffic in the endocytic pathway. Indeed, several yeast FYVE proteins, including Vps27p, Vac1p and Fab1p, have been shown to regulate post-Golgi endosomal traffic [5,73], while EEA1 and Rabenosyn-5 have been shown to play roles in endosome fusion [62,65,74]. A recent study that investigated the distribution of two copies of the FYVE domain of Hrs tagged to a green fluorescent protein (GFP) reporter has revealed that this fusion protein is enriched in early endosomes and inner membrane folds of multivesicular bodies, supporting the hypothesis that the FYVE domain does indeed serve as a membrane-targeting module [12]. Other observations in support of this hypothesis include the establishment that the EEA1 FYVE domain is essential for its endosomal localization and the stabilization of its interaction with the small GTPase Rab5 [65]. Mutations of the EEA1 FYVE domain have been found to result in the mislocalization of the protein concomitant with a loss of function of the protein. The same finding has been reported for Smad anchor for receptor activation (SARA), which is involved in the transforming growth factor (TGF)β signalling pathway [75]. Endofin, a SARA-related protein, is associated with the early endosome via its FYVE domain-mediated interaction with PtdIns3*P* [63]. However, not all FYVE domain proteins are dependent on the FYVE domain for their association with the endosomal membranes, and not all FYVE domain proteins have obvious functions in membrane trafficking. Firstly, the FYVE domain of Hrs is inefficient at targeting the protein to the endosomes and tandem FYVE domains are required [12]. The FYVE domain of Hrs has in fact been shown in one study not to be the motif responsible for the intracellular distribution of the protein. Instead, the 100 amino acid residues at the C-terminus of Hrs are responsible for determining its subcellular localization [76]. The FYVE domain of Rabip4 is neither necessary nor sufficient for endosomal targeting; the endosomal targeting of Rabip4 is mediated instead by its RUN domain [61]. Similarly, Vps27p is not dependent on the FYVE domain for its membrane association even though an intact FYVE domain is required for its function [77]. Fgd1, a GEF for Rho family members whose mutations are associated with the inherited disease faciogenital dysplasia, also appears to be independent of the FYVE domain for its localization [78]. In addition, this protein is not localized in endosomes but in the plasma membrane and the Golgi complex. Frabin (Fgd1-related F-actin-binding protein) is not localized in endosomes but co-localizes with F-actin beneath the plasma membrane [79]. Other FYVE domain proteins that are not restricted to endosomes include double FYVE-containing protein 1 ('DFCP1'), which is found in cytoplasmic vesicles, the endoplasmic reticulum and the Golgi apparatus [80], and PIKfyve, which co-localizes mainly with the Golgi apparatus and late endosomes [81]. A protein with two tandem FYVE domains (i.e. TAFF1, for TAndem FYVE Fingers-1) has recently been shown to localize to the Golgi in a manner that is independent of either of its two FYVE domains [82]. Instead of a targeting role, it seems that the FYVE domains in these cases may execute a regulatory role on the functional activities of these proteins, possibly via conformational changes or protein–protein interactions upon their interaction with PtdIns3*P*.

The PH domain

The PH domain was first identified as a region of sequence homology of approx. 100–120 amino acid residues that is duplicated in the platelet protein kinase C substrate pleckstrin [49,50]. It was later found to be present in a multitude of proteins involved in diverse processes, including cellular signalling [in protein kinases, such as protein kinase B (PKB)/Akt, phoshoinositide-dependent kinase 1 (PDK1), and Bruton's tyrosine kinase (BTK)], cytoskeletal organization (in spectrin and α-actinin), and membrane trafficking [in GEFs such as Vav, ARF nucleotide binding site opener (ARNO), general receptor for phosphoinositides-1 (GRP1)/cytohesin-1] and in GTPases (such as dynamin) [6,14,49,50]. The PH domain has been shown to be a binding motif for PIs. This is reflected by the need of many PH domain-containing proteins for membrane association in order to function. The PH domain is composed of approx. 100–120 residues, but there is limited sequence similarity between PH domains of different molecules. They are defined instead by a conserved tertiary structure. The alignment of multiple sequences from PH domains has been used to identify features common among PH domains. These include seven blocks of sequences that correspond to secondary structural elements, a similar distribution of charged and hydrophobic residues and five highly conserved residues [83].

In contrast with FYVE domains, PH domains show a broad range of PI-binding specificities [4,84]. Four major groups of PH domains have been classified [85,86]. The group I PH domains have high affinity for PtdIns(3,4,5)P_3 and are present in BTK, Ras GAP1 and GRP1. The PH domain of BTK binds to PtdIns(3,4,5)P_3 with high affinity [87], and this interaction serves both to target the protein to the plasma membrane as well as to activate the kinase activity [88]. As an ARF6GEF, GRP1 binds preferentially to PtdIns(3,4,5)P_3 through its PH domain. PH domains of group II have preferential affinities for PtdIns(4,5)P_2, and are represented by PLCδ. Group III PH domains, as represented by those in PKB/Akt, PDK1 and dual adaptor for phosphotyrosine and 3′-phosphoinositides ('DAPP1') [89], bind to both PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3. GRP1 homologues, such as ARNO and cytohesin-1, also contain PH domains that bind PtdIns(3,4)P_2 and/or PtdIns(3,4,5)P_3 *in vitro* [90,91]. The fourth group of PH domains, represented by that of dynamin, have low affinity for PIs. In addition, a recent report has identified other novel PH domains with specificities for PtdIns(3,4)P_2 (TAPP1 and TAPP2, for tandem PH-domain-containing protein 1 and 2 respectively), PtdIns4*P* (FAPP1, for phosphoinositol-four-phosphate adaptor protein 1), PtdIns3*P* (PEPP1, for phosphoinositol-three-phosphate-binding PH-domain protein 1), and PtdIns(3,5)P_2 (Centaurin-β2) through *in vitro* binding assays [84].

The three-dimensional structures of at least eight PH domains have been determined by X-ray and/or NMR methods [14]. Studies of the PH domains of β-spectrin, pleckstrin-1, dynamin-1, PLCδ, BTK and others have revealed that the three-dimensional structures of PH domains are highly conserved. The PH domain folds into a core consisting of seven β-strands that form two sandwiched β-sheets (β1–β4 and β5–β7) followed by a C-terminal

α-helix. The phospholipid binding surface is usually formed by conserved residues in the β1- and β2-strands and the intervening loop (the β1–β2 loop). The basic residues in the β1- and β2-strands and the β1–β2 loop, conserved in groups I, II and III but not group IV PH domains, play a key role in interaction with PIs. In fact, a motif termed putative PtdIns(3,4,5)P_3 binding motif ('PPBM') has been mapped to the β1- and β2-strands and the β1–β2 loop and consists of Lys-Xaa-Sma-Xaa$_{6-11}$-Arg/Lys-Xaa-Arg-Hyd-Hyd (where Xaa is any amino acid, Sma is an amino acid with a small side-chain, and Hyd is a hydrophobic amino acid) [92]. Furthermore, group I PH domains contain a relatively larger loop with more basic residues linking the β1- and β2-strands. The loops linking other β-strands are likely to be involved in PI interaction. The loops connecting β-strands are highly variable in length and sequence, thereby accounting for diverse specificities and affinities for PI binding by the different PH domains. For example, structural studies of the recognition of PtdIns(3,4,5)P_3 by the PH domain of GRP1 reveal that this specificity is also conferred by two additional β-strands inserted between β6 and β7 of the PH domain [93,94].

Several mechanisms by which the association of PH domains with PIs influences cellular activities have been proposed [6,14,46]. Firstly, the PH domains are proposed to be important for the recruitment of proteins to cellular membranes. The PH domain in PDK1 is thought to be important in co-localizing it with PKB/Akt at the plasma membrane for the phosphorylation and full activation of PKB/Akt [95]. For BTK, a protein involved in the B-cell maturation pathway, the PH domain is found to be important in targeting the protein to the plasma membrane for its activating phosphorylations [96,97]. The importance of PI binding for BTK function is reflected by the association of mutations in the BTK PH domain, which result in impaired binding to PtdIns(3,4,5)P_3, with the inherited B-cell defect X-linked agammaglobulinaemia in humans and a similar X-linked immunodeficiency in mice [3,98,99]. The PH domain of GRP1 is important for its targeting to membrane ruffles where ARF6 is enriched [100]. The PH domain of ARNO, when expressed as a fusion protein with GFP, has been shown to mediate translocation to the plasma membrane upon stimulation, supporting a role for membrane targeting via the PH domain in this protein [101]. In addition, the PH domain has also been proposed to have a direct stimulatory action on protein activity upon association with PIs [88]. Experiments performed on PKB/Akt have led to the suggestion that PI binding and membrane recruitment can induce a conformational change in the protein so that it may be phosphorylated by PDK1 [102,103]. The PH domain in GEFs, such as Vav, is also suggested to be involved in stimulating GEF activity via association with PtdIns(3,4,5)P_3 [104].

The ENTH domain

The ENTH domain was identified as a region of approx. 140 residues conserved in several proteins involved in endocytosis and/or cytoskeletal organization, such as epsin 1, epsin 2, epsin 3, adaptor protein 180 (AP180), clathrin assembly lymphoid myeloid leukaemia protein (CALM) and the yeast Sla2p, Ent1p, Ent2p, Ent3p, Ent4p, yAP180A and yAP180B [51,52]. The ENTH domain is usually located at the N-terminus. Recently, it was established that the ENTH domain of epsin, AP180 and CALM interacts selectively with PtdIns(4,5)P_2 [105,106], suggesting that one of the roles of PtdIns(4,5)P_2 in endocytosis [107–109] is to function as docking sites for ENTH domain-containing proteins. The tertiary structures of the ENTH domains of epsin [110] and CALM [105] have been resolved. The epsin ENTH domain consists of eight α-helices while that of CALM consist of ten α-helices, the first seven of which superimpose on those of epsin. The PtdIns(4,5)P_2 binding site on CALM is a surface-positive patch with the phosphates of PtdIns(4,5)P_2 perched on the tips of the side chains of three lysine residues (Lys^{28}, Lys^{38} and Lys^{40}) and one histidine residue (His^{41}), and is present in the α1- and α2-helices and the loop in-between these two helices. This $KX_9KX(K/R)(H/Y)$ binding motif is present in other members of the AP180 family but not in epsins, suggesting that epsins have a different binding structure. Indeed, the binding site for PtdIns(4,5)P_2 in epsins has been mapped to Arg^8, Arg^{63}, Lys^{76} and possibly His^{73}. Arg^8 is present in the non-structured region preceding the first α-helix, Arg^{63} is located in the α3-helix, and His^{73} and Lys^{76} are located in the α4-helix. Huntingtin interacting protein (HIP)1 was identified by its ability to interact with huntingtin [111], the product of the Huntington disease gene [112], while HIP1R is a widely expressed protein related to HIP1 [113–115]. Interestingly, both HIP1 and HIP1R contain N-terminal ENTH domains harbouring the $K(X)_9KX(K/R)(H/Y)$ PtdIns(4,5)P_2 motif and are structurally homologous to Sla2p, which is involved in the actin cytoskeleton and endocytosis in yeast [116], suggesting that the interaction of HIP1 and HIP1R with PtdIns(4,5)P_2 may provide spatial/functional regulation of huntingtin. In addition, it suggests that HIP1, HIP1R and possibly huntingtin itself may also co-ordinate cytoskeletal organization in relation to endocytosis [114].

IDENTIFICATION OF THE PX DOMAIN

The PX domain averaging 100–120 amino acids was first identified through analysis of two of the cytosolic components of the NADPH oxidase, p47^{phox} and p40^{phox} [53]. These two proteins show significant sequence identity in the first $\sim$ 120 residues at their N-terminal regions. Database searches with this region revealed a spectrum of other proteins also containing this conserved domain. Due to the presence of a polyproline motif (PXXP) in the middle of the PX domain that is characteristic of SH3 domain binding motifs, it was speculated that some PX domains may be interacting partners of SH3 domain proteins.

Our interest in the PX domain began with the report on the identification of sorting nexin (SNX)1, a protein that binds the lysosomal targeting signal of the epidermal growth factor (EGF) receptor and enhances its degradation [117]. A search of databases with the SNX1 sequence revealed a region of 83 amino acids showing approx. 30% identity with Mvp1p, a suppressor of Vps1 mutants deficient in trafficking of carboxypeptidase Y (CPY) to the vacuole. The region shared by SNX1 and Mvp1p [which we termed the SNX1–Mvp1p domain (SM domain)] was suggested to be a membrane-targeting domain responsible for interaction with other proteins. Using the SM domain to search both the expressed sequence tag and protein databases, we have uncovered approx. 16 novel proteins or SM domain proteins (SDPs), including SDP1 (SNX9), SDP3 (SNX3), SDP7 (SNX16) and SDP8 (SNX12). At about the same time, several groups reported the identification of SNX2–SNX15 [118–122]. Our recent database searches have revealed an additional 12 members (SNX18–SNX29) of the sorting nexin family (Figures 3 and 4).

