the results of methylation interference experiments performed on the GMEB[13] and DEAF-1[3] SAND domain containing proteins. Attractively, it also seems to explain the importance of the ^{653}KDWK^{656} motif and other side chains implicated in binding, which are all poised for interactions with the major groove of the duplex.

Why hasn't anything like the SAND domain been seen before? One can speculate that this fold may not be ideally suited for DNA binding. Helix H3 is very short in length, and it seems likely that most interactions with the duplex will be mediated by the preceding loop. Perhaps loop burial by SAND domains does not yield a sufficiently large enough molecular interface to achieve highly specific nucleotide binding. This would be consistent with the finding that many SAND domain-containing proteins interact with low-complexity nucleotide sequences and the requirement that they multimerize for tight binding. Almost all SAND domains are located in multidomain proteins that contain a varied arrangement of chromosome-associated modules, homooligomerization domains, other DNA binding domains, protein–protein interaction modules and domains that mediate transcriptional repression or activation (Fig. 1*a*)[14–20]. It thus appears that highly specific regulation of gene expression requires the combined efforts of several of these modules targeted to the correct location by SAND domains, perhaps with the assistance of other DNA binding modules. A more complete understanding of how SAND domains recognize DNA awaits the structure determination of a SAND domain–DNA complex and biochemical studies to probe the determinants of its affinity and specificity. The results of the Sattler group[1] leave no doubt that this molecular complex will be unique.

Acknowledgments

J.M.W. and R.T.C. are supported by grants from the National Institutes of Health and Department of Energy.

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The double life of PX domains

Kenneth E. Prehoda and Wendell A. Lim

Recent structural and cell biological studies show that the previously uncharacterized PX domain can bind both phospholipids and SH3 domains, suggesting that this module may play a critical role in coordinating membrane localization and protein complex assembly during cell signaling.

Eukaryotic signaling proteins are made up of discrete modular domains, most of which mediate interactions with other signaling molecules. Each of these protein domains is generally assumed to have a single, dedicated function. For example, Src homology 3 (SH3) domains bind to proline-rich sequences in target proteins, whereas Pleckstrin Homology (PH) domains bind to acidic phospholipids. However, growing evidence suggests that many of these domains may be multifunctional, thereby increasing the complexity of connections they can mediate within the web of signaling pathways in the cell. Recent studies, including one by Hiroaki *et al.*[1] in the June issue of *Nature Structural Biology* and a number of reports in the July issue of *Nature Cell Biology*[2–5], provide two different functional views of the previously uncharacterized Phox Homology (PX) domain. Together these findings suggest that PX domains may be bifunctional — participating in both protein and phospholipid recognition. These results raise the intriguing possibility that PX domains may couple these two functions to coordinate both phospholipid- and protein-mediated signals.

The PX domain as an SH3 domain ligand

The PX domain was originally identified in the protein p47^{phox}, a component of phagocyte NADPH oxidase[6]. This multiprotein complex generates the microbicidal agent superoxide in neutrophils and other phagocytic cells activated by infectious stimuli[7]. Under basal conditions, the activity of this enzyme is tightly regulated because of the highly toxic nature of superoxide. Although the exact mechanism of regulation is unknown, activation involves translocation of several cytosolic components, including p47^{phox}, to the membrane where they form an active complex with other catalytic oxidase components[8].

SH3 domains play a critical role in the regulated assembly and activation of the NADPH oxidase. p47^{phox} has two SH3

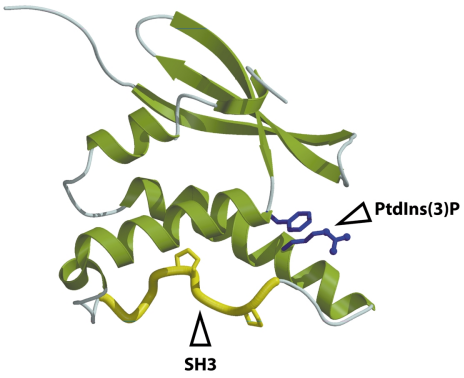

Fig. 1 Structure of the p47^{phox} PX domain solved by Hiroaki *et al.*[1]. The PX domain adopts a novel fold, shown here in a ribbon drawing. The proline-rich sequence, implicated in binding to an SH3 domain in p47^{phox}, is shown in yellow. In the Vam7 PX domain, PtdIns(3)P is thought to bind in an area near Tyr 42 (Phe 44 in p47^{phox}; shown in blue) based on chemical shift perturbation studies[3]. A conserved Arg residue (Arg 90) required for p47^{phox} phosphoinositide binding[2] is also shown.

domains, and translocation of p47^{phox} to the membrane requires an interaction between one of these SH3 domains and a proline-rich motif in p22^{phox}, a membrane oxidase component[8]. Interestingly, prior to activation, the p47^{phox} SH3 domains may be masked by intramolecular interactions. Several potential internal Pro-X-X-Pro motifs, the hallmark SH3 binding sequences, are found within p47^{phox} itself, including one in the PX domain. This Pro-X-X-Pro motif is highly conserved in all PX domains, leading to the proposal by Ponting[5] in 1996 that these domains might serve as ligands for SH3 domains. Nonetheless, this hypothesis has remained untested, and the PX domain has remained an 'orphan' module, until now.

