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Mechanistic similarities in docking of the FYVE and PX domains to phosphatidylinositol 3-phosphate containing membranes

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Abstract

Phosphatidylinositol 3-phosphate [PtdIns(3)P], a phospholipid produced by PI 3-kinases in early endosomes and multivesicular bodies, often serves as a marker of endosomal membranes. PtdIns(3)P recruits and activates effector proteins containing the FYVE or PX domain and therefore regulates a variety of biological processes including endo- and exocytosis, membrane trafficking, protein sorting, signal transduction and cytoskeletal rearrangement. Structures and PtdIns(3)P binding modes of several FYVE and PX domains have recently been characterized, unveiling the molecular basis underlying multiple cellular functions of these proteins. Here, structural and functional aspects and current mechanisms of the multivalent membrane anchoring by the FYVE and PX domains are reviewed and compared.

Keywords

FYVE domain; PX domain; phosphoinositide; phosphatidylinositol 3-phosphate; membrane

Introduction

Phosphatidylinositol 3-phosphate [PtdIns(3)P] is one of the seven phosphorylated derivatives of PtdIns, a major lipid component of cellular membranes (PtdIns constitutes ~8% of all phospholipids). PtdIns(3)P is produced by PI 3-kinases, which phosphorylate the D3 position of the myo-inositol ring of PtdIns. While only one PI 3-kinase, Vps34p, has been identified in yeast, three different classes of PI 3-kinases (I, II and III) are found in mammals, all with the ability to generate PtdIns(3)P *in vitro* (reviewed in [1–3]). Among them, the mammalian homolog of Vps34p produces the bulk of PtdIns(3)P and appears to specifically phosphorylate PtdIns but not PtdIns(4)P or PtdIns(4,5)P₂. PtdIns(3)P is found primarily in membranes of early endosomes, phagosomes and the internal vesicles of multivesicular bodies in mammalian cells, and in vacuolar and endosomal membranes in yeast. Although PtdIns(3)P is constitutively present at a ~200 μM concentration in human cells [4], its level is modulated by a relatively fast turnover, which occurs largely through internalization into multivesicular bodies and lysosomes (or yeast vacuoles) [5] and by the action of lipid kinases and phosphatases. For example, PtdIns(3)P can be converted into PtdIns(3,4)P₂ and PtdIns(3,5)P₂ by a putative 4-kinase and the 5-kinase PIKfyve [6–9], respectively, or dephosphorylated by the myotubularin family of phosphatases [10]. PtdIns(3)P serves as a reliable marker of endosomes and recruits

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cytosolic effector proteins involved in regulation of endocytotic machinery and trafficking to the endosomal membranes. A number of such effectors has been identified, the majority of which contain PtdIns(3)P-binding FYVE and PX domains, although C2 domain of Tollip [11] and PH domain of PEPP1 [12] are also able to recognize this PI *in vitro* (Fig. 1). In this review we will focus on the molecular mechanisms of docking of the FYVE and PX domain-containing proteins to PtdIns(3)P-enriched membranes.

FYVE domain

The FYVE (Fab1, YOTB, Vac1 and EEA1) domain is a zinc binding finger found in eukaryotic proteins involved in membrane trafficking and phosphoinositide metabolism [13]. This conserved ~70-residue module specifically recognizes PtdIns(3)P and targets many cytosolic proteins to PtdIns(3)P-enriched endosomal membranes [14–16]. The FYVE domain is defined by the three conserved sequences: the N-terminal WxxD, the central RR/KHHCR, and the C-terminal RVC motifs that form a compact PtdIns(3)P binding site and distinguish FYVE from other structurally related RING and PHD fingers.

FYVE domain-containing proteins have diverse biological functions. One of the largest subfamily of FYVE proteins is involved in endocytotic transport and regulates fusion of endosomal membranes with other endocytic vesicles and organelles. This includes the most characterized FYVE domain protein EEA1 [17–21], Rabenosin-5 [22], Rabip4 [23], Hrs [24–27] and the yeast proteins Vac1p [28] and Vps27p [29]. Similar to their non-catalytic relatives, a number of enzymes contain the FYVE domain and localize to endosomal membranes, including PIKfyve [8,30], yeast kinase Fab1 [7,31], MTMR3 and MTMR4 phosphatases [10, 32] and Pib1p ubiquitin ligase [33]. Another group of the FYVE domain proteins, such as Hrs and SARA, play roles in signal transduction [34–40].

Structure of the FYVE domain

Three-dimensional structures of the *S. cerevisiae* Vps27p, *Drosophila* Hrs, human EEA1 and Leishmania Major Lm5-1 FYVE domains have been determined by X-ray crystallography and NMR spectroscopy [41–44] (and unpublished data, PDB code 1Z2Q). All structures reveal a similar overall fold consisting of two double-stranded antiparallel β sheets and a C-terminal α -helix (Fig. 2). An additional N-terminal α -helical turn is seen in the EEA1 FYVE domain structure, and a short α -helix connecting β 2 and β 3 is present in the structures of Lm5-1 and EEA1. The functionally critical β 1 strand spans three residues of the RR/KHHCR motif and pairs with the β 2 strand, which links the two zinc clusters. The β 1 strand is preceded by an exposed hydrophobic protrusion, a so called membrane interaction loop (MIL), which penetrates into the bilayers upon binding of the FYVE domain to PtdIns(3)P-containing membranes. The FYVE domain fold is stabilized by tetrahedral coordination of two zinc ions, which are bound by four CxxC motifs in a cross-braced topology. One zinc ion is coordinated by the first and the third cysteine motifs, whereas second zinc ion is bound by the second and the fourth motifs in all human proteins. In yeast Vps27p, the fourth Cys residue is replaced by His. Zinc coordination is required for structural stability and biological activity of the FYVE finger, as mutation of any of the zinc coordinating residues [14,15,33,45,46] or zinc removal with chelators [15,47] results in the loss of the domain structure and function.