THE PX DOMAIN EXISTS IN DIVERSE PROTEINS

Although present in a diverse range of proteins, the PX domain-containing proteins can be loosely divided into three subclasses (Figure 3). Within the first category are small proteins in which the PX domain itself represents more than half of the polypeptide and these include SNX3, SNX10, SNX12, SNX22, SNX23,

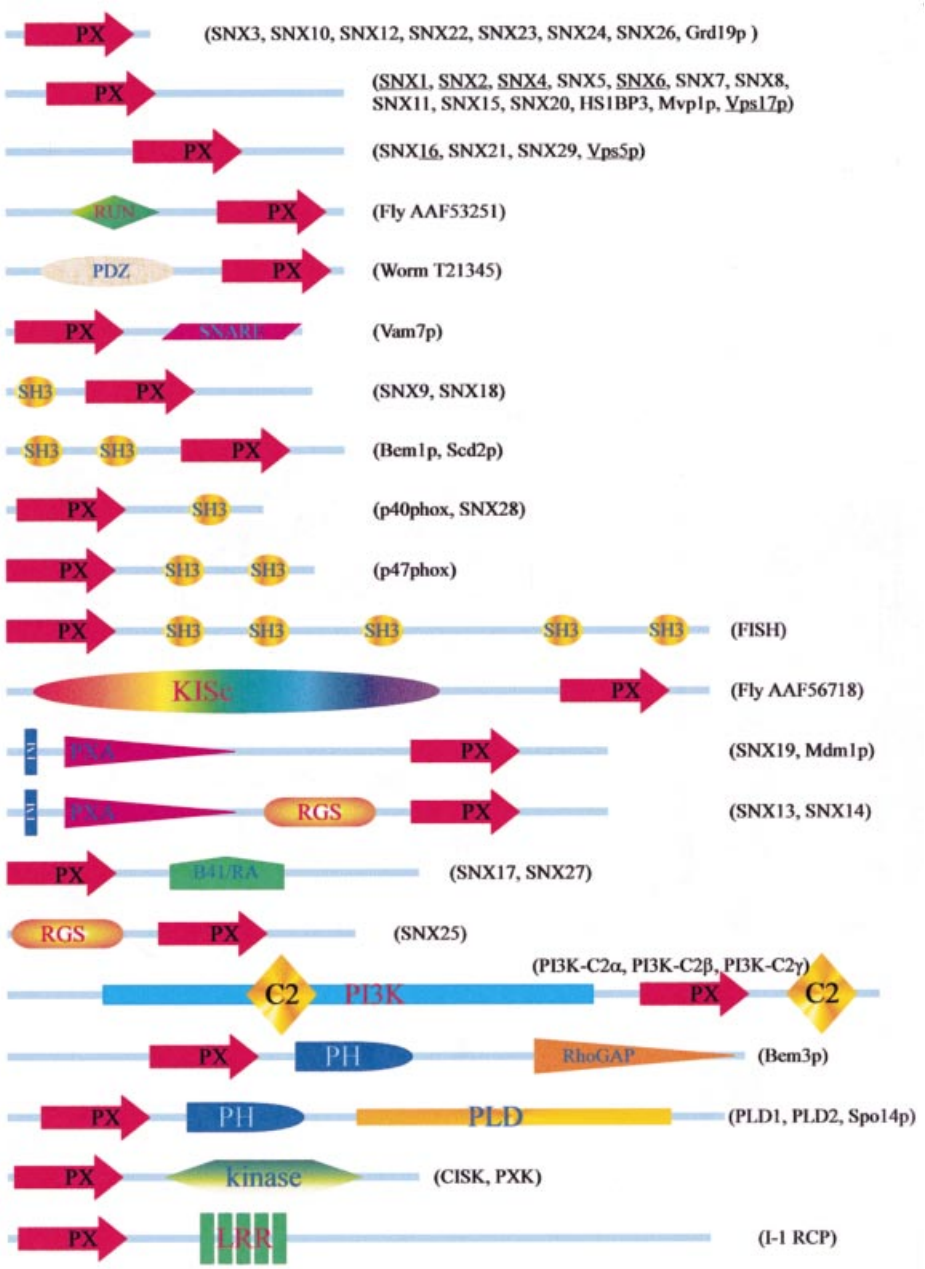

Figure 3 Summary of diverse PX domain-containing proteins

The schematic frameworks of various PX domain-containing proteins are indicated. The proteins of subclass II (see the text) that are predicted to contain coiled-coil regions are underlined.

SNX24, SNX26 and yeast Grd19p. Proteins in the second subclass contain longer flanking sequences but lack other known motifs and include SNX1, SNX2, SNX4, SNX5, SNX6, SNX7, SNX8, SNX11, SNX15, SNX16, SNX20, SNX21, SNX29, haemopoietic-specific protein 1 (HS1) SH3 domain binding protein (HS1BP3) and yeast Vps5p, Vps17p and Mvp1p. The flanking sequences of some of the subclass II proteins are predicted to form coiled-coil structures (such as SNX1, SNX2, SNX4, SNX6, SNX16, Vps5p and Vps17p). In addition to the presence of a single copy of the PX domain, proteins in the third category contain other characterized domains, such as the SH3 domain (SNX9/SH3PX1, SNX18, SNX28, $p40^{phox}$, $p47^{phox}$, FISH, Bem1p and Scd2p), the PI3K domain (PI3K-C2α, P13K-C2β, and PI3K-C2γ), both the PH and PLD domains (PLD1, PLD2 and yeast Spo14p), the protein kinase domain [cytokine-independent survival kinase (CISK) and PX domain-containing protein kinase (PXK)], the soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) domain (Vam7p), the regulator of G-protein signalling (RGS) domain (SNX25), both the PX domain-associated (PXA) and transmembrane domains (SNX19 and Mdm1p), the trans-membrane, PXA and RGS domains (SNX13 and SNX14), the B41 domain (SNX17 and SNX27), both the PH and RhoGAP domains (Bem3p), Leu-rich repeats [imidazoline-1 receptor

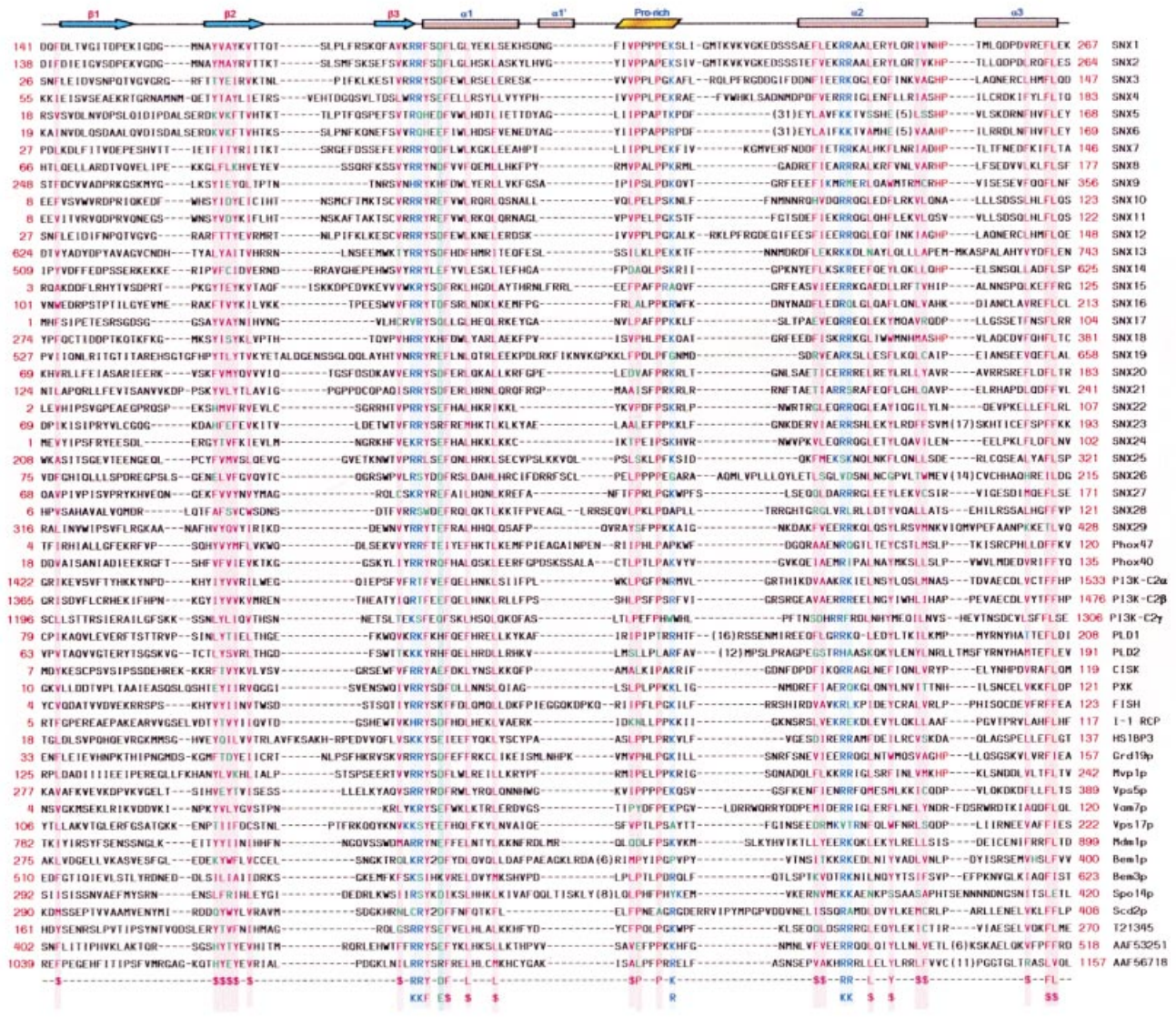

Figure 4 Alignment of amino acid sequences of various PX domains

The amino acid sequences of the PX domains of various proteins are aligned. The name of each protein is indicated on the right. The secondary structure of the PX domains is indicated on the top. Conserved hydrophobic residues are shown in pink, while conserved basic residues are shown in blue. Highly conserved residues (FL) are shown in red. The consensus is shown at the bottom, in which $ stands for hydrophobic residues. The numbers located in-between residues indicate the numbers of residues not shown. The GenBank® accession numbers are as follows: SNX1, Q13596; SNX2, O60749; SNX3, O60493; SNX4, O95219; SNX5, AF121855; SNX6, AF121856; SNX7, AF121857; SNX8, AF121858; SNX9, AF121859; SNX10, AF121860; SNX11, AF121861; SNX12, AF171229; SNX13, AB018256; SNX14, AY044865; SNX15, Q9NRS6; SNX16, P57768; SNX17, Q15036; SNX18, AAK82415; SNX19, AF395843; SNX20, AF395844; SNX21, AF395845; SNX22, AY044653; SNX23, AY044654; SNX24, AY044655; SNX25, AY044656; SNX26, AY044864; SNX27, AY044866; SNX28, AF399754; SNX29, AF399755; Phox47, XP_016956; Phox40, Q15080; PI3K-C2α, NP_002636; PI3K-C2β, O00750; PI3K-C2γ, O75747; PLD1, Q13393; PLD2, AF035483; CISK, AAG34115; PXK, AF399753; FISH, NP_032044; I-1 RCP, AAC33104; HS1BP3, NP_067404; Grd19p, Q08826; Mvp1p, AAA67884; Vps5p, Q92331; Vam7p, P32912; Vps17p, S60994; Mdm1p, NP_013603; Bem1p, P29366; Bem3p, P32873; Spo14p, S38103; Scd2p, P40996; *Caenorhabditis elegans* T21345; Fly AAF53251; and Fly AAF56718.

candidate protein (I-1 RCP)], the RUN domain (fly AAF53251), the PDZ domain (worm T21345), and the kinesin domain (fly AAF56717). The multidomain proteins may exhibit the most diverse range of functions.

THE PX DOMAIN AS A NOVEL MOTIF FOR INTERACTION WITH PIs

Despite its identification 5 years ago and its presence in many proteins implicated in diverse biological processes, the physiological function of the PX domain was only revealed by several independent studies published recently [123–128]. During our biochemical and cell biological characterization of SNX3, we found that it is enriched in the early endosome and intermediates involved in transport from the early to the recycling endosome. The observation that its endosomal association was abolished by treatment with wortmannin, an inhibitor of PI3Ks [6,129], suggests that its endosomal targeting is dependent on the continuous generation of 3′-PIs. This observation prompted us to investigate the molecular mechanism underlying this 3′-PI-dependent endosomal association of SNX3. Our studies revealed

that the PX domain of SNX3 is capable of directly interacting with PtdIns3*P* but not other PIs. Furthermore, SNX7 and SNX16 were similarly shown to interact preferentially with PtdIns3*P*. In addition, we observed that overexpression of SNX3 causes a clustering of endosomal compartments (early endosomes marked by EEA1, recycling endosomes marked by transferrin receptor, and late endosomes marked by syntaxin 7). Importantly, this endosomal clustering effect of SNX3 was abolished by mutations in the PX domain that prevented its endosomal targeting. These observations suggest that the PX domain of SNXs and other proteins functions as a novel motif for direct interaction with PtdIns3*P* for membrane targeting and/or regulation of functional activities [127].

SNAREs are a superfamily of proteins involved in late stages of vesicle docking and fusion. Yeast Vam7p is a 25 kDa synaptosome-associated protein ('SNAP-25')-like SNARE involved in docking and fusion at the vacuoles. One unique property of Vam7p that is not shared by other known SNAREs is the presence of a PX domain in its N-terminal portion, and previous studies have shown that its PX domain is important for its function [130]. In a recent study, it was shown that endosomal/vacuolar association of Vam7p is dependent on continuous production of PtdIns3*P*, because inactivation of Vps34p (the major PI3K in yeast) results in redistribution of Vam7p to the cytosol [123]. A direct interaction between PtdIns3*P* and the PX domain of Vam7p was also established. This study suggests that the PX domain of Vam7p mediates its targeting to the vacuolar membrane via selective interaction with PtdIns3*P*.

The concept that the PX domain is a PI-binding module was independently shown by two other studies investigating the mechanisms of action of two components ($p40^{phox}$ and $p47^{phox}$) of the NADPH oxidase complex, a key defensive component of neutrophils [124,125]. It was revealed that the PX domains of $p40^{phox}$ and $p47^{phox}$ interact specifically with PtdIns3*P* and PtdIns(3,4)P_2 respectively. In another study, the PX domain of class II PI3K was shown to interact specifically with PtdIns(4,5)P_2 [126]. The PX domain of CISK was independently shown to bind PtdIns(3,5)P_2, PtdIns(3,4,5)P_3 and, to a lesser extent, PtdIns(4,5)P_2 [128]. These six independent studies firmly establish that the PX domain is a structural motif that interacts with PtdIns3*P*, PtdIns(3,4)P_2 and PtdIns(4,5)P_2, and possibly other PIs. The diverse PX domain-containing proteins are thus potential effectors of various PIs and a novel mechanism for PIs to regulate various cellular events has been uncovered.

STRUCTURAL BASIS FOR PX DOMAIN-MEDIATED INTERACTION WITH PIs

As seen from the alignment of the amino acid sequences of the majority of the known mammalian PX domain proteins and selected PX domain proteins from other species (Figure 4), the PX domain consists of several subdomains with conserved hydrophobic residues in which several (at least five) conserved basic residues are distributed. The solution structure of the PX domain of $p47^{phox}$ has recently been resolved to adopt a novel fold [131]. The secondary structure and the structural basis for the PX domain of Vam7p to interact with PtdIns3*P* and the lipid bilayer have also been investigated by NMR spectroscopy. The PX domain is generally organized into a flat and compact shape consisting of three β-strands followed by three α-helices (Figure 5). The three β-strands form an antiparallel β-sheet. There is another α-helix (tentatively referred to as $\alpha1'$ here) downstream of $\alpha1$-helix in some proteins (such as $p47^{phox}$) but not others (such as Vam7p and SNX3). Two of the five basic residues of the PX domain are present in the most conserved RR(F/Y)(S)(D/E)F motif located in the $\beta3$–$\alpha1$ region. The other two conserved basic residues are located in the $\alpha2$-helix. The conserved basic residues [particularly those in the RR(F/Y)(S)(D/E)F motif] are predicted to form a basic binding pocket for interaction with the negatively charged phosphate groups of PIs. Located in-between the $\alpha1$-helix (or $\alpha1'$-helix) and the $\alpha2$-helix is a conserved proline-rich motif (Hyd-Pro-p-Hyd/p-Pro-Xaa-Bas; where Hyd is a hydrophobic residue and Bas is a basic residue). This represents a conserved SH3 domain-binding motif. The hydrophobic loop between the proline-rich motif and the $\alpha2$-helix is implicated in interactions with the membrane. Thus the membrane targeting of the PX domain not only relies on the direct specific interaction of the basic pocket with the head group of PtdIns3*P* but also on non-specific hydrophobic interaction of the hydrophobic loop (in-between the proline-rich motif and the $\alpha2$-helix) with the membrane.

The NMR structural studies of PX domains have been complemented by site-directed mutagenesis studies. The most conserved motif in the PX domain, RR(F/Y)S(D/E)F, was found to be very sensitive to mutations. Point mutations within this region in most PX domains, including those in Vam7p, SNX3, $p40^{phox}$ and $p47^{phox}$, were found to significantly reduce/abolish the interaction with PIs and membrane targeting [123–125,127]. Mutations of other basic residues have also been shown to affect PI binding.

The existence of a proline-rich motif within most PX domains is an interesting feature, because the proline-rich motif might potentially mediate interaction with SH3 domains or other related domains. In the resolved structure of the PX domain of human $p47^{phox}$, the proline-rich motif is positioned on the surface of the compact structure and is readily exposed for interaction with other proteins. In fact, the PX domain of $p47^{phox}$ has been shown to interact selectively with the second, but not the first, SH3 domain in its C-terminal region [131]. Moreover, a PX-domain-containing protein (HS1BP3) has been identified by virtue of its interaction with the SH3 domain of HS1 [132]. Whether this interaction is mediated by the proline-rich motif in the PX domain remains to be determined.

DIVERSE FUNCTIONS OF PX DOMAIN-CONTAINING PROTEINS

Since the PX domain is present in diverse proteins involved in many different biological processes, it is reasonable to speculate that PIs may regulate (spatially, temporally and/or functionally) these events via interaction with the PX domains in these proteins (Figure 6). In addition to events that are described in more detail (see below), it has also been shown recently that SNX5 may interact with Fanconi anaemia protein A (FANCA) via a region downstream of the PX domain [119]. It thus seems possible that SNX5 may regulate the distribution and/or functional activity of FANCA and other proteins belonging to the eight complementation groups involved in Fanconi anaemia, because FANCA has been shown to exist in a complex with Fanconi anaemia proteins C, G and F [133]. I-1 RCP is a PX domain protein that was identified using antisera directed against candidate receptor proteins to screen a cDNA library, and could be a functional component of the imidazoline-1 receptor [134]. Additionally, SNX2 has recently been shown to interact with human formin-binding protein 17 ('FBP17'), which contains a C-terminal SH3 domain and an N-terminal CDC15 ('cell division cycle 15') homologous domain [135].

Membrane traffic

The discovery that the FYVE domain is a PtdIns3*P*-binding module has attributed the role of PI3Ks in traffic to FYVE

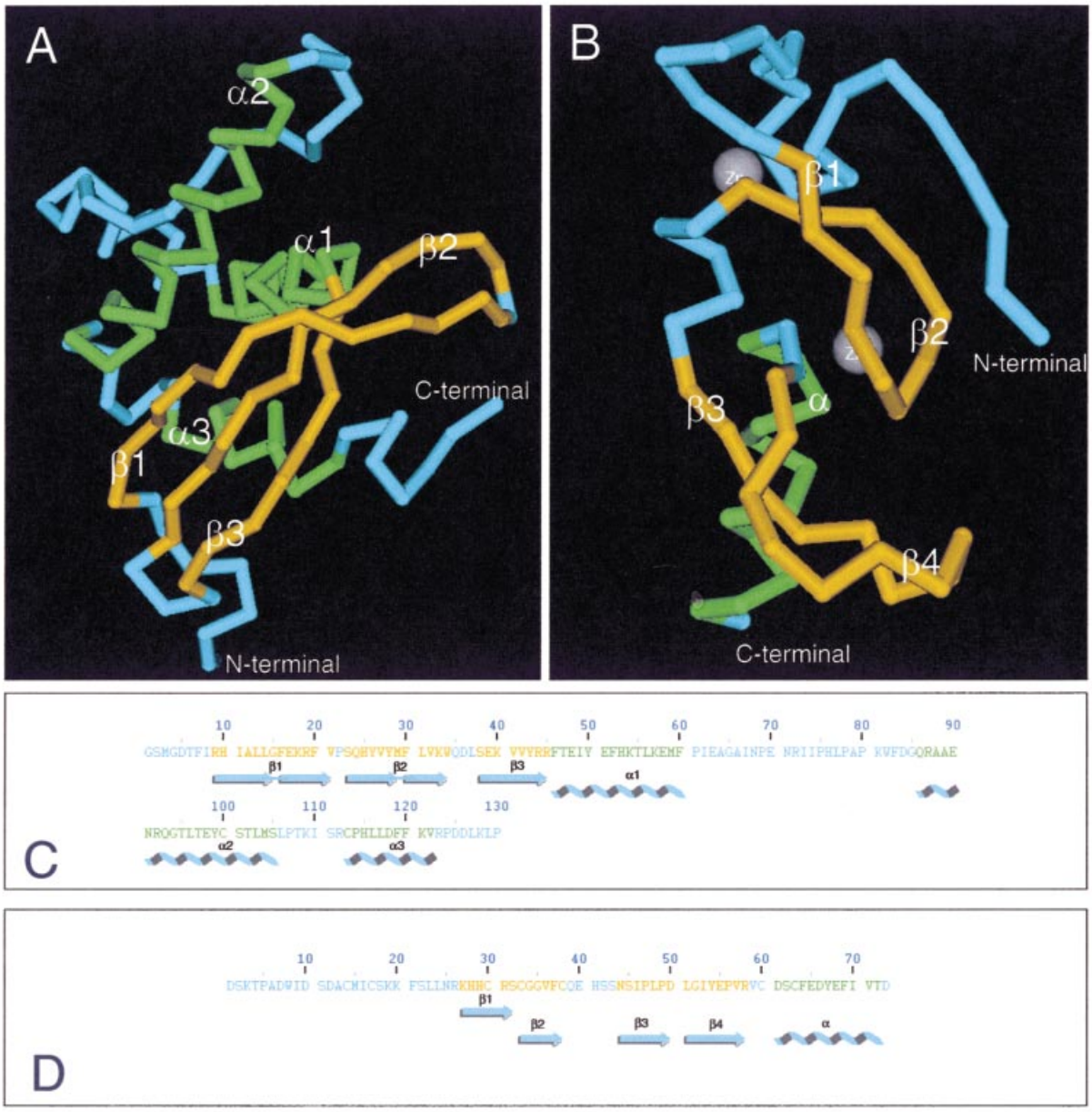

Figure 5 Secondary and tertiary structures of the PX domain of p47^{phox} and the FYVE domain of Vps27p

The secondary and tertiary structures of the PX domain of p47^{phox} (Molecular Modelling Database Id, 16715; Protein Data Bank Id, 1GD5) and the FYVE domain of Vps27p (Molecular Modelling Database Id, 10336; Protein Data Bank Id, 1VFY) are retrieved from the National Center for Biotechnology and Information structure at http://www3.ncbi.nlm.nih.gov/Entrez/ and viewed using the Cn3D 3.0 program (with the Style setting at Neighbor). (**A**) Tertiary structure of the PX domain of p47^{phox}. (**B**) Tertiary structure of the FYVE domain of Vps27p. (**C**) Secondary structure of the PX domain of p47^{phox}. (**D**) Secondary structure of the FYVE domain of Vps27p. Dark yellow and green represent regions adopting β-strands and α-helices respectively, while the connecting regions are shown in light blue. As shown, the PX domain consists of three β-strands followed by three α-helices, whereas the FYVE domain consists of four β-strands followed by an α-helix. As discussed in the text, the PH domain consists mainly of seven β-strands followed by an α-helix, whereas the ENTH domain is mostly composed of 7–10 α-helices that form a solenoid structure. In all cases, the PI binding sites are mainly composed of positively charged residues that interact with the phosphate groups of PIs.

domain proteins [5,13,57,60]. Most of the FYVE proteins identified so far act at the docking and fusion stages of membrane transport. For example, EEA1, the prototypical FYVE domain protein, functions as a tethering molecule to regulate endosomal docking and fusion [136]. Hrs and yeast Vac1p and Vps27p have also been shown to interact with the SNARE machinery to mediate membrane docking and fusion [73,137,138]. On the other hand, PI3Ks have also been shown to play a role in cargo sorting and vesicle formation, suggesting that there are other PtdIns3*P* effectors in addition to the FYVE proteins [139,140]. The identification of the PX domain as another module for interaction with PIs suggests that some of these PX domain-containing proteins, such as the SNXs, may meet this requirement to function as adaptors and/or cargo sorters.

Several PX domain-containing proteins (such as Mvp1p, Grd19p, Vps5p/Grd2p and Vps17p) in yeast have been characterized to function in protein traffic. The late Golgi compartment of yeast is involved in traffic to both the surface and to the

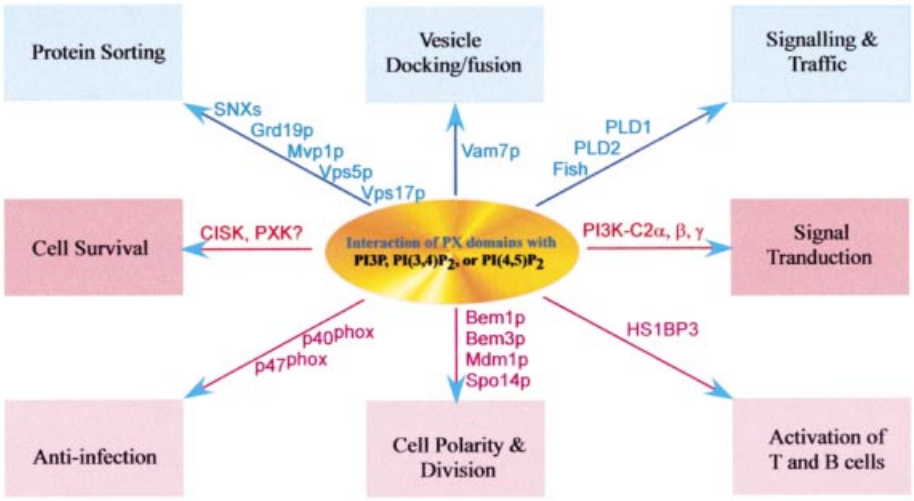

Figure 6 Diverse cellular functions regulated by interactions of PIs with PX domain-containing proteins

vacuole via endosomes. It contains several integral membrane proteins [dipeptidyl aminopeptidase A (DPAP A), Kex2p, Kex1p and Vps10] whose Golgi retention depends on continuous retrieval from endosomes to the Golgi. The *Mvp1* gene was isolated as a multicopy suppressor of dominant-negative *vps1* mutations [141]. Both Vps1p (a dynamin-like GTPase) [142,143] and Mvp1p are involved in sorting proteins in the late Golgi for delivery to the vacuole. Since Mvp1p co-localizes with Vps1p in the Class E endocytic compartment of Vps27 mutant cells, they could also participate in sorting events in endosomes for proteins destined for the vacuoles and/or proteins destined for retrieval back to the late Golgi. Grd19p was identified from mutants which show defective retrieval of a chimaeric protein (A-ALP, which consists of the cytoplasmic domain of DPAP A fused to the transmembrane and luminal domains of alkaline phosphatase) that behaves like DPAP A [144]. Grd19p consists essentially of a PX domain. Similar to Mvp1p and Vps1p, Grd19p is also localized to the Class E compartment and is suggested to be a component of the retrieval machinery by direct interaction with certain late Golgi proteins, such as DPAP A, at pre-vacuolar endosomal structures [144]. Grd19p is also important for retrieval of Kex2p, but not Vps10p, and therefore does not play a role in sorting soluble enzymes to the vacuole. Vps17p, identified using mutants where 95% CPY was secreted [145], is found in a membrane fraction that also contains Vps5p/Grd2p, a homologue of human SNX1 [146]. Similarly to Vps17p, Vps5p/Grd2p was identified as a protein responsible for normal traffic to the vacuole, and a defective Vps5p results in the secretion of CPY with Vps10p being mis-delivered to and rapidly degraded in the vacuole. Vps5p/Grd2p was also independently identified in the aforementioned screen for defective endosome-to-Golgi transport of A-ALP [147]. Furthermore, Vps17p and Vps5p were shown to exist with Vps26p, Vps29p and Vps35p in a protein complex referred to as retromer, which is involved in the retrieval of Golgi proteins, such as Vps10p and Kex2p, from pre-vacuolar endosomes [148]. Human orthologues of Vps26p, Vps29p and Vps35p have recently been identified and are known to form a complex with SNX1 [149].

Isolated by its ability to bind the lysosomal targeting signal of the EGF receptor, SNX1 is found to be present in endosomal structures which also contain the EGF receptor following ligand-induced internalization [117]. Overexpression of SNX1 dramatically decreases the half-life of the EGF receptor, suggesting that it may be involved in trafficking from endosomes to the lysosome. Recently, yeast two-hybrid screens using Hrs have identified SNX1 as an interacting protein. Hrs and the EGF receptor compete for the same binding site on SNX1 and overexpression of Hrs or its SNX1 binding domain inhibited ligand-induced degradation of the EGF receptor, suggesting that Hrs may modulate lysosomal traffic of the EGF receptor by sequestering SNX1 [150]. In addition to its presence in a protein complex containing Vps26, Vps29 and Vps35 [149], SNX1 has also been shown to oligomerize [151] and/or interact with other sorting nexins, such as SNX2, SNX4, SNX6 and SNX15, but not SNX3 [118,120,121]. The significance of these interactions remains to be investigated. SNX1, SNX2, SNX4 and SNX6 have been shown to interact with several members of the receptor tyrosine kinase family, such as the EGF receptor, the platelet-derived growth factor (PDGF) receptor, the insulin receptor and the long form of the leptin receptor, while SNX15 interacts preferentially with the PDGF receptor. In addition, SNX2, SNX4 and SNX5, but not SNX1 and SNX3, have been shown to interact strongly with the type II TGF-β receptor. Interaction with activin-like receptor (ALK)5 has been shown for SNX2 and SNX6. Only SNX2 has been shown to interact with ALK4. So far, interaction of SNXs with ALK2 and ALK3 has not been reported.