Molecular mechanism of the FYVE domain docking to PtdIns(3)P-containing membranes

FYVE domains bind PtdIns(3)P and direct a wide variety of cytosolic proteins to membranes during cell signaling and trafficking. Although the specific recognition of PtdIns(3)P remains a major distinguishing feature of the FYVE finger [14–16], the overall mechanism of

membrane anchoring is found to be multivalent and involves non-specific electrostatic contacts with acidic lipids other than PtdIns(3)P, activation of the histidine switch, hydrophobic insertion into the bilayers, and in some cases oligomerization of proteins (Fig. 3). Each of these binding components uniquely contributes to the FYVE domain specificity and affinity for PtdIns(3)P embedded in membranes.

PtdIns(3)P binding

The ability of FYVE finger to specifically recognize PtdIns(3)P was first observed by *in vitro* liposome binding experiments and substantiated by the protein's subcellular localization *in vivo* [14–16]. Removal of zinc or mutations of residues that coordinate the lipid or metal abolish the FYVE domain association with PtdIns(3)P-containing liposomes [14,15,45,47]. The recruitment of FYVE domain proteins to endosomes and yeast vacuoles can also be blocked by elimination of PI3K or by the presence of kinase inhibitor wortmannin [14,15,22,30,48,49]. The crystal [44] and NMR [43] structures of the EEA1 FYVE domain complexed with inositol 1,3-bisphosphate and dibutanoyl PtdIns(3)P, respectively, show that the PtdIns(3)P head group is coordinated by the N-terminal WxxD, the central basic RR/KHHCR and the C-terminal RVC motifs that together comprise a small concave binding pocket.

The histidine switch

Recent reports suggest that recruitment of the EEA1 FYVE domain to PtdIns(3)P-enriched membranes is pH-dependent and may be regulated by alterations in intracellular pH [50]. The FYVE domain affinity for PtdIns(3)P is considerably increased in the acidic media, and similar pH sensitivity is observed for the *in vivo* localization of EGFP-EEA1 in mammalian and yeast cells [50]. Lowering the cytosolic pH strengthens the anchoring of EEA1 to endosomal membranes, whereas increasing the pH disrupts the phosphoinositide binding and leads to cytoplasmic redistribution of EEA1. The pH dependency is attributed to a histidine switch comprising of a pair of adjacent His residues in the RR/KHHCR motif [50]. Apparently, the EEA1 FYVE domain binds PtdIns(3)P when both histidine residues are positively charged and releases the lipid upon their deprotonation. Based on the estimated affinities and the lipid's physiological concentrations, the EEA1 FYVE domain exists mainly in a bound state at low pH (6.0–6.6). At the cytosolic pH level of 7.3, only half of the protein is active, while essentially no activity is expected under more basic conditions. Because the two histidine residues are conserved among all PtdIns(3)P-binding FYVE domains, it is conceivable that FYVE modules other than EEA1 also exhibit the pH sensitivity. The pH-dependence can influence the function of FYVE proteins in cells with unusual cytosolic pH levels and in normal cells during physiological processes that involve changing of pH [51–59].

Non-specific electrostatic interactions

Mammalian early endosomes are enriched in acidic phospholipids other than PtdIns(3)P, such as phosphatidylserine (PtdSer) or phosphatidic acid [60] that contribute to the association of FYVE domains with endosomal membranes. The calculated electrostatic properties of the Vps27p, Hrs, EEA1, FENS-1 and Endofin FYVE domains suggest that membrane recruitment of these proteins is facilitated by non-specific electrostatic interactions involving basic residues of the FYVE fingers and negatively charged phospholipids in the membrane [61,62]. The calculations show a strong positive potential surrounding the MIL, which may drive the initial membrane association of the proteins and facilitate PtdIns(3)P binding. Furthermore, non-specific electrostatic interactions continue to play a role after PtdIns(3)P is fully bound and stabilize anchoring of the EEA1 FYVE domain to PtdIns(3)P-enriched dodecylphosphocholine (DPC) micelles, amplifying the binding affinity by three fold [63]. NMR titrations suggest that basic residues located around the PtdIns(3)P binding site and the MIL are involved in the

interaction with PtdSer [63]. Conservation of the basic residues suggests that the non-specific electrostatic contacts are a common feature of the FYVE fingers.