The selective interaction of SNX15 with the PDGF receptor is dependent on the presence of its PX domain [121]. Overexpression of SNX15 disrupts the organization of endosomes, resulting in the appearance of large amorphous structures containing markers of the early endosome, the late endosome and the lysosome. However, it does not affect the localization of a marker of the recycling endosome. The appearance of these abnormal structures affects the recycling of both *trans*-Golgi network 38 kDa protein ('TGN38') and furin from the endosomes to the *trans*-Golgi network, resulting in their mislocalization. Furin is the endopeptidase responsible for the processing of PDGF and insulin receptors into their mature forms. Overexpression of SNX15 therefore decreases the appearance of mature forms of insulin

and PDGF receptors [121,152]. Taken together, these results suggest an involvement of SNX15, and possibly also SNX1, SNX2, SNX4 and SNX6, in the sorting of various proteins in the early endosome for delivery to the late endosome and the *trans*-Golgi network.

SNX3 is similarly enriched in the early endosome and also in intermediates involved in transport from the early to the recycling endosome, although it does not interact with SNX1, SNX2, SNX4, SNX6 or SNX15. Micro-injection of anti-SNX3 antibodies affects the transport of transferrin receptor from the early to the recycling endosome. Although overexpression of SNX3 and SNX15 both led to the formation of abnormal endosomal structures [127,152], only the structures induced by SNX3, but not by SNX15, contain components of the recycling endosome, suggesting that these two SNXs could mediate two distinct pathways from the early endosome. Our current hypothesis is that SNX3 is involved in traffic from the early to the recycling endosome. Although more detailed analysis is necessary, it seems likely that SNXs are potentially important components of the sorting machinery of the endocytic pathway and combinatory use of different members of this family may regulate the traffic of different proteins at distinct sites along the endosomal pathway.

P-selectin is a transmembrane domain protein stored intracellularly in platelets and endothelial cells, and is translocated to the surface to mediate interaction with leucocytes upon activation [153]. Interaction of SNX17 with the cytoplasmic domain of P-selectin was recently demonstrated, indicating that SNX17 may regulate the traffic and/or function of P-selectin [154].

In addition to their roles in cargo sorting and selective traffic in the endosomes, PX domain-containing proteins may also regulate docking and fusion of transport vesicles with the acceptor compartment. A role of the PX domain of Vam7p, which functions as a SNARE of the vacuole, has been shown [123,130].

Cell signalling and/or cell survival

In a study designed to identify novel mediators of interleukin (IL)-3-dependent survival mechanisms in haematopoietic cells, CISK (identical to SGK3, the serum- and glucocorticoid-induced protein kinase 3) was characterized [155,156]. It contains an N-terminal PX domain followed by a kinase domain. CISK can be activated *in vitro* by PDK1 and *in vivo* in response to signals triggering the activation of PI3Ks. The kinase domain of CISK shows more than 50 % identity with PKB/Akt and is capable of phosphorylating both BAD (Bcl-xL/Bcl-2 associated death promoter) and the transcription factor FKHRL1 (forkhead drosophila homologue rhabdomyosarcoma like 1), substrates of PKB/Akt, leading to the suggestion that CISK may act in parallel with PKB/Akt. In contrast with the PH domain-mediated targeting of PKB/Akt to the plasma membrane, CISK is distributed to endosomal structures in a PX domain-dependent manner, suggesting that CISK is involved in different but complementary signalling pathways. The PX domain of CISK has been shown to interact with PtdIns(3,4)P_2, PtdIns(3,4,5)P_3 and, to a lesser extent, PtdIns (4,5)P_2 [128].

PX domains are also present in proteins involved in phosphorylation and hydrolysis of phospholipids. The first of these and probably the most extensively studied are the PI3Ks, which have been implicated in the regulation of diverse cellular processes by phosphorylation of the 3′-OH of PIs [2,157,158]. All three members of class II PI3Ks (also called the CPK class) have a PX domain located in their C-terminal region in-between the catalytic domain and the C2 domain (Figure 3). It is highly possible that PIs execute regulatory effects over class II PI3Ks via their interaction with the PX domain, although the PX domain seems not to be important for subcellular targeting of PI3K-C2α [159]. Recent studies suggest that class II PI3Ks may couple PI metabolism to membrane traffic [160].

Both isoforms of mammalian PLD (PLD1 and PLD2) contain a PX domain in their N-termini as does the yeast PLD1 (Spo14p) [161,162]. PLDs have been implicated in a wide variety of cell processes ranging from vesicle trafficking via interaction with ARFs to cell signalling through activation of mitogen-activated protein (MAP) kinase cascades. The PX domain has not been shown to be important for many activities of PLDs, since removal of this domain does not disrupt its catalytic activity or its interaction with ARF. It remains, however, possible that physiological regulation of PLDs may be dependent on the interaction of PIs with the PX domain. For instance, the PX domain of PLD1 can be phosphorylated by protein kinase C in the loop between the first and second α-helices [163]. This phosphorylation may regulate the PX domain–PI interaction, which in turn controls the membrane association and/or function of the enzyme.

FISH is a protein containing an N-terminal PX domain followed by five SH3 domains. It was isolated due to its phosphorylation in Src-transformed fibroblasts or by treatment with several growth factors, which activate members of the Rho family of small GTPases and cause changes in the actin cytoskeleton. It is possible that FISH might be involved in signalling by tyrosine kinases [164]. SNX9 (or SH3PX1) contains a single SH3 domain followed by a PX domain, and has been shown to interact with the cytoplasmic domain of the metalloprotease disintegrins MDC9 and MDC15 (where an MDC protein is a metalloprotease, disintegrin, cysteine-rich protein), which exhibit diverse functions in the cell. It is possible that interaction of PIs with SNX9/SH3PX1 may influence the intracellular transport, processing and/or signalling of MDC9 and MDC15 [165].

Anti-infection mediated by neutrophils and other professional phagocytic cells

NADPH oxidase in neutrophils and other phagocytic cells is responsible for the generation of superoxide (O^{2-}) as the precursor to other reactive oxygen species (such as hydrogen peroxide) involved in defence against bacterial and fungal infection [153,166]. This defence machinery is composed of several components including $p22^{phox}$, $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ and $gp91^{phox}$. The importance of the NADPH oxidase complex in anti-infection is evidenced by the fact that mutations in or loss of function of any one of these proteins lead to chronic granulomatous diseases [167,168]. At the resting stage, only $p22^{phox}$ and $gp91^{phox}$ (forming the transmembrane electron carrier flavocytochrome b_{558}) are associated with membranes of the surface, endocytic structures and/or phagosomal compartments, while the other components are found in the cytosol. Among the cytosolic components, $p40^{phox}$ contains one SH3 domain, whereas $p47^{phox}$ and $p67^{phox}$ contain two SH3 domains. In addition, $p40^{phox}$ and $p47^{phox}$ contain a PX domain in their N-terminal regions (Figure 3). The C-terminal region of $p47^{phox}$ contains many sites for phosphorylation. $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$ form a cytosolic complex through several intermolecular interactions mediated by SH3 domains and proline-rich motifs [169–172]. Upon stimulation, this cytosolic complex is delivered to membranes and interacts with $gp91^{phox}$–$p22^{phox}$ to form an active enzymic complex mediating the generation of superoxide. Phosphorylation of $p47^{phox}$ is important for translocation of this complex to the membrane and for activation of the oxidase [173,174]. Phosphorylation by protein kinase C may cause conformational

changes in $p47^{phox}$, which presumably allow its SH3 domain to interact with a proline-rich motif in $p22^{phox}$ [153]. NADPH oxidase activity is also under the control of the small GTPases Rac2 and, to a lesser extent, Rac1. Their GTP-bound activated forms interact directly with $p67^{phox}$ (residues 170–199) and p21(Cdc42Hs/Rac)-activated kinase ('PAK'), which in turn phosphorylates $p67^{phox}$ residues adjacent to the Rac-binding site [175]. Recently, *in vitro* binding and mutational studies of the PX domain of $p47^{phox}$ have shown that the second SH3 domain is able to interact with the proline-rich motif within its N-terminal PX domain [131]. It is tempting to speculate that phosphorylation of $p47^{phox}$ changes the conformation of the protein by disrupting this intramolecular interaction, so that other interactions [such as its PX domain interaction with PtdIns(3,4)P_2 and its SH3 domain interaction with the proline-rich motif in membrane $p22^{phox}$] that mediate membrane targeting of the cytosolic complex could occur.

The membrane recruitment of the cytoslic $p40^{phox}$–$p47^{phox}$–$p67^{phox}$ complex and activation of the enzyme are regulated by PI3K activity. The mechanism has, however, remained elusive. Two recent papers have shown that PI3Ks might regulate this process through the two effectors ($p40^{phox}$ and $p47^{phox}$) of the cytosolic complex [124,125] via their PX domain-mediated interaction with PIs. The PX domains of $p40^{phox}$ and $p47^{phox}$ bind to PI3P and PtdIns(3,4)P_2 respectively, and these lipid–protein interactions result in membrane targeting of the cytosolic complex and activation of the NADPH oxidase. The importance of this lipid–protein interaction is highlighted by the observation that a point mutation ($Arg^{57} \rightarrow Gln$) in the PX domain of $p40^{phox}$ abolishes interaction with PI3P, which in turn abolishes the membrane targeting of $p40^{phox}$. In addition, a point mutation of the conserved Arg^{42} in the PX domain of $p47^{phox}$, giving a mutant protein which cannot bind PtdIns(3,4)P_2, is associated with chronic granulomatous disease [168]. It is possible that the inability to bind PIs due to a point mutation in the PX domain is one molecular basis for the development of the disease. Although the mechanism of how two different PIs [$p40^{phox}$ interaction with PtdIns3P and $p47^{phox}$ interaction with PtdIns(3,4)P_2] co-ordinate/regulate the assembly of the NADPH oxidase complex on specific membranes needs further investigation, it is obvious that the PX domains of $p40^{phox}$ and $p47^{phox}$ could provide spatial clues and/or activation signals for this process.

Activation of T- and B-cells

HS1 is a 486-residue protein expressed in haemapoietic and lymphoid lineage cells [176] and becomes tyrosine phosphorylated upon activation of antigen receptors in both T- and B-cells. It may participate in antigen receptor signalling through interaction with Lck in T-cells [177] and with Syk and Lyn in B-cells [178]. T- and B-cells deficient in HS1 are defective in activation-mediated proliferation [179]. HS1 has also been shown to bridge growth-factor-receptor-bound protein 2 ('Grb2') to Lck in T-cells [180] and to participate in activation-triggered apoptosis of B-cells [181]. In addition, HS1 has been implicated in the signalling cascades of IL-5 receptors [182], Fc receptors [183] and erythropoietin receptors [184]. HS1 may thus participate in activation, differentiation and apoptosis of haemopoietic and lymphoid lineage cells. HS1 contains several interesting features and has a 37-amino-acid motif repeated four times in tandem at the N-terminus, a proline-rich motif, and a C-terminal SH3 domain. HS1 is a major substrate phosphorylated by tyrosine kinases during T- and B-cell activation and has been reported to translocate into the nucleus in a kinase-dependent manner [181]. HS1BP3 is a widely expressed 396-residue protein identified by its capability to interact with the SH3 domain of HS1 [132]. HS1BP3 contains an N-terminal PX domain followed by an acidic region, a basic region, a leucine zipper and several proline-rich motifs in the C-terminus. Overexpression of mutant HS1BP3 lacking an intact PX domain and the C-terminal region in T-cells results in a strong inhibition of anti-CD3ϵ antibody-induced IL-2 production, suggesting that HS1BP3 may be involved in T-cell activation in a PX domain-dependent manner. Based on the discovery that the PX domain functions as a PI-binding motif, one possible function of HS1BP3 could be to target HS1 to the membrane for its interaction with antigen receptors and/or tyrosine kinases in T- and B-cells. Once tyrosine-phosphorylated, HS1 may translocate to the nucleus to modulate other downstream effects. Whether membrane targeting of the HS1–HS1BP3 complex is triggered by activation of the antigen receptors or occurs constitutively will be an interesting issue to address. Whatever the case, the interaction of HS1BP3 with a major substrate of tyrosine kinases associated with antigen receptor-mediated activation in T- and B-cells indicates that this PX domain protein may be an important regulator of signalling cascades during the activation of T- and B-cells.

Cell polarity and cytoskeleton

In budding yeast, cell polarization occurs during vegetative growth and during the mating process [185]. The PX domain protein Bem1p is involved in the establishment of polarity during both processes. Originally identified by a screen for mutants defective in bud formation [186], Bem1p has a similar domain structure as $p47^{phox}$, but in reverse order, with two N-terminal SH3 domains followed by a C-terminal PX domain (Figure 3). Scd2p is the fission yeast homologue of Bem1p [187]. Screens for synthetic lethality or two-hybrid interaction with Bem1p have identified Cdc24p and Bem2p [188], which act as a GEF and putative GAP respectively for Rho-type GTPase Cdc42p. Cdc24p, Cdc42p and Bem2p have also been shown to be required for bud emergence [189–191]. In addition, Bem1p has been shown to interact with a number of other proteins (such as Ste20p, Ste5p, Ste11p, Ste7p and Fus3p) that are involved in the signalling cascade initiated by binding of mating pheromones with surface receptors [192–194]. Bem1p also interacts with the apparently functionally redundant SH3 domain-containing proteins Boi1p and Boi2p [195]. While Boi1p and Boi2p are shown to be involved in bud emergence, their exact role remains unclear. However, their fission yeast homologue Pob1p is essential for cell elongation and separation and may recruit proteins required for formation of the cell wall [196]. Recently it has been suggested that the function of Bem1p (Scd2p) may be to concentrate proteins involved in cell polarity establishment and signalling kinases to discrete sites, facilitating polarization and the Ste20p-mediated MAP kinase signalling cascade [187,192,197]. Bem3p, isolated as a multicopy suppressor of Bem2p, contains a PX domain in the middle of the protein and a RhoGAP domain toward the C-terminus that is capable of stimulating the hydrolysis of GTP on Cdc42p [198,199]. Another PX domain protein involved in the process of cell polarity is Mdm1p, which was identified from studies of mutants defective in the inheritance of nuclei and mitochondria into developing daughter cells [200]. Mdm1p shows some identity with vimentin and epidermal keratin, and readily assembles into 10 nm filaments [201]. Mutations in the PX domain of Mdm1p cause loss of inheritence of nuclei and/or mitochondria [202]. Interestingly, Mdm1p also contains a PXA domain, observed to be present in some of the PX domain proteins (SNX13, SNX14 and SNX19). However,

the function of this domain has not yet been established. Perhaps it is required for protein–protein interaction and/or filament formation. In summary, interaction of PIs with the PX domains of at least three proteins (Bem1p, Bem3p and Mdm1p) could potentially co-ordinate the establishment of cell polarity, bud emergence and/or inheritance of organelles.