Hydrophobic insertion

In addition to the stereospecific recognition of PtdIns(3)P and non-specific electrostatic contacts with acidic lipids, the exposed MIL residues of the FYVE domain penetrate the bilayer. Insertion of the hydrophobic residues at the tip of this loop into membranes upon interaction with PtdIns(3)P was initially suggested based on the crystal structure of Vps27p FYVE domain [41] and on the micelle-induced changes in NMR resonances of the EEA1 FYVE finger [47]. It was further corroborated by monolayer penetration [62,64], liposome binding [62,65], computational modeling [61] and NMR studies with membrane mimetic micelle systems [43,63].

Recent computational and monolayer penetration studies have shown that binding of PtdIns(3)P facilitates penetration of the FYVE domains and increases their membrane residence time by decreasing the positive charge surrounding the MIL [61,62]. NMR experiments using spin label probes incorporated at various positions within micelles and intermolecular nuclear Overhauser effects indicate that the VT sequence at the tip of the MIL in the EEA1 FYVE domain inserts into the hydrophobic core of DPC micelles [63,66]. The inserted residues are surrounded by polar and charged residues located at the level of the lipid's headgroups and properly positioned for the non-specific electrostatic contacts. Conformational changes in the MIL accompany the micelle interaction, in which hydrophobic residues of the loop tend to move deeper into the non-polar core of micelles, whereas hydrophilic residues move toward the aqueous interface, hence stretching the MIL [66]. Substitution of the membrane-inserting residues of the FYVE domains abolishes or significantly decreases the membrane association and disrupts the normal biological functions of these proteins [47,62,67].

Multivalent binding

The multiple anchoring resulting from binding the PtdIns(3)P headgroup, non-specific electrostatic interactions and insertion of a set of aliphatic or aromatic residues provides the strength and selectivity that are necessary for the proper localization and function of the FYVE fingers. Thus, FYVE domains bind PtdIns(3)P-enriched acidic vesicles several orders of magnitude stronger than soluble lipids or isolated inositol headgroups [44,45]. The FYVE domain of EEA1 exhibits a 130 μ M affinity for a short dibutanoyl form of PtdIns(3)P [44,63,67], while a 50 nM affinity is measured for a long chain lipid embedded in acidic liposomes [45]. Other FYVE proteins including FENS-1, Endofin, *Drosophila* Hrs and yeast Vps27p bind PtdIns(3)P-containing liposomes with comparable affinities of 0.6 nM, 1 nM, 25 nM and 32 nM, respectively [46,62,64]. However, the soluble inositol 1,3-bisphosphate head group is recognized by these FYVE domains three orders of magnitude weaker [64]. Even in the neutral vesicles, human Hrs FYVE domain prefers intact lipid in a bilayer over the isolated headgroup [65]. Likewise, the EEA1 FYVE domain affinity is significantly increased in the presence of DPC micelles [63]. The endosomal localization of EEA1 is lost when the VT residues of the MIL are mutated [47]. Similarly, replacement of corresponding hydrophobic residues of the Vps27p or Hrs FYVE domains results in a seven to twenty fold reduction of their affinities for the membrane-bound PtdIns(3)P [62]. Consequently, the hydrophobic insertion in concert with electrostatic interactions stabilizes anchoring of the FYVE domains to membranes (Fig. 3).

Oligomerization

Membrane localization of a number of FYVE proteins is enhanced by bivalent or multivalent PtdIns(3)P interactions. For example, a synthetic construct containing a pair of covalently linked Hrs FYVE domains shows much higher affinity for PtdIns(3)P-containing membranes [48]. While a singly expressed GFP-fusion FYVE domain of Hrs appears to be cytosolic, it

translocates to endosomal membranes when a region adjacent to the FYVE finger is dimerized [67]. DFCP1 naturally contain a FYVE domain tandem [46,68], and the stable dimers and higher oligomers are intrinsically formed by SARA [67]. EEA1 forms a parallel coiled coil homodimer that juxtaposes two C-terminal FYVE domains allowing for simultaneous interactions with two PtdIns(3)P headgroups [44,69]. Increased avidity due to the dimerization of FYVE domain-containing proteins represent another way to enhance the membrane binding by the FYVE fingers.

PX domain

The PX domain was first identified in and named after the two phagocyte NADPH oxidase (phox) subunits, p40^{phox} and p47^{phox} [70]. Since then, it has been found in at least 47 mammalian and 15 yeast signaling proteins, protein kinases, PI kinases and phospholipases (reviewed in [71]). The PX domain consists of ~130 residues that are folded in a highly conserved three dimensional structure despite little sequence similarity between the family members. A proline-rich region (PXXP), involved in the interaction with SH3 domains, and a set of basic residues, shown to coordinate PIs, comprise the most conserved elements. Of all PIs, PtdIns(3)P appears to be a primary target of the PX domain-containing proteins as the majority of them are found associated with PtdIns(3)P-enriched endosomes and vacuoles [Vam7p, sorting nexins (SNXs), p40^{phox}, Grd19p], although interactions with PtdIns(3,4)P₂, PtdIns(3,5)P₂, PtdIns(4,5)P₂ and PtdIns(3,4,5)P₃ have also been reported for p47^{phox}, SNXs, PI3K-C2 α , CISK, FISH and PLD1 [72–81].