CONCLUSIONS

The establishment of the PX domain as a novel PI-binding module has revealed a large number of proteins that can potentially act as effectors of various PIs. In contrast with the FYVE domain, which specifically interacts with PtdIns3*P*, the PX domain is more like the PH domain in that it displays broader substrate specificity. Collectively, the PH, FYVE, ENTH and PX domain-containing proteins probably account for several hundreds of the proteins in mammalian cells. In addition, as many of these proteins can interact with other proteins to form functional complexes and/or relay signalling cascades, we may speculate that thousands of proteins are potentially regulated directly or indirectly by various PIs. The molecular mechanisms determining the spatial distribution/production of different PIs in the membrane will need more investigation. One obvious possibility is that regulation of the spatial distribution and/or activation of various lipid kinases and phosphatases will determine the spatial concentrations of different PIs, although it is also possible that different PIs may have some intrinsic properties of segregating with specific membrane compartments or micro-domains. Additionally, an interesting question is whether individual PI-interacting proteins have unique modes of spatial and temporal recruitment on to particular membrane domains or whether all PI-binding proteins sharing the same motif are recruited in a similar manner to the same membrane domain. The regulation of the biochemical properties of PI-interacting domains by other structural domains within the same protein or by other proteins may add additional layers for controlling these PI-binding proteins. The mechanisms that govern the spatial and temporal recruitment of various PI-interacting proteins in relation to trafficking, signalling and other events will thus be one of the important focuses for future investigation. The diverse cellular processes that are regulated and/or executed by these PI-interacting proteins and their associates may share some common principles in the mechanism of action in addition to refined loops of regulation specific for each particular case. Understanding the common pathways that regulate the metabolism of PIs and the shared molecular mechanisms underlying the action of these PI-interacting proteins, in conjunction with detailed biochemical, functional and mechanistic studies of each of these PI-interacting proteins, will provide additional and comprehensive insights into the molecular events underlying multiple cellular processes.

Note added in proof (received 8 November 2001)

Two recent studies [204,205] have further confirmed the conclusion that the PX domain is a PI-interacting motif. The crystal structure of the $p40^{phox}$ PX domain bound to PtdIns3*P* has been resolved, revealing that the PX domain embraces the 3-phosphate of PtdIns3*P* with a positively charged pocket [206].

We thank Dr Bor Luen Tang, Dr Parenjeet Singh and Dr Shi Hong for their help in preparing the manuscript.

REFERENCES

1 Sprong, H., van der Sluijs, P. and van Meer, G. (2001) How proteins move lipids and lipids move proteins. Nat. Rev. Mol. Cell Biol. **2**, 504–513
2 Fruman, D. A., Meyers, R. E. and Cantley, L. C. (1998) Phosphoinositide kinases. Annu. Rev. Biochem. **67**, 481–507
3 Fruman, D. A., Rameh, L. E. and Cantley, L. C. (1999) Phosphoinositide binding domains: embracing 3-phosphate. Cell (Cambridge, Mass.) **97**, 817–820
4 Martin, T. F. (1998) Phosphoinositide lipids as signaling molecules: common themes for signal transduction, cytoskeletal regulation and membrane trafficking. Annu. Rev. Cell Dev. Biol. **14**, 231–264
5 Odorizzi, G., Babst, M. and Emr, S. D. (2000) Phosphoinositide signaling and the regulation of membrane trafficking in yeast. Trends Biochem. Sci. **25**, 229–235
6 Vanhaesebroeck, B., Leevers, S. J., Khatereh, A., Timms, J., Katso, R., Driscoll, P. C., Woscholski, R., Parker, P. J. and Waterfield, M. D. (2001) Synthesis and function of 3-phosphorylated inositol lipids. Annu. Rev. Biochem. **70**, 535–602
7 Cozier, G. E., Lockyer, P. J., Reynolds, J. S., Kupzig, S., Bottomley, J. R., Millard, T. H., Banting, G. and Cullen, P. J. (2000) GAP1IP4BP contains a novel group I pleckstrin homology domain that directs constitutive plasma membrane association. J. Biol. Chem. **275**, 28261–28268
8 Czech, M. P. (2000) PIP2 and PIP3: complex roles at the cell surface. Cell (Cambridge, Mass.) **100**, 603–606
9 Gray, A., Van Der Kaay, J. and Downes, C. P. (1999) The pleckstrin homology domains of protein kinase B and GRP1 (general receptor for phosphoinositides-1) are sensitive and selective probes for the cellular detection of phosphatidylinositol 3,4-bisphosphate and/or phosphatidylinositol 3,4,5-trisphosphate *in vivo*. Biochem. J. **344**, 929–936
10 Stauffer, T. P., Ahn, S. and Meyer, T. (1998) Receptor-induced transient reduction in plasma membrane PtdIns(4,5)P_2 concentration monitored in living cells. Curr. Biol. **12**, 343–346
11 Varnai, P. and Balla, T. (1998) Visualization of phosphoinositides that bind pleckstrin homology domains: calcium- and agonist-induced dynamic changes and relationship to myo-[^{3}H]inositol-labeled phosphoinositide pools. J. Cell Biol. **143**, 501–510
12 Gillooly, D. J., Morrow, I. C., Lindsay, M., Gould, R., Bryant, N. J., Gaullier, J. M., Parton, R. G. and Stenmark, H. (2000) Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells. EMBO J. **19**, 4577–4588
13 Gillooly, D. J., Simonsen, A. and Stenmark, H. (2001) Cellular functions of phosphatidylinositol 3-phosphate and FYVE domain proteins. Biochem. J. **355**, 249–258
14 Lemmon, M. A. and Ferguson, K. M. (2000) Signal-dependent membrane targeting by pleckstrin homology (PH) domains. Biochem. J. **350**, 1–18
15 Schu, P. V., Takegawa, K., Fry, M. J., Stack, J. H., Waterfield, M. D. and Emr, S. D. (1993) Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. Science (Washington, D.C.) **260**, 88–91
16 Christoforidis, S., Miaczynska, M., Ashman, K., Wilm, M., Zhao, L., Yip, S.-C., Waterfield, M. D., Backer, J. M. and Zerial, M. (1999) Phosphatidylinositol-3-OH kinases are Rab5 effectors. Nat. Cell Biol. **1**, 249–252
17 Panaretou, C., Domin, J., Cockcroft, S. and Waterfield, M. D. (1997) Characterization of p150, an adaptor protein for the human phosphatidylinositol (PtdIns) 3-kinase. Substrate presentation by phosphatidylinositol transfer protein to the p150.PtdIns 3-kinase complex. J. Biol. Chem. **272**, 2477–2485
18 Barylko, B., Gerber, S. H., Binns, D. D., Grichine, N., Khvotchev, M., Südhof, T. C. and Albanesi, J. P. (2001) A novel family of phosphatidylinositol 4-kinases conserved from yeast to humans. J. Biol. Chem. **276**, 7705–7708
19 Minogue, S., Anderson, J. S., Waugh, M. G., dos Santos, M., Corless, S., Cramer, R. and Hsuan, J. J. (2001) Cloning of a human type II phosphatidylinositol 4-kinase reveals a novel lipid kinase family. J. Biol. Chem. **276**, 16635–16640
20 Cooke, F. T., Dove, S. K., McEwen, R. K., Painter, G., Holmes, A. B., Hall, M. N., Michell, R. H. and Parker, P. J. (1998) The stress-activated phosphatidylinositol 3-phosphate 5-kinase Fab1p is essential for vacuole function in *S. cerevisiae*. Curr. Biol. **8**, 1219–1222
21 Gary, J. D., Wurmser, A. E., Bonangelino, C. J., Weisman, L. S. and Emr, S. D. (1998) Fab1p is essential for PtdIns(3)P 5-kinase activity and the maintenance of vacuolar size and membrane homeostasis. J. Cell Biol. **143**, 65–79
22 Odorizzi, G., Babst, M. and Emr, S. D. (1998) Fab1p PtdIns(3)P 5-kinase function essential for protein sorting in the multivesicular body. Cell (Cambridge, Mass.) **95**, 847–858
23 Sbrissa, D., Ikonomov, O. C. and Shisheva, A. (1999) PIKfyve, a mammalian ortholog of yeast Fab1p lipid kinase, synthesizes 5-phosphoinositides. Effect of insulin. J. Biol. Chem. **274**, 21589–21597
24 Godi, A., Pertile, P., Meyers, R., Marra, P., Di Tullio, G., Iurisci, C., Luini, A., Corda, D. and De Matteis, M. A. (1999) ARF mediates recruitment of PtdIns-4-OH kinase-beta and stimulates synthesis of PtdIns(4,5)P_2 on the Golgi complex. Nat. Cell Biol. **1**, 280–287
25 Jones, D. H., Morris, J. B., Morgan, C. P., Kondo, H., Irvine, R. F. and Cockcroft, S. (2000) Type I phosphatidylinositol 4-phosphate 5-kinase directly interacts with ADP-ribosylation factor 1 and is responsible for phosphatidylinositol 4,5-bisphosphate synthesis in the Golgi compartment. J. Biol. Chem. **275**, 13962–13966

26 Honda, A., Nogami, M., Yokozeki, T., Yamazaki, M., Nakamura, H., Watanabe, H., Kawamoto, K., Nakayama, K., Morris, A. J., Frohman, M. A. and Kanaho, Y. (1999) Phosphatidylinositol 4-phosphate 5-kinase alpha is a downstream effector of the small G protein ARF6 in membrane ruffle formation. Cell (Cambridge, Mass.) **99**, 521–532

27 Laporte, J., Blondeau, F., Buj-Bello, A. and Mandel, J.-L. (2001) The myotubularin family: from genetic disease to phosphoinositide metabolism. Trends Genet. **17**, 221–228

28 Maehama, T., Taylor, G. S. and Dixon, J. E. (2001) PTEN and myotubularin: novel phosphoinositide phosphatases. Annu. Rev. Biochem. **70**, 247–279

29 Woscholski, R. and Parker, P. J. (2000) Inositol phosphatases – constructive destruction of phosphoinositides and inositol phosphates. In Biology of Phosphoinositides, vol. 27 (Cockcroft, S., ed.), pp. 320–338, Oxford University Press, Oxford

30 Khvotchev, M. and Südhof, T. C. (1998) Developmentally regulated alternative splicing in a novel synaptojanin. J. Biol. Chem. **273**, 2306–2311

31 Seet, L.-F., Cho, S., Hessel, A. and Dumont, D. J. (1998) Molecular cloning of multiple isoforms of synaptojanin 2 and assignment of the gene to mouse chromosome 17A2-3.1. Biochem. Biophys. Res. Commun. **247**, 116–122

32 Robinson, L. J. and Martin, T. F. (1998) Docking and fusion in neuroscience. Curr. Opin. Cell Biol. **10**, 483–492

33 Rhee, S. G. (2001) Regulation of phosphoinositide-specific phospholipase C. Annu. Rev. Biochem. **70**, 281–312

34 Kearns, B. G., Alb, Jr, J. G. and Bankaitis, V. (1998) Phosphatidylinositol transfer proteins: the long and winding road to physiological function. Trends Cell Biol. **8**, 276–282

35 Cockcroft, S. (1999) Mammalian phosphatidylinositol transfer proteins: emerging roles in signal transduction and vesicular traffic. Chem. Phys. Lipids **98**, 23–33

36 Cleves, A. E., Novick, P. J. and Bankaitis, V. A. (1989) Mutations in the SAC1 gene suppress defects in yeast Golgi and yeast actin function. J. Cell Biol. **109**, 2939–2950

37 Bankaitis, V. A., Aitken, J. R., Cleves, A. E. and Dowhan, W. (1990) An essential role for a phospholipid transfer protein in yeast Golgi function. Nature (London) **347**, 561–562

38 Fullwood, Y., dos Santos, M. and Hsuan, J. J. (1999) Cloning and characterization of a novel human phosphatidylinositol transfer protein, rdgBβ. J. Biol. Chem. **274**, 31553–31558

39 Lev, S., Hernandez, J., Martinez, R., Chen, A., Plowman, G. and Schlessinger, J. (1999) Identification of a novel family of targets of PYK2 related to Drosophila retinal degeneration B (rdgB) protein. Mol. Cell. Biol. **19**, 2278–2288

40 Attree, O., Olivos, I. M., Okabe, I., Bailey, L. C., Nelson, D. L., Lewis, R. A., McInnes, R. R. and Nussbaum, R. L. (1992) The Lowe's oculocerebrorenal syndrome gene encodes a protein highly homologous to inositol polyphosphate-5-phosphatase. Nature (London) **358**, 239–242

41 Maehama, T. and Dixon, J. E. (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J. Biol. Chem. **273**, 13375–13378

42 Bolino, A., Muglia, M., Conforti, F. L., LeGuern, E., Salih, M. A., Georgiou, D. M., Christodoulou, K., Hausmanowa-Petrusewicz, I., Mandich, P., Schenone, A. et al. (2000) Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. Nat. Genet. **25**, 17–19

43 Weimar, W. R., Lane, P. W. and Sidman, R. L. (1982) Vibrator (vb): a spinocerebellar system degeneration with autosomal recessive inheritance in mice. Brain Res. **251**, 357–364

44 Hamilton, B. A., Smith, D. J., Mueller, K. L., Kerrebrock, A. W., Bronson, R. T., van Berkel, V., Daly, M. J., Kruglyak, L., Reeve, M. P., Nemhauser, J. L. et al. (1997) The vibrator mutation causes neurodegeneration via reduced expression of PITP alpha: positional complementation cloning and extragenic suppression. Neuron **18**, 711–722

45 Cremona, O., Di Paolo, G., Wenk, M. R., Luthi, A., Kim, W. T., Takei, K., Daniell, L., Nemoto, Y., Shears, S. B., Flavell, R. A. et al. (1999) Essential role of phosphoinositide metabolism in synaptic vesicle recycling. Cell (Cambridge, Mass.) **99**, 179–188

46 Hurley, J. H. and Meyer, T. (2001) Subcellular targeting by membrane lipids. Curr. Opin. Cell Biol. **13**, 146–152

47 Leevers, S. J., Vanhaesebroeck, B. and Waterfield, M. D. (1999) Signalling through phosphoinositide 3-kinases: the lipids take centre stage. Curr. Opin. Cell Biol. **11**, 219–225

48 Stenmark, H., Aasland, R., Toh, B. H. and D'Arrigo, A. (1996) Endosomal localization of the autoantigen EEA1 is mediated by a zinc-binding FYVE finger. J. Biol. Chem. **271**, 24048–24054

49 Gibson, T. J., Hyvonen, M., Musacchio, A., Saraste, M. and Birney, E. (1994) PH domain: the first anniversary. Trends Biochem. Sci. **19**, 349–353

50 Musacchio, A., Gibson, T., Rice, P., Thompson, J. and Saraste, M. (1993) The PH domain: a common piece in the structural patchwork of signalling proteins. Trends Biochem. Sci. **18**, 343–348

51 Kay, B. K., Yamabhai, M., Wendland, B. and Emr, S. D. (1999) Identification of a novel domain shared by putative components of the endocytic and cytoskeletal machinery. Protein Sci. **8**, 435–438

52 Rosenthal, J. A., Chen, H., Slepnev, V. I., Pellegrini, L., Salcini, A. E., Di Fiore, P. P. and De Camilli, P. (1999) The epsins define a family of proteins that interact with components of the clathrin coat and contain a new protein module. J. Biol. Chem. **274**, 33959–33965

53 Ponting, C. P. (1996) Novel domains in NADPH oxidase subunits, sorting nexins, and PtdIns 3-kinases: binding partners of SH3 domains? Protein Sci. **5**, 2353–2357

54 Thorner, J. W. (2001) Greasing the wheels of secretory transport. Nat. Cell Biol. **3**, E196–E198

55 Wishart, M. J., Taylor, G. S. and Dixon, J. E. (2001) Phoxy lipids: revealing PX domains as phosphoinositide binding modules. Cell (Cambridge, Mass.) **105**, 817–820

56 Burd, C. G. and Emr, S. D. (1998) Phosphatidylinositol(3)-phosphate signaling mediated by specific binding to RING FYVE domains. Mol. Cell **2**, 157–162

57 Corvera, S., D'Arrigo, A. and Stenmark, H. (1999) Phosphoinositides in membrane traffic. Curr. Opin. Cell Biol. **11**, 460–465

58 Gaullier, J. M., Simonsen, A., D'Arrigo, A., Bremnes, B., Stenmark, H. and Aasland, R. (1998) FYVE fingers bind PtdIns(3)P. Nature (London) **394**, 432–433

59 Patki, V., Lawe, D. C., Corvera, S., Virbasius, J. V. and Chawla, A. (1998) A functional PtdIns(3)P-binding motif. Nature (London) **394**, 433–434

60 Stenmark, H. and Aasland, R. (1999) FYVE-finger proteins – effectors of an inositol lipid. J. Cell Sci. **112**, 4175–4183

61 Mari, M., Macia, E., Le Marchand-Brustel, Y. and Cormont, M. (2001) Role of FYVE-finger and the run domain for the subcellular localization of RABIP4. J. Biol. Chem. **276**, 42501–42508

62 Nielsen, E., Christoforidis, S., Uttenweiler-Joseph, S., Miaczynska, M., Dewitte, F., Wilm, M., Hoflack, B. and Zerial, M. (2000) Rabenosyn-5, a novel rab5 effector, is complexed with hVPS45 and recruited to endosomes through a FYVE finger domain. J. Cell Biol. **151**, 601–612

63 Seet, L.-F. and Hong, W. (2001) Endofin, an endosomal FYVE domain protein. J. Biol. Chem. **276**, 42445–42454

64 Shin, M. E., Ogburn, K. D., Varban, O. A., Gilbert, P. M. and Burd, C. G. (2001) FYVE domain targets Pib1p ubiquitin ligase to endosome and vacuolar membranes. J. Biol. Chem. **276**, 41388–41393

65 Simonsen, A., Lippe, R., Christoforidis, S., Gaullier, J.-M., Brech, A., Callaghan, J., Toh, B.-H., Murphy, C., Zerial, M. and Stenmark, H. (1998) EEA1 links PI(3)K function to Rab5 regulation of endosome fusion. Nature (London) **394**, 494–498

66 Gaullier, J.-M., Rønning, E., Gillooly, D. J. and Stenmark, H. (2000) Interaction of the EEA1 FYVE finger with phosphatidylinositol 3-phosphate and early endosomes. Role of conserved residues. J. Biol. Chem. **275**, 24595–24600

67 Raiborg, C., Bremnes, B., Mehlum, A., Gillooly, D. J., Stang, E. and Stenmark, H. (2001) FYVE and coiled-coil domains determine the specific localisation of Hrs to early endosomes. J. Cell Sci. **114**, 2255–2263

68 Driscoll, P. C. (2001) Solving the FYVE domain-PtdIns(3)P puzzle. Nat. Struct. Biol. **8**, 287–290

69 Misra, S. and Hurley, J. H. (1999) Crystal structure of a phosphatidylinositol 3-phosphate-specific membrane-targeting motif, the FYVE domain of Vps27p. Cell (Cambridge, Mass.) **97**, 657–666

70 Mao, Y., Nickitenko, A., Duan, X., Lloyd, T. E., Wu, M. N., Bellen, H. and Quiocho, F. A. (2000) Crystal structure of the VHS and FYVE tandem domains of Hrs, a protein involved in membrane trafficking and signal transduction. Cell (Cambridge, Mass.) **100**, 447–456

71 Kutaleladze, T. G., Ogburn, K. D., Watson, W. T., de Beer, T., Emr, S. D., Burd, C. G. and Overduin, M. (1999) Phosphatidylinositol 3-phosphate recognition by the FYVE domain. Mol. Cell **3**, 805–811

72 Kutataladze, T. and Overduin, M. (2001) Structural mechanism of endosome docking by the FYVE domain. Science (Washington, D.C.) **291**, 1793–1796

73 Wurmser, A. E., Gary, J. D. and Emr, S. D. (1999) Phosphoinositide 3-kinases and their FYVE domain-containing effectors as regulators of vacuolar/lysosomal membrane trafficking pathways. J. Biol. Chem. **274**, 9129–9132

74 Christoforidis, S., McBride, H. M., Burgoyne, R. D. and Zerial, M. (1999) The Rab5 effector EEA1 is a core component of endosome docking. Nature (London) **397**, 621–626

75 Tsukazaki, T., Chiang, T. A., Davison, A. F., Attisano, L. and Wrana, J. L. (1998) SARA, a FYVE domain protein that recruits Smad2 to the TGF-β receptor. Cell (Cambridge, Mass.) **95**, 779–791

76 Hayakawa, A. and Kitamura, (2000) Early endosomal localization of Hrs requires a sequence within the proline- and glutamine-rich region but not the FYVE finger. J. Biol. Chem. **275**, 29636–29642

77 Piper, R. C., Cooper, A. A., Yang, H. and Stevens, T. H. (1995) *VPS27* controls vacuolar and endocytic traffic through a prevacuolar compartment in *Saccharomyces cerevisiae*. J. Cell Biol. **131**, 603–617

78 Estrada, L., Caron, E. and Gorski, J. L. (2001) *Fgd1*, the Cdc42 guanine nucleotide exchange factor responsible for faciogenital dysplasia, is localized to the subcortical actin cytoskeleton and Golgi membrane. Hum. Mol. Genet. **10**, 485–495

79 Obaishi, H., Nakanishi, H., Mandai, K., Satoh, K., Satoh, A., Takahashi, K., Miyahara, M., Nishioka, H., Takaishi, K. and Takai, Y. (1998) Frabin, a novel FGD-related actin filament-binding protein capable of changing cell shape and activating c-Jun N-terminal kinase. J. Biol. Chem. **273**, 18697–18700

80 Derubeis, A. R., Young, M. F., Jia, L., Robey, P. G. and Fisher, L. W. (2000) Double FYVE-containing protein 1 (DFCP1): isolation, cloning and characterization of a novel FYVE finger protein from a human bone marrow cDNA library. Gene **255**, 195–203

81 Shisheva, A., Rusin, B., Ikonomov, O. C., DeMarco, C. and Sbrissa, D. (2001) Localization and insulin-regulated relocation of phosphoinositide 5-kinase PIKfyve in 3T3-L1 adipocytes. J. Biol. Chem. **276**, 11859–11869

82 Cheung, P. C., Trinkle-Mulcahy, L., Cohen, P. and Lucocq, J. M. (2001) Characterization of a novel phosphatidylinositol 3-phosphate-binding protein containing two FYVE fingers in tandem that is targeted to the Golgi. Biochem. J. **355**, 113–121

83 Rong, S. B., Hu, Y., Enyedy, I., Powis, G., Meuillet, E. J., Wu, X., Wang, R., Wang, S. and Kozikowski, A. P. (2001) Molecular modeling studies of the Akt PH domain and its interaction with phosphoinositides. J. Med. Chem. **44**, 898–908

84 Dowler, S., Currie, R. A., Campbell, D. G., Deak, M., Kular, G., Downes, C. P. and Alessi, D. R. (2000) Identification of pleckstrin-homology-domain-containing proteins with novel phosphoinositide-binding specificities. Biochem. J. **351**, 19–31

85 Kavran, J. M., Klein, D. E., Lee, A., Falasca, M., Isakoff, S. J., Skolnik, E. Y. and Lemmon, M. A. (1998) Specificity and promiscuity in phosphoinositide binding by pleckstrin homology domains. J. Biol. Chem. **273**, 30497–30508

86 Rameh, L. E., Arvidsson, A.-K., Carraway, III, K. L., Couvillon, A. D., Rathbun, G., Cromptoni, A., VanRenterghem, B., Czech, M. P., Ravichandran, K. S., Burakoff, S. J. et al. (1997) A comparative analysis of the phosphoinositide binding specificity of pleckstrin homology domains. J. Biol. Chem. **272**, 22059–22066

87 Scharenberg, A. M., El-Hillal, O., Fruman, D. A., Beitz, L. O., Li, Z., Lin, S., Gout, I., Cantley, L. C., Rawlings, D. J. and Kinet, J. P. (1998) Phosphatidylinositol-3,4,5-trisphosphate (PtdIns-3,4,5-P_3)/Tec kinase-dependent calcium signaling pathway: a target for SHIP-mediated inhibitory signals. EMBO J. **17**, 1961–1972

88 Saito, K., Scharenberg, A. M. and Kinet, J. P. (2001) Interaction between the Btk PH domain and phosphatidylinositol-3,4,5-trisphosphate directly regulates Btk. J. Biol. Chem. **276**, 16201–16206

89 Dowler, S., Currie, R. A., Downes, C. P. and Alessi, D. R. (1999) DAPP1: a dual adaptor for phosphotyrosine and 3-phosphoinositides. Biochem. J. **342**, 7–12

90 Meacci, E., Tsai, S. C., Adamik, R., Moss, J. and Vaughan, M. (1997) Cytohesin-1, a cytosolic guanine nucleotide-exchange protein for ADP-ribosylation factor. Proc. Natl. Acad. Sci. U.S.A. **94**, 1745–1748

91 Chardin, P., Paris, S., Antonny, B., Robineau, S., Beraud-Dufour, S., Jackson, C. L. and Chabre, M. (1996) A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains. Nature (London) **384**, 481–484

92 Isakoff, S. J., Cardozo, T., Andreev, J., Li, Z., Ferguson, K. M., Abagyan, R., Lemmon, M. A., Aronheim, A. and Skolnik, E. Y. (1998) Identification and analysis of PH domain-containing targets of phosphatidylinositol 3-kinase using a novel *in vivo* assay in yeast. EMBO J. **17**, 5374–5387

93 Lietzke, S. E., Bose, S., Cronin, T., Kharlund, J., Chawla, A., Czech, M. P. and Lambright, D. G. (2000) Structural basis of 3-phosphoinositide recognition by pleckstrin homology domains. Mol. Cell **6**, 385–394

94 Ferguson, K. M., Kavran, J. M., Sankaran, V. G., Fournier, E., Isakoff, S. J., Skolnik, E. Y. and Lemmon, M. A. (2000) Structural basis for discrimination of 3-phosphoinositides by pleckstrin homology domains. Mol. Cell **6**, 373–384

95 Vanhaesebroeck, B. and Alessi, D. R. (2000) The PI3K-PDK1 connection: more than just a road to PKB. Biochem. J. **346**, 561–576

96 August, A., Sadra, A., Dupont, B. and Hanafusa, H. (1997) Src-induced activation of inducible T-cell kinase (ITK) requires phosphatidylinositol 3-kinase activity and the Pleckstrin homology domain of inducible T-cell kinase. Proc. Natl. Acad. Sci. U.S.A. **94**, 11227–11232

97 Li, Z., Wahl, M. I., Eguinoa, A., Stephens, L. R., Hawkins, P. T. and Witte, O. N. (1997) Phosphatidylinositol 3-kinase-γ activates Bruton's tyrosine kinase in concert with Src family kinases. Proc. Natl. Acad. Sci. U.S.A. **94**, 13820–13825

98 Fukuda, M., Kojima, T., Kabayama, H. and Mikoshiba, K. (1996) Mutation of the pleckstrin homology domain of Bruton's tyrosine kinase in immunodeficiency impaired inositol 1,3,4,5-tetrakisphosphate binding capacity. J. Biol. Chem. **271**, 30303–30306

99 Suzuki, H., Terauchi, Y., Fujiwara, M., Aizawa, S., Yazaki, Y., Kadowaki, T. and Koyasu, S. (1999) Xid-like immunodeficiency in mice with disruption of the p85alpha subunit of phosphoinositide 3-kinase. Science (Washington, D.C.) **283**, 393–397