The PX domain proteins play fundamental roles in endocytosis, protein sorting, membrane trafficking, transcription, cell polarity and signaling (reviewed in [71,82]). The SNXs, found in both yeast and mammalian cells, comprise the largest family of PX proteins. Mammalian SNXs are involved in endosomal sorting and recycling, and in internalization, transport and lysosomal degradation of epidermal growth factor and other receptors [76,82–84]. Yeast SNXs, Mvp1p and Grd19p are required for regulation of protein retrieval and recycling traffic from prevacuolar/late endosomes to the late Golgi [85–87]. The t-SNARE Vam7p mediates fusion of multiple transport intermediates with the vacuole [88]. The subunits of neutrophilic NADPH oxidase complex, p40^{phox} and p47^{phox} are implicated in phagocyte-mediated destruction of ingested microbes [70,89]. Phospholipase PLD1 catalyzes the hydrolysis of phosphatidylcholine and produces choline and phosphatidic acid, a lipid second messenger [90]. The cytokine-independent survival kinase (CISK), PI 3-kinases and the adaptor protein FISH play a role in cell signaling [71,79,80].

Structure of the PX domain

The atomic-resolution crystal and solution structures of nine (Bem1p, CISK, Grd19p, p40^{phox}, p47^{phox}, PI3K-C2 α , SNX12, SNX22 and Vam7p) PX domains in the free and PtdIns(3)P- or PtdIns(3,4)P₂-bound states have been determined (ref. [91–98] and unpublished data, PDB ID 2CZO, 2CSK, 2ETT, 2AR5 and 1KQ6). All structures show a similar fold consisting of the N-terminal three-stranded β -sheet, packed against a helical subdomain composed of three to four α -helices (Fig. 4). An additional 3_{10} helix is seen in the structures of the p40^{phox}, CISK and PI3K-C2 α PX domains and another $\alpha 0$ helix is formed by residues N-terminal to the β -sheet in p40^{phox} [91,96,99]. The $\alpha 1$ and $\alpha 2$ are connected by a long variable loop, which in p40^{phox}, p47^{phox} and CISK contains a type II polyproline helix [91,92,96]. Strand $\beta 1$ has a β -bulge that twists the β sheet, forming one wall of the lipid binding pocket.

Molecular mechanism of the PX domain docking to PtdIns(3)P-containing membranes

The major function of the PX domain is to recruit trafficking and signaling proteins to membranes enriched in PtdIns(3)P or other PIs. As in the case of the FYVE domain, the PX domain targeting to PtdIns(3)P-containing bilayers involves multiple interactions. The specific recognition of the inositol headgroup is often accompanied by non-specific electrostatic contacts with acidic membrane surfaces, hydrophobic insertion into the bilayers and oligomerization of proteins (Fig. 5).

PtdIns(3)P binding

The PX domain was identified as a novel PI binding module simultaneously and independently by several groups [72–76]. The p40^{phox}, SNX3 and Vam7p PX domains were found to specifically recognize PtdIns(3)P, whereas p47^{phox} PX domain was shown to interact with PtdIns(3,4)P₂. The PtdIns(3)P binding was detected by liposome binding assay, protein-lipid overlay, surface plasmon resonance and NMR experiments and by *in vivo* localization of these proteins to endosomal and vacuolar membranes [72–76]. Since then a number of other PX domains have been identified that interact preferentially with PtdIns(3)P, including those of SNX1 [100], SNX16 [101], SNX17 [102] and FISH [80]. All 15 yeast PX domain proteins specifically recognize PtdIns(3)P [77]. Four of them, Grd19p/SNX3, Mdm1, Vam7p and Ypt35p/Yhr105wp, bind with high affinities (~2–3 μM) while the rest bind weaker ($K_d > 100$ μM). A strong interaction of the PX domain of p40^{phox}, SNX3 and Vam7p is sufficient for targeting of these proteins to PtdIns(3)P-enriched endosomes and vacuoles [72–74,76]. The membrane association of Vam7p and p40^{phox} is disrupted by mutations of the conserved residues in the PtdIns(3)P binding site or by inhibition of PI 3-kinase [72,74,91]. The PtdIns(3)P molecule in the p40^{phox} PX domain complex is positioned in a relatively narrow and deep (7 Å) groove formed by three regions, the loop connecting β3 and α1, the long loop preceding α2, and the N-terminal halves of β2 and α2 [91] (Fig. 4). Conserved residues of these regions form hydrogen bonds to the 3-phosphate, 1-phosphate and hydroxyl groups of PtdIns(3)P.

Non-specific electrostatic interactions

Membrane association of the PX domains is often facilitated by non-specific electrostatic interactions. Initial binding of p40^{phox} and p47^{phox} PX domains to the negatively charged membrane surfaces is shown to enhance specific recognition of a PI, which in turn induces a hydrophobic insertion into the bilayers [92,103]. In the case of the p47^{phox} PX domain, additional amplification is provided by binding of PtdSer or phosphatidic acid in a separate lipid binding site [92]. Basic residues located around the insertion loop and the PtdIns(3)P binding pocket of the Vam7p PX domain make electrostatic contacts with the acidic membrane surfaces [104]. These contacts enhance the binding affinity as the replacement of a single basic R73 residue diminishes the Vam7p PX domain membrane anchoring and penetration. The basic residues surrounding the insertion loop and the PI binding site are conserved in many PX domain sequences suggesting that the non-specific interactions with acidic membrane lipids are a general characteristic of the PX domains. This was exemplified by the recent studies on membrane docking of the PLD1 [105] and PI3K-C2α [99] PX domains.

Hydrophobic insertion

Several mechanistic studies have revealed that PtdIns(3)P binding induces the membrane penetration of surface hydrophobic residues of the variable α1–α2 loop (or membrane interaction loop (MIL)). The X-ray reflectivity experiments show that the p40^{phox} PX domain penetrates into the lipid layer by 9 Å, with the side chains of Tyr94 and Val95 inserted deepest [106]. Mutations of the Tyr94 and Val95 residues substantially reduce (by 6- to 27-fold) the

membrane binding affinity and the extent of penetration as measured by SPR and monolayer surface pressure studies [103]. Corresponding hydrophobic residues in the p47^{phox} PX domain, Ile65 and Trp80, also penetrate membranes [103]. Chemical shift perturbation analysis, monolayer surface tension, liposome binding and spin-label experiments indicate that the Val70, Leu71 and Trp75 residues of the Vam7p insert into membranes, and among them, Leu71 plays a major role in the membrane anchoring [74,104]. Replacement of the MIL residues disrupts localization of the EGFP- Vam7p, p40^{phox} and p47^{phox} PX domains to vacuolar and endosomal membranes, demonstrating that the hydrophobic penetration is essential for membrane targeting [92,103,104]. Modeling the structure of the PtdIns(3)P-bound p40^{phox} PX domain next to the membrane surface shows that the penetrating MIL residues are well positioned for the insertion. Furthermore, the membrane penetration places the basic Lys92 and Lys98 residues near the phosphate groups of the membrane lipids, providing favorable electrostatic contacts [106]. Theoretical calculations indicate that PtdIns(3)P binding reduces the strong positive electrostatic potential surrounding Tyr94 and Val95, which promotes their membrane penetration by decreasing the dehydration penalty [103,106]. Alignment of the PX domain sequences shows some conservation of the hydrophobic residues despite the fact that the $\alpha 1$ – $\alpha 2$ loop is highly variable. Thus, the Bem1, CISK, CPK, FISH, Grd19p, p40^{phox}, p47^{phox} and SNX3 contain hydrophobic and aromatic VPYV, IFG, MVLG, VYVGV, ILF, ILL, WFDG and LPF sequences, respectively, in place of the hydrophobic residues of p40^{phox}, p47^{phox} and Vam7p, and the MIL occupies analogous conformations in all PX domain structures [91–96].

Oligomerization

Membrane recruitment of the low-affinity PX domain proteins may be enhanced by the formation of dimers or oligomers that would increase the affinity through the synergistic binding to multiple PtdIns(3)P headgroups. Most sorting nexins have coiled coil regions, which mediate homo- and hetero-oligomeric interactions necessary for targeting to endosomal membranes [83,107,108]. In SNX1, the C-terminal BAR domain mediates its dimerization and is required for the high affinity binding of this protein to PtdIns(3)P-containing membranes [98,107].

Conclusion

Recent structural, biophysical and cellular studies have provided a detailed outline of the mechanism used by the FYVE domain and PX domain proteins to target PtdIns(3)P-enriched membranes. A number of elements in the multivalent anchoring are shared by these structurally unrelated modules (Fig. 6). In both domains the stereospecific PtdIns(3)P head group recognition is facilitated by non-specific electrostatic contacts with other acidic lipids, followed by a hydrophobic insertion into the bilayers. These interactions can be further stabilized by dimerization or oligomerization of the proteins. However several differences are apparent. While the FYVE domains exclusively and strongly bind PtdIns(3)P, the PX domains exhibit a wide range of specificities and affinities. The FYVE domain interaction with PtdIns(3)P is regulated by a histidine switch and is pH-dependent, whereas the PX domain interaction is not. In contrast to the lipid-binding FYVE domain, the PX domain is shown to bind other protein modules, such as SH3 domains [95], which may also regulate the PI binding and functions of the PX domain-containing proteins.

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Abbreviations

PtdIns(3)P, phosphatidylinositol 3-phosphate
 PtdIns, phosphatidylinositol
 PI, phosphoinositide
 PX, phox homology
 MIL, membrane interaction loop
 PtdSer, phosphatidylserine
 NMR, nuclear magnetic resonance
 EGFP, enhanced green fluorescent protein
 DPC, dodecylphosphocholine
 EEA1, early endosome antigen 1
 Hrs, hepatocyte growth factor-regulated tyrosine kinase substrate
 SARA, Smad anchor for receptor activation
 SNXs, sorting nexins
 SNARE, soluble N-ethylmaleimide-sensitive fusion protein attachment receptor