100 Langille, S. E., Patki, V., Klarlund, J. K., Buxton, J. M., Holik, J. J., Chawla, A., Corvera, S. and Czech, M. P. (1999) ADP-ribosylation factor 6 as a target of guanine nucleotide exchange factor GRP1. J. Biol. Chem. **274**, 27099–27104

101 Venkateswarlu, K., Oatey, P. B., Tavare, J. M. and Cullen, P. J. (1998) Insulin-dependent translocation of ARNO to the plasma membrane of adipocytes requires phosphatidylinositol 3-kinase. Curr. Biol. **8**, 463–466

102 Franke, T. F., Kaplan, D. R., Cantley, L. C. and Toker, A. (1997) Direct regulation of the Akt proto-oncogene product by phosphatidylinositol 3,4-bisphosphate. Science (Washington, D.C.) **275**, 665–668

103 Klippel, A., Kavanaugh, W. M., Pot, D. and Williams, L. T. (1997) A specific product of phosphatidylinositol 3-kinase directly activates the protein kinase Akt through its pleckstrin homology domain. Mol. Cell. Biol. **17**, 338–344

104 Han, J., Luby-Phelps, K., Das, B., Shu, X., Xia, Y., Mosteller, R. D., Krishna, U. M., Falck, J. R., White, M. A. and Broek, D. (1998) Role of substrates and products of PI 3-kinase in regulating activation of Rac-related guanosine triphosphatases by Vav. Science (Washington, D.C.) **279**, 558–560

105 Ford, M. G., Pearse, B. M., Higgins, M. K., Vallis, Y., Owen, D. J., Gibson, A., Hopkins, C. R., Evans, P. R. and McMahon, H. T. (2001) Simultaneous binding of PtdIns(4,5)P_2 and clathrin by AP180 in the nucleation of clathrin lattices on membranes. Science (Washington, D.C.) **291**, 1051–1055

106 Itoh, T., Koshiba, S., Kigawa, T., Kikuchi, A., Yokoyama, S. and Takenawa, T. (2001) Role of the ENTH domain in phosphatidylinositol-4,5-bisphosphate binding and endocytosis. Science (Washington, D.C.) **291**, 1047–1051

107 D'Hondt, K., Heese-Peck, A. and Riezman, H. (2000) Protein and lipid requirements for endocytosis. Annu. Rev. Genet. **34**, 255–295

108 Jost, M., Simpson, F., Kavran, J. M., Lemmon, M. A. and Schmid, S. L. (1988) Phosphatidylinositol-4,5-bisphosphate is required for endocytic coated vesicle formation. Curr. Biol. **8**, 1399–1402

109 Martin, T. F. (2001) PI(4,5)P_2 regulation of surface membrane traffic. Curr. Opin. Cell Biol. **13**, 493–499

110 Hyman, J., Chen, H., Di Fiore, P. P., De Camilli, P. and Brunger, A. T. (2000) Epsin 1 undergoes nucleocytosolic shuttling and its Eps15 interactor NH_2-terminal homology (ENTH) domain, structurally similar to *Armadillo* and HEAT repeats, interacts with the transcription factor promyelocytic leukemia Zn^{2+} finger protein (PLZF). J. Cell Biol. **149**, 537–546

111 Wanker, E. E., Rovira, C., Scherzinger, E., Hasenbank, R., Walter, S., Tait, D., Colicelli, J. and Lehrach, H. (1997) HIP-I: a huntingtin interacting protein isolated by the yeast two-hybrid system. Hum. Mol. Genet. **6**, 487–495

112 Walling, H. W., Baldassare, J. J. and Westfall, T. C. (1998) Molecular aspects of Huntington's disease. J. Neurosci. Res. **54**, 301–308

113 Chopra, V. S., Metzler, M., Rasper, D. M., Engqvist-Goldstein, A. E., Singaraja, R., Gan, L., Fichter, K. M., McCutcheon, K., Drubin, D., Nicholson, D. W. and Hayden, M. R. (2000) HIP12 is a non-proapoptotic member of a gene family including HIP1, an interacting protein with huntingtin. Mamm. Genome **11**, 1006–1015

114 Engqvist-Goldstein, A. E., Kessels, M. M., Chopra, V. S., Hayden, M. R. and Drubin, D. G. (1999) An actin-binding protein of the Sla2/Huntingtin interacting protein 1 family is a novel component of clathrin-coated pits and vesicles. J. Cell Biol. **147**, 1503–1518

115 Seki, N., Muramatsu, M., Sugano, S., Suzuki, Y., Nakagawara, A., Ohhira, M., Hayashi, A., Hori, T. and Saito, T. (1998) Cloning, expression analysis, and chromosomal localization of HIP1R, an isolog of huntingtin interacting protein (HIP1). J. Hum. Genet. **43**, 268–271

116 Wesp, A., Hicke, L., Palecek, J., Lombardi, R., Aust, T., Munn, A. L. and Riezman, H. (1997) End4p/Sla2p interacts with actin-associated proteins for endocytosis in *Saccharomyces cerevisiae*. Mol. Biol. Cell **8**, 2291–2306

117 Kurten, R. C., Cadena, D. L. and Gill, G. N. (1996) Enhanced degradation of EGF receptors by a sorting nexin, SNX1. Science (Washington, D.C.) **272**, 1008–1010

118 Haft, C. R., de la Luz Sierra, M., Barr, V. A., Haft, D. H. and Taylor, S. I. (1998) Identification of a family of sorting nexin molecules and characterization of their association with receptors. Mol. Cell. Biol. **18**, 7278–7287

119 Otsuki, T., Kajigaya, S., Ozawa, K. and Liu, J. M. (1999) SNX5, a new member of the sorting nexin family, binds to the Fanconi anemia complementation group A protein. Biochem. Biophys. Res. Commun. **265**, 630–635

120 Parks, W. T., Frank, D. B., Huff, C., Renfrew Haft, C., Martin, J., Meng, X., de Caestecker, M. P., McNally, J. G., Reddi, A., Taylor, S. I., Roberts, A. B., Wang, T. and Lechleider, R. J. (2001) Sorting nexin 6, a novel SNX, interacts with the transforming growth factor-β family of receptor serine-threonine kinases. J. Biol. Chem. **276**, 19332–19339

121 Phillips, S. A., Barr, V. A., Haft, D. H., Taylor, S. I. and Haft, C. R. (2001) Identification and characterization of snx15, a novel sorting nexin involved in protein trafficking. J. Biol. Chem. **276**, 5074–5084

122 Teasdale, R. D., Loci, D., Houghton, F., Karlsson, L. and Gleeson, P. A. (2001) A large family of endosome-localized proteins related to sorting nexin 1. Biochem. J. **358**, 7–16

123 Cheever, M. L., Sato, T. K., de Beer, T., Kutateladze, T. G., Emr, S. D. and Overduin, M. (2001) Phox domain interaction with PtdIns(3)P targets the Vam7 t-SNARE to vacuole membranes. Nat. Cell Biol. **3**, 613–618

124 Ellson, C. D., Gobert-Gosse, S., Anderson, K. E., Davidson, K., Erdjument-Bromage, H., Tempst, P., Thuring, J. W., Cooper, M. A., Lim, Z. Y., Holmes, A. B. et al. (2001) PtdIns(3)P regulates the neutrophil oxidase complex by binding to the PX domain of p40phox. Nat. Cell Biol. **3**, 679–682

125 Kanai, F., Liu, H., Field, S. J., Akbary, H., Matsuo, T., Brown, G. E., Cantley, L. C. and Yaffe, M. B. (2001) The PX domains of p47phox and p40phox bind to lipid products of PI(3)K. Nat. Cell Biol. **3**, 675–678

126 Song, X., Xu, W., Zhang, A., Huang, G., Liang, X., Virbasius, J. V., Czech, M. P. and Zhou, G. W. (2001) Phox homology domains specifically bind phosphatidylinositol phosphates. Biochemistry **40**, 8940–8944

127 Xu, Y., Hortsman, H., Seet, L. F., Wong, S. H. and Hong, W. (2001) SNX3 regulates endosomal function through its PX-domain-mediated interaction with PtdIns(3)P. Nat. Cell Biol. **3**, 658–666

128 Xu, J., Liu, D., Gill, G. and Zhou, S. (2001) Regulation of cytokine-independent survival kinase (CISK) by the Phox homology domain and phosphoinositides. J. Cell Biol. **154**, 699–705

129 Arcaro, A. and Wymann, M. P. (1993) Wortmannin is a potent phosphatidylinositol 3-kinase inhibitor: the role of phosphatidylinositol 3,4,5-trisphosphate in neutrophil responses. Biochem. J. **296**, 297–301

130 Sato, T. K., Darsow, T. and Emr, S. D. (1998) Vam7p, a SNAP-25-like molecule, and Vam3p, a syntaxin homolog, function together in yeast vacuolar protein trafficking. Mol. Cell. Biol. **18**, 5308–5319

131 Hiroaki, H., Ago, T., Ito, T., Sumimoto, H. and Kohda, D. (2001) Solution structure of the PX domain, a target of the SH3 domain. Nat. Struct. Biol. **8**, 526–530

132 Takemoto, Y., Furuta, M., Sato, M., Kubo, M. and Hashimoto, Y. (1999) Isolation and characterization of a novel HS1 SH3 domain binding protein, HS1BP3. Int. Immunol. **11**, 1957–1964

133 Medhurst, A. L., Huber, P. A., Waisfisz, Q., de Winter, J. P. and Mathew, C. G. (2000) Direct interactions of the five known Fanconi anaemia proteins suggest a common functional pathway. Hum. Mol. Genet. **15**, 423–429

134 Piletz, J. E., Ivanov, T. R., Sharp, J. D., Ernsberger, P., Chang, C. H., Pickard, R. T., Gold, G., Roth, B., Zhu, H., Jones, J. C. et al. (2000) Imidazoline receptor antisera-selected (IRAS) cDNA: cloning and characterization. DNA Cell Biol. **19**, 319–329

135 Fuchs, U., Rehkamp, G., Haas, O. A., Slany, R., Konig, M., Bojesen, S., Bohle, R. M., Damm-Welk, C., Ludwig, W. D., Harbott, J. and Borkhardt, A. (2001) The human formin-binding protein 17 (FBP17) interacts with sorting nexin, SNX2, and is an MLL-fusion partner in acute myelogeneous leukemia. Proc. Natl. Acad. Sci. U.S.A. **98**, 8756–8761

136 McBride, H. M., Rybin, V., Murphy, C., Giner, A., Teasdale, R. and Zerial, M. (1999) Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin 13. Cell (Cambridge, Mass.) **98**, 377–386

137 Tall, G. G., Hama, H., DeWald, D. B. and Horazdovsky, B. F. (1999) The phosphatidylinositol 3-phosphate binding protein Vac1p interacts with a Rab GTPase and a Sec1p homologue to facilitate vesicle-mediated vacuolar protein sorting. Mol. Biol. Cell **10**, 1873–1889

138 Bean, A. J., Seifert, R., Chen, Y. A., Sacks, R. and Scheller, R. H. (1997) Hrs-2 is an ATPase implicated in calcium-regulated secretion. Nature (London) **385**, 826–829

139 Davidson, H. W. (1995) Wortmannin causes mistargeting of procathepsin D. Evidence for the involvement of a phosphatidylinositol 3-kinase in vesicular transport to lysosomes. J. Cell Biol. **130**, 797–805

140 Spiro, D. J., Boll, W., Kirchhausen, T. and Wessling-Resnick, M. (1996) Wortmannin alters the transferrin receptor endocytic pathway *in vivo* and *in vitro*. Mol. Biol. Cell **7**, 355–367

141 Ekena, K. and Stevens, T. H. (1995) The *Saccharomyces cerevisiae* MVP1 gene interacts with VPS1 and is required for vacuolar protein sorting. Mol. Cell. Biol. **15**, 1671–1678

142 Vater, C. A., Raymond, C. K., Ekena, K., Howald-Stevenson, I. and Stevens, T. H. (1992) The VPS1 protein, a homolog of dynamin required for vacuolar protein sorting in *Saccharomyces cerevisiae*, is a GTPase with two functionally separable domains. J. Cell Biol. **119**, 773–786

143 Wilsbach, K. and Payne, G. S. (1993) Vps1p, a member of the dynamin GTPase family, is necessary for Golgi membrane protein retention in *Saccharomyces cerevisiae*. EMBO J. **12**, 3049–3059

144 Voos, W. and Stevens, T. H. (1998) Retrieval of resident late-Golgi membrane proteins from the prevacuolar compartment of *Saccharomyces cerevisiae* is dependent on the function of Grd19p. J. Cell Biol. **140**, 577–590

145 Köhrer, K. and Emr, S. D. (1993) The yeast *VPS17* gene encodes a membrane associated protein required for the sorting of soluble vacuolar hydrolases. J. Biol. Chem. **268**, 559–569

146 Horazdovsky, B. F., Davies, B. A., Seaman, M. N., McLaughlin, S. A., Yoon, S. and Emr, S. D. (1997) A sorting nexin-1 homologue, Vps5p, forms a complex with Vps17p and is required for recycling the vacuolar protein-sorting receptor. Mol. Biol. Cell **8**, 1529–1541

147 Nothwehr, S. F. and Hindes, A. E. (1997) The yeast VPS5/GRD2 gene encodes a sorting nexin-1-like protein required for localizing membrane proteins to the late Golgi. J. Cell Sci. **110**, 1063–1072

148 Seaman, M. N., McCaffery, J. M. and Emr, S. D. (1998) A membrane coat complex essential for endosome-to-Golgi retrograde transport in yeast. J. Cell Biol. **142**, 665–681

149 Haft, C. R., de la Luz Sierra, M., Bafford, R., Lesniak, M. A., Barr, V. A. and Taylor, S. I. (2000) Human orthologs of yeast vacuolar protein sorting proteins Vps26, 29, and 35: assembly into multimeric complexes. Mol. Biol. Cell **11**, 4105–4116

150 Chin, L.-S., Raynor, M. C., Wei, X., Chen, H.-Q. and Li, L. (2001) Hrs interacts with sorting nexin 1 and regulates degradation of epidermal growth factor receptor. J. Biol. Chem. **276**, 7069–7078

151 Kurten, R. C., Eddington, A. D., Chowdhury, P., Smith, R. D., Davidson, A. D. and Shank, B. B. (2001) Self-assembly and binding of a sorting nexin to sorting endosomes. J. Cell Sci. **114**, 1743–1756

152 Barr, V. A., Phillips, S. A., Taylor, S. I. and Haft, C. R. (2001) Overexpression of a novel sorting nexin, SNX15, affects endosome morphology and protein trafficking. Traffic **1**, 904–916

153 Burg, N. D. and Pillinger, M. H. (2001) The neutrophil: function and regulation in innate and humoral immunity. Clin. Immunol. **99**, 7–17

154 Florian, V., Schlüter, T. and Bohnensack, R. (2001) A new member of the sorting nexin family interacts with the C-terminus of P-selectin. Biochem. Biophys. Res. Commun. **281**, 1045–1050

155 Kobayashi, T., Deak, M., Morrice, N. and Cohen, P. (1999) Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. Biochem. J. **344**, 189–197

156 Liu, D., Yang, X. and Zhou, S. (2000) Identification of CISK, a new member of the SGK kinase family that promotes IL-3-dependent survival. Curr. Biol. **10**, 1233–1236

157 Rameh, L. E. and Cantley, L. C. (1999) The role of phosphoinositide 3-kinase lipid products in cell function. J. Biol. Chem. **274**, 8347–8350

158 Cantrell, D. A. (2001) Phosphoinositide 3-kinase signaling pathways. J. Cell Sci. **114**, 1439–1445

159 Domin, J., Gaidarov, I., Smith, M. E., Keen, J. H. and Waterfield, M. D. (2000) The class II phosphoinositide 3-kinase PI3K-C2α is concentrated in the trans-Golgi network and present in clathrin-coated vesicles. J. Biol. Chem. **275**, 11943–11950

160 Gaidarov, I., Smith, M. E., Domin, J. and Keen, J. H. (2001) The class II phosphoinositide 3-kinase C2alpha is activated by clathrin and regulates clathrin-mediated membrane trafficking. Mol. Cell **7**, 443–449

161 Gomez-Cambronero, J. and Keire, P. (1998) Phospholipase D: a novel major player in signal transduction. Cell Signalling **10**, 387–397

162 Liscovitch, M., Czarny, M., Fiucci, G. and Tang, X. (2000) Phospholipase D: molecular and cell biology of a novel gene family. Biochem. J. **345**, 401–415

163 Kim, Y., Han, J. M., Park, J. B., Lee, S. D., Oh, Y. S., Chung, C., Lee, T. G., Kim, J. H., Park, S. K., Yoo, J. S. et al. (1999) Phosphorylation and activation of phospholipase D1 by protein kinase C *in vivo*: determination of multiple phosphorylation sites. Biochemistry **38**, 10344–10351

164 Lock, P., Abram, C. L., Gibson, T. and Courtneidge, S. A. (1998) A new method for isolating tyrosine kinase substrates used to identify fish, an SH3 and PX domain-containing protein, and Src substrate. EMBO J. **17**, 4346–4357

165 Howard, L., Nelson, K. K., Maciewicz, R. A. and Blobel, C. P. (1999) Interaction of the metalloprotease disintegrins MDC9 and MDC15 with two SH3 domain-containing proteins, endophilin I and SH3PX1. J. Biol. Chem. **274**, 31693–31699

166 Babior, B. M. (1999) NADPH oxidase: an update. Blood **93**, 1464–1476

167 Lekstrom-Himes, J. A. and Gallin, J. I. (2000) Immunodeficiency diseases caused by defects in phagocytes. N. Engl. J. Med. **343**, 1703–1714

168 Noack, D., Rae, J., Cross, A. R., Ellis, B. A., Newburger, P. E., Curnutte, J. T. and Heyworth, P. G. (2001) Autosomal recessive chronic granulomatous disease caused by defects in NCF-1, the gene encoding the phagocyte p47-phox: mutations not arising in the NCF-1 pseudogenes. Blood **97**, 305–311

169 De Mendez, I., Adams, A. G., Sokolic, R. A., Malech, H. L. and Leto, T. L. (1996) Multiple SH3 domain interactions regulate NADPH oxidase assembly in whole cells. EMBO J. **15**, 1211–1220

170 Fuchs, A., Dagher, M.-C. and Vignais, P. V. (1995) Mapping the domains of interaction of $p40^{phox}$ with both $p47^{phox}$ and $p67^{phox}$ of the neutrophil oxidase complex using the two-hybrid system. J. Biol. Chem. **270**, 5695–5697

171 Grizot, S., Grandvaux, N., Fieschi, F., Fauré, J., Massenet, C., Andrieu, J.-P., Fuchs, A., Vignais, P. V., Timmins, P. A., Dagher, M.-C. and Pebay-Peyroula, E. (2001) Small angle neutron scattering and gel filtration analysis of neutrophil NADPH oxidase cytosolic factors highlight the role of the C-terminal end of $p47^{phox}$ in the association with $p40^{phox}$. Biochemistry **40**, 3127–3133

172 Leusen, J. H., Verhoeven, A. J. and Roos, D. (1996) Interactions between the components of the human NADPH oxidase: intrigues in the phox family. J. Lab. Clin. Med. **128**, 461–476

173 Park, H.-S., Kim, I. S. and Park, J.-W. (1999) Phosphorylation induces conformational changes in the leukocyte NADPH oxidase subunit $p47^{phox}$. Biochem. Biophys. Res. Commun. **259**, 38–42

174 Johnson, J. L., Park, J.-W., El Benna, J., Faust, L. P., Inanami, O. and Babior, B. M. (1998) Activation of $p47^{PHOX}$, a cytosolic subunit of the leukocyte NADPH oxidase. J. Biol. Chem. **273**, 35147–35152

175 Ahmed, S., Prigmore, E., Govind, S., Veryard, C., Kozma, R., Wientjes, F. B., Segal, A. W. and Lim, L. (1998) Cryptic Rac-binding and p21(Cdc42Hs/Rac)-activated kinase phosphorylation sites of NADPH oxidase component p67(phox). J. Biol. Chem. **273**, 15693–15701

176 Kitamura, D., Kaneko, H., Miyagoe, Y., Ariyasu, T. and Watanabe, T. (1989) Isolation and characterization of a novel human gene expressed specifically in the cells of hematopoietic lineage. Nucleic Acids Res. **17**, 9367–9379

177 Takemoto, Y., Furuta, M., Li, X. K., Strong-Sparks, W. J. and Hashimoto, Y. (1995) LckBP1, a proline-rich protein expressed in haematopoietic lineage cells, directly associates with the SH3 domain of protein tyrosine kinase p56lck. EMBO J. **14**, 3403–3414

178 Yamanashi, Y., Okada, M., Semba, T., Yamori, T., Umemori, H., Tsunasawa, S., Toyoshima, K., Kitamura, D., Watanabe, T. and Yamamoto, T. (1993) Identification of HS1 protein as a major substrate of protein-tyrosine kinase(s) upon B-cell antigen receptor-mediated signaling. Proc. Natl. Acad. Sci. U.S.A. **90**, 3631–3635

179 Taniuchi, I., Kitamura, D., Maekawa, Y., Fukuda, T., Kishi, H. and Watanabe, T. (1995) Antigen-receptor induced clonal expansion and deletion of lymphocytes are impaired in mice lacking HS1 protein, a substrate of the antigen-receptor-coupled tyrosine kinases. EMBO J. **14**, 3664–3678

180 Takemoto, Y., Furuta, M., Sato, M., Findell, P. R., Ramble, W. and Hashimoto, Y. (1998) Growth factor receptor-bound protein 2 (Grb2) association with hemopoietic specific protein 1: linkage between Lck and Grb2. J. Immunol. **161**, 625–630

181 Yamanashi, Y., Fukuda, T., Nishizumi, H., Inazu, T., Higashi, K., Kitamura, D., Ishida, T., Yamamura, H., Watanabe, T. and Yamamoto, T. (1997) Role of tyrosine phosphorylation of HS1 in B cell antigen receptor-mediated apoptosis. J. Exp. Med. **185**, 1387–1392

182 Sato, S., Katagiri, T., Takaki, S., Kikuchi, Y., Hitoshi, Y., Yonehara, S., Tsukada, S., Kitamura, D., Watanabe, T., Witte, O. and Takatsu, K. (1994) IL-5 receptor-mediated tyrosine phosphorylation of SH2/SH3-containing proteins and activation of Bruton's tyrosine kinase and Janus 2 kinases. J. Exp. Med. **180**, 2101–2111

183 Fukamachi, H., Yamada, N., Miura, T., Kato, T., Ishikawa, M., Gulbins, E., Altman, A., Kawakami, Y. and Kawakami, T. (1994) Identification of a protein, SPY75, with repetitive helix-turn-helix motifs and an SH3 domain as a major substrate for protein tyrosine kinase(s) activated by FcRI cross-linking. J. Immunol. **152**, 642–652

184 Ingley, E., Sarna, M. K., Beaumont, J. G., Tilbrook, P. A., Tsai, S., Takemoto, Y., Williams, J. H. and Klinken, S. P. (2000) HS1 interacts with Lyn and is critical for erythropoietin-induced differentiation of erythroid cells. J. Biol. Chem. **275**, 7887–7893

185 Elion, E. A. (2000) Pheromone response, mating and cell biology. Curr. Opin. Microbiol. **3**, 573–581

186 Chenevert, J., Corrado, K., Bender, A., Pringle, J. and Herskowitz, I. (1992) A yeast gene (*BEM1*) necessary for cell polarization whose product contains two SH3 domains. Nature (London) **356**, 77–79

187 Chang, E., Bartholomeusz, G., Pimental, R., Chen, J., Lai, H., Wang, L. L., Yang, P. and Marcus, S. (1999) Direct binding and *in vivo* regulation of the fission yeast p21-activated kinase Shk1 by the SH3 domain protein Scd2. Mol. Cell. Biol. **19**, 8066–8074

188 Peterson, J., Zheng, Y., Bender, L., Myers, A., Cerione, R. and Bender, A. (1994) Interactions between the bud emergence proteins Bem1p and Bem2p and Rho-type GTPases in yeast. J. Cell Biol. **127**, 1395–1406

189 Sloat, B. F., Adams, A. and Pringle, J. R. (1981) Roles of the *CDC24* gene product in cellular morphogenesis during the *Saccharomyces cerevisiae* cell cycle. J. Cell Biol. **89**, 395–405

190 Adams, A. E. M., Johnston, D. I., Longnecker, R. M., Sloat, B. F. and Pringle, J. R. (1990) *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. J. Cell Biol. **111**, 131–142

191 Bender, A. and Pringle, J. R. (1991) Use of a screen for synthetic lethal and multicopy suppressee mutants to identify two new genes involved in morphogenesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**, 1295–1305

192 Bose, I., Irazoqui, J. E., Moskow, J. J., Bardes, E. S., Zyla, T. R. and Lew, D. J. (2001) Assembly of scaffold mediated complexes containing cdc42p, the exchange factor cdc24p, and the effector cla4p required for cell cycle-regulated phosphorylation of cdc24p. J. Biol. Chem. **276**, 7176–7186

193 Leeuw, T., Fourest-Lieuvin, A., Wu, C., Chenevert, J., Clark, K., Whiteway, M., Thomas, D. Y. and Leberer, E. (1995) Pheromone response in yeast: Association of Bem1p with proteins of the MAP kinase cascade and actin. Science (Washington, D.C.) **270**, 1210–1213

194 Lyons, D. M., Mahanty, S. K., Choi, K. Y., Manandhar, M. and Elion, E. A. (1996) The SH3-domain protein Bem1 coordinates mitogen-activated protein kinase cascade activation with cell cycle control in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16**, 4095–4106

195 Bender, L., Lo, H. S., Lee, H., Kokojan, V., Peterson, J. and Bender, A. (1996) Associations among PH and SH3 domain-containing proteins and Rho-type GTPases in yeast. J. Cell Biol. **133**, 879–894

196 Toya, M., Iino, Y. and Yamamoto, M. (1999) Fission yeast Pob1p, which is homologous to budding yeast Boi proteins and exhibits subcellular localization close to actin patches, is essential for cell elongation and separation. Mol. Biol. Cell **10**, 2745–2757

197 Moskow, J. J., Gladfelter, A. S., Lamson, R. E., Pryciak, P. M. and Lew, D. J. (2000) Role of Cdc42p in pheromone-stimulated signal transduction in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **20**, 7559–7571

198 Zheng, Y., Cerione, R. and Bender, A. (1994) Control of the yeast bud-site assembly GTPase Cdc42. J. Biol. Chem. **269**, 2369–2372

199 Zheng, Y., Hart, M. J., Shinjo, K., Evans, T., Bender, A. and Cerione, R. A. (1993) Biochemical comparisons of the *Saccharomyces cerevisiae* Bem2 and Bem3 proteins. J. Biol. Chem. **268**, 24629–24634

200 McConnell, S. J. and Yaffe, M. P. (1992) Nuclear and mitochondrial inheritance in yeast depends on novel cytoplasmic structures defined by the MDM1 protein. J. Cell Biol. **118**, 385–395

201 McConnell, S. J. and Yaffe, M. P. (1993) Intermediate filament formation by a yeast protein essential for organelle inheritance. Science (Washington, D.C.) **260**, 687–689

202 Fisk, H. A. and Yaffe, M. P. (1997) Mutational analysis of Mdm1p function in nuclear and mitochondrial inheritance. J. Cell Biol. **138**, 485–494

203 Appel, S., Reichwald, K., Zimmermann, W., Reis, A., Rosenthal, A. and Hennies, H. C. (2001) Identification and localization of a new human myotubularin-related protein gene, mtmr8, on 8p22-p23. Genomics **75**, 6–8

204 Ago, T., Takeya, R., Hiroaki, H., Kuribayashi, F., Ito, T., Kohda, D. and Sumimoto, H. (2001) The PX domain as a novel phosphoinositide-binding module. Biochem. Biophys. Res. Commun. **287**, 733–738

205 Virbasius, J. V., Song, X., Pomerleau, D. P., Zhan, Y., Zhou, G. W. and Czech, M. P. (2001) Activation of the Akt-related cytokine-independent survival kinase requires interaction of its phox domain with endosomal phosphatidylinositol 3-phosphate. Proc. Natl. Acad. Sci. U.S.A., in the press

206 Bravo, J., Karathanassis, D., Pacold, C. M., Pacold, M. E., Ellson, C. D., Anderson, K. E., Butler, P. J., Lavenir, I., Perisic, O., Hawkins, P. T. et al. (2001) The crystal structure of the PX domain from p40(phox) bound to phosphatidylinositol 3-phosphate. Mol. Cell **8**, 829–839