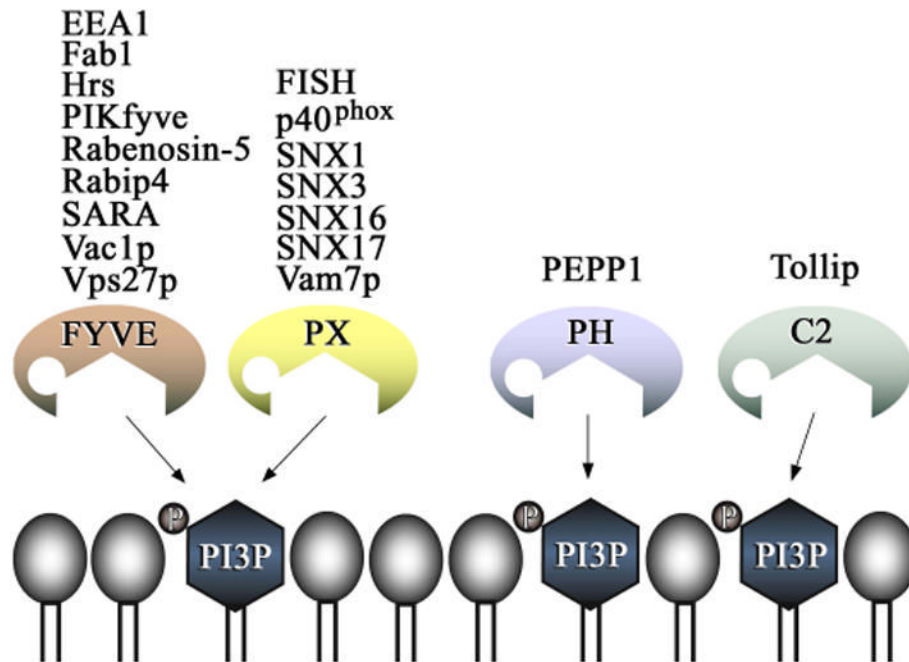


Fig. 1. PtdIns(3)P binding domains. Signaling domains are shown as colored shapes with proteins containing these domains listed above.

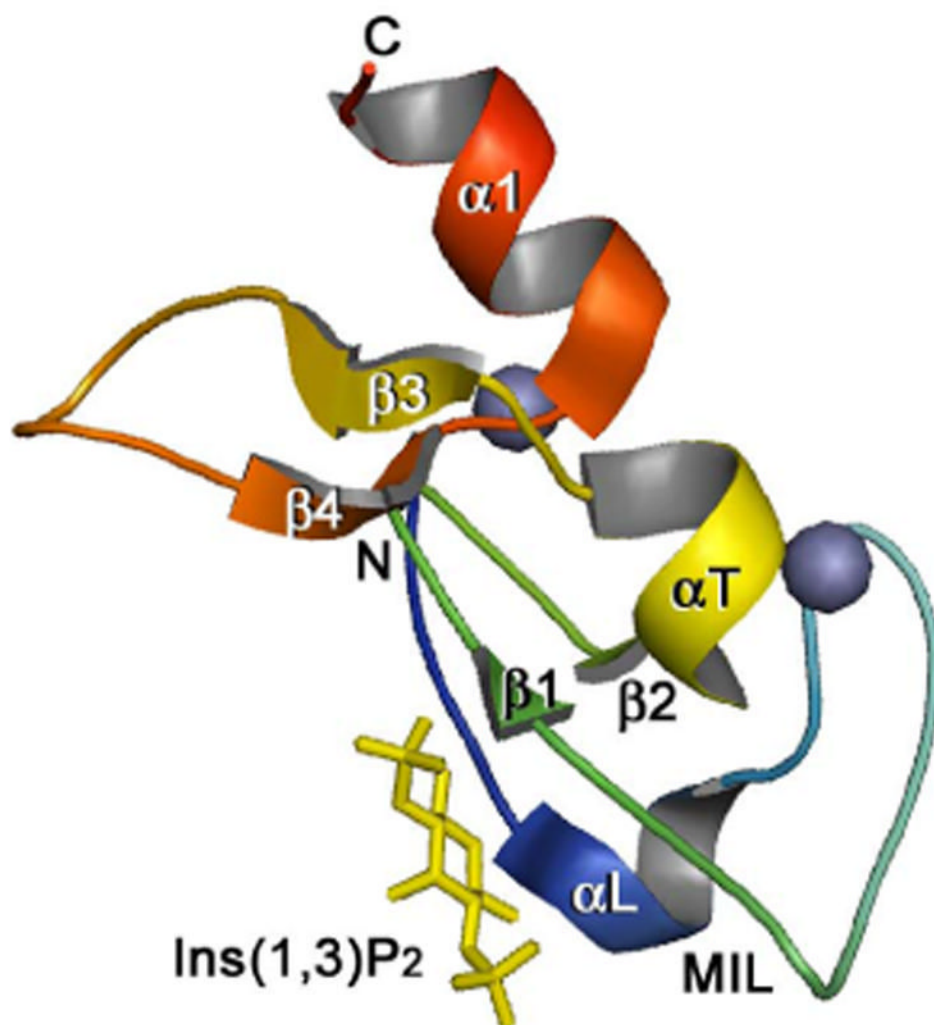


Fig. 2. The crystal structure of the EEA1 FYVE domain complexed with inositol 1,3-bisphosphate, a head group of PtdIns(3)P [44] (PDB code 1JOC, chain A residues 1346–1410 of the EEA1 dimer are shown). The backbone ribbon is colored using a graded scheme from the blue N-terminus to the red C-terminus. The zinc ions are shown as gray spheres. This figure and Figure 4 were prepared with PyMOL.

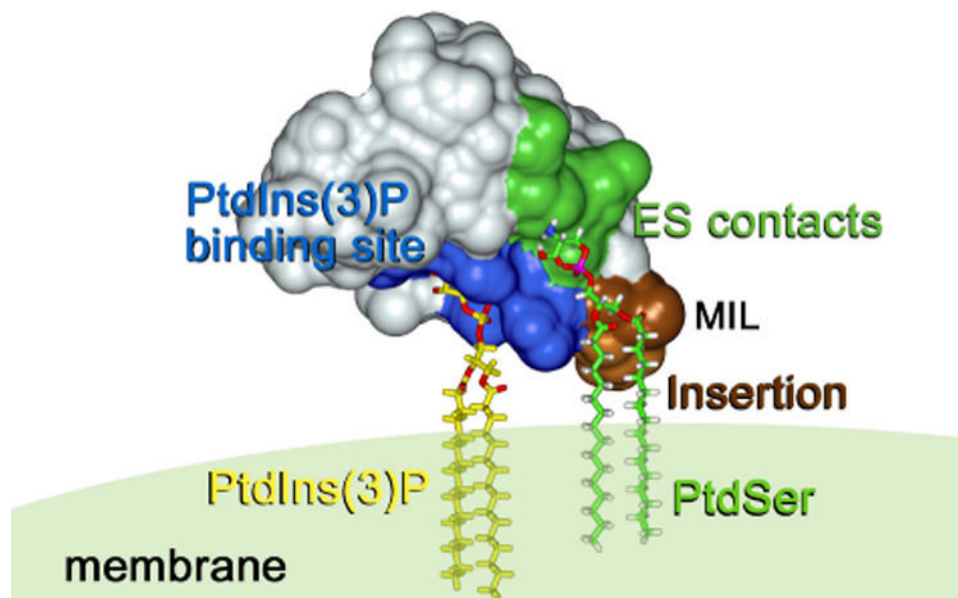


Fig. 3. A model of the FYVE domain anchoring to PtdIns(3)P- and PtdSer-containing membranes (NMR structure of the EEA1 FYVE domain [43], PDB code 1HYI). Basic residues of the PtdIns(3)P-binding pocket, the membrane interaction loop (MIL) and residues that are involved in electrostatic (ES) contacts with PtdSer are colored in blue, brown and green, respectively. PtdIns(3)P and PtdSer are shown as stick models. This figure and Figure 5 were prepared with InsightII.

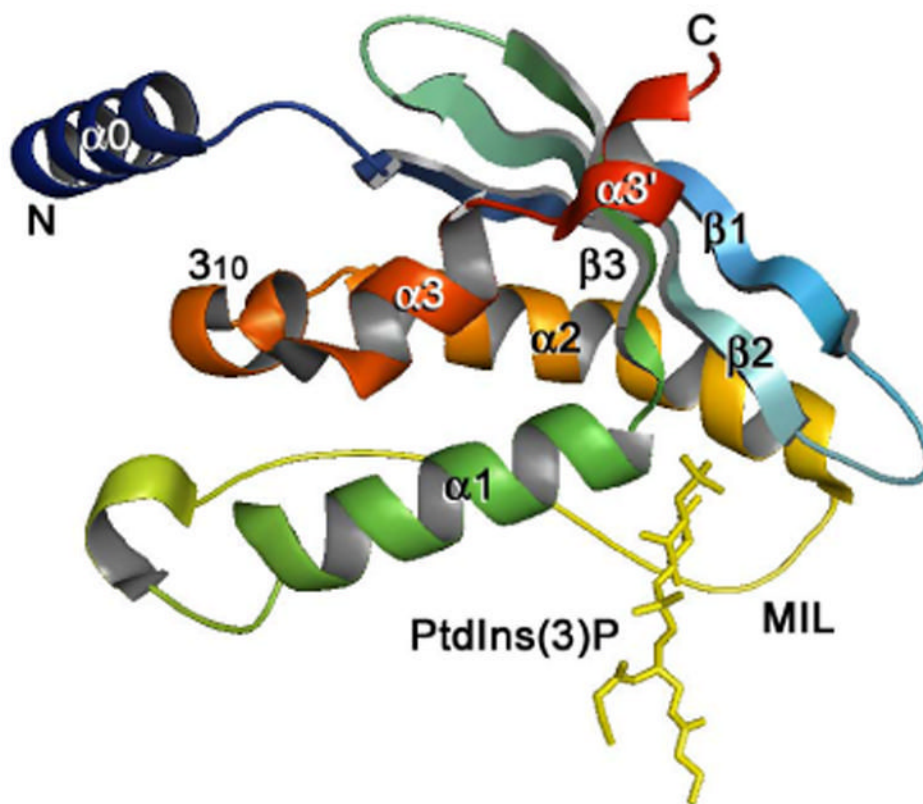


Fig. 4. The crystal structure of the PtdIns(3)P-bound PX domain of p40^{phox} [91] (PDB code 1H6H). The backbone ribbon is colored using a graded scheme from the blue N-terminus to the red C-terminus. PtdIns(3)P is depicted as a stick model and colored yellow.

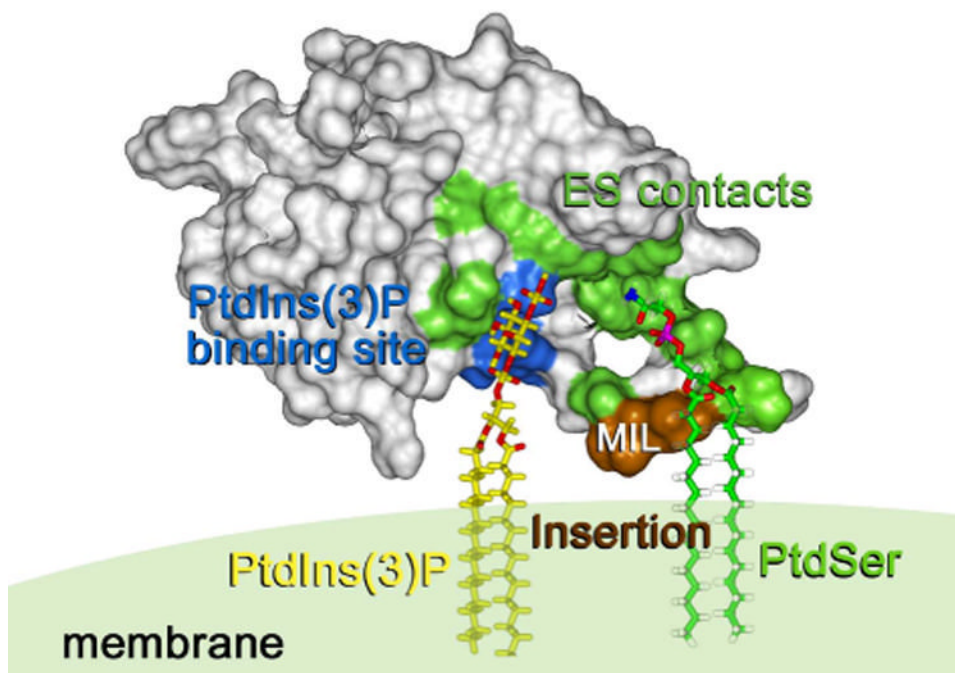


Fig. 5. A model of the PX domain docking to PtdIns(3)P- and PtdSer-containing membranes (NMR structure of the Vam7p PX domain [94], PDB code 1KMD). Basic residues of the PtdIns(3)P-binding pocket, the membrane interaction loop (MIL) and residues that are in contact with PtdSer are colored in blue, brown and green, respectively.

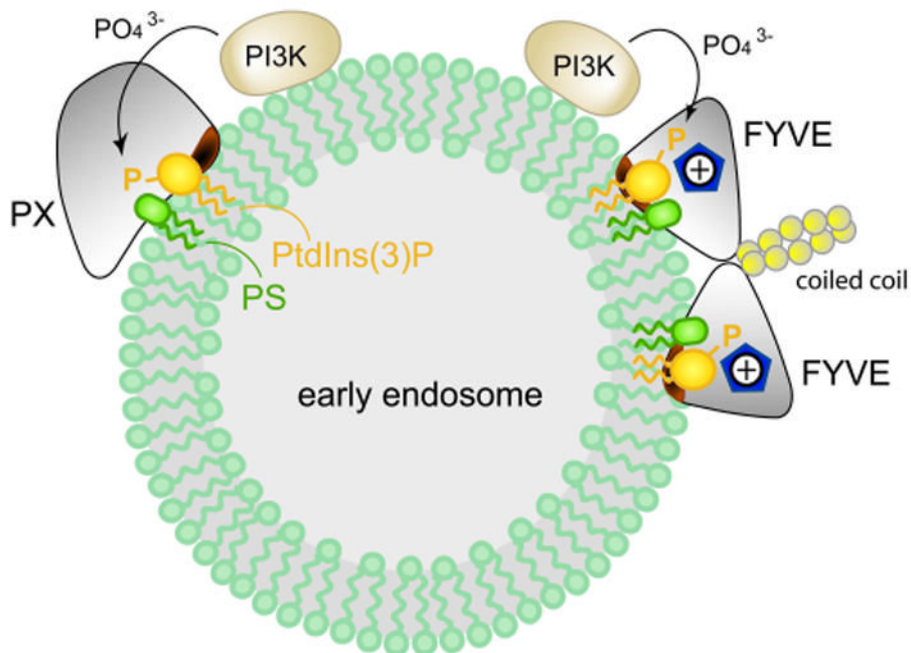


Fig. 6. Multivalent mechanism of the membrane targeting by the FYVE domain and PX domain proteins. Schematics of anchoring of a monomeric PX domain and a dimeric FYVE domain to PtdIns(3)P- and PtdSer-containing endosomal membranes. Binding of either module to PtdIns(3)P (dark yellow) is facilitated by non-specific electrostatic contacts with acidic lipids (PtdSer, green) and accompanied by a hydrophobic insertion (brown) into the bilayer. Dimerization/oligomerization through the adjacent coiled coil or other regions may juxtapose FYVE or PX domains for the simultaneous interactions with multiple head groups of PtdIns(3)P. The histidine switch required for the FYVE domain binding is shown as a blue pentagon. A signal is initiated by phosphorylation of PtdIns at the D3 position by PI3K.