Hiroaki *et al.*[1] have determined the structure of the PX domain from p47^{phox}, and they show that the domain can indeed serve as a ligand for one of the p47^{phox} SH3 domains. The PX domain adopts a novel fold, with the Pro-X-X-Pro motif conveniently lying on the domain surface. Mutation of these Pro residues disrupts SH3 binding, suggesting that they are recognized in a manner similar to canonical SH3–peptide interactions. Moreover, chemical shift studies indicate that the PX domain binds at the same site on SH3 domains as canonical peptide ligands.

However, there are several unusual properties of the motif found in PX domains that differ from canonical SH3–peptide interactions. First, the Pro-X-X-Pro motif in the PX domain structure is distorted from the polyproline type II helix observed for ideal SH3 ligands. Moreover, one of the key Pro residues in the PX domain appears, at the resolution of this structure, to be partially occluded from solvent, suggesting that a conformational change would be required for binding to occur. The NMR data presented by Hiroaki *et al.*[1] agree with this possibility; significant changes in the PX domain spectra are observed when the SH3 domain is added. This is consistent with the relatively low affinity (K_d ~100 μM) observed for the SH3–PX interaction. A structure of a PX–SH3 complex will be required to determine the molecular details of the interaction and its similarity to canonical SH3 interactions.

The PX domain binds phosphoinositides

Three new independent studies reveal a different side of the PX domain — in this case, as a phospholipid binding module[2–4]. Phosphoinositide signaling has long been suspected to play a role in NADPH oxidase activation. For example, inhibition of phosphoinositide 3-kinase (PI3K) blocks superoxide production[9]. How phosphoinositide signals are detected by the oxidase components, however, has been a mystery because they lack any of the well-characterized lipid binding modules, such as PH and FYVE domains.

In the recent papers, several groups have examined whether PX domains might mediate lipid recognition. These studies reveal that the PX domain from p47^{phox} specifically binds phosphatidylinositol-3,4-bisphosphate (PtdIns(3,4)P_2), whereas the PX domain from another oxidase component, p40^{phox}, specifically binds phosphatidylinositol-3-phosphate (PtdIns(3)P). A conserved Arg residue in PX domains, which by mutagenesis was found to be required for phospholipid binding, was determined to be the site of a mutation causing the immunodeficiency disorder chronic granulomatous disease, in which patients are unable to generate superoxide[10].

In a different report, Cheever *et al.*[11] uncovered an essential role of a PX domain in vesicular membrane fusion to vacuoles. Membrane fusion events are, in general, mediated by the SNARE machinery, in which a protein on the vesicle (a v-SNARE) binds to its counterpart on the target membrane (a t-SNARE)[11]. Other proteins are recruited to the complex, which ultimately leads to membrane fusion. According to this mechanism, the correct pairing of t- and v-SNAREs leads to specific fusion of vesicles with their intended targets. Of critical importance, then, is how a SNARE becomes associated with a particular membrane. Many SNAREs associate with a membrane through transmembrane segments in their sequence. However, the SNAP-25 family of t-SNAREs lack such a segment and must interact with membrane components to properly localize[11].

Cheever *et al.*[3] now show that the yeast protein Vam7, a SNAP-25-type t-SNARE that mediates vesicular membrane fusion with the vacuole, is localized to the vacuole by its PX domain. The authors show that PtdIns(3)P is required for recruitment, and that the Vam7 PX domain directly binds PtdIns(3)P.

Can PX domains coordinate lipid and SH3-based signals?

These studies provide a view of the PX domain as a bifunctional protein module. At least three different PX domains have now been shown to recognize a unique phosphoinositide species. Even more intriguing is the composite result that at least some PX domains, such as the p47^{phox} domain, can bind both phosphoinositides and SH3 domains.

Several important questions remain to be resolved. Is this dual function a general feature of PX domains? Sequence evidence suggest that it may be, given that the Pro-X-X-Pro motif and the putative lipid binding residues appear to be very well conserved throughout the family. Can an individual PX domain bind both protein and lipid ligands simultaneously? The structural evidence presented in these papers suggests that this may be possible.

Chemical shift mapping studies[3] and mutagenesis[2] implicate a deep surface pocket in phospholipid binding (Fig. 1). This putative binding pocket does not overlap with the putative SH3 binding site observed in the p47^{phox} structure. Interestingly, the putative SH3 and phospholipid binding sites, although not overlapping, are in very close proximity. Together with the evidence for structural plasticity of the PX domain, these observations raise the intriguing possibility that SH3 and phospholipid binding could be coupled. In support of this hypothesis, a residue in the proline-rich region of the Vam7 PX domain is found to undergo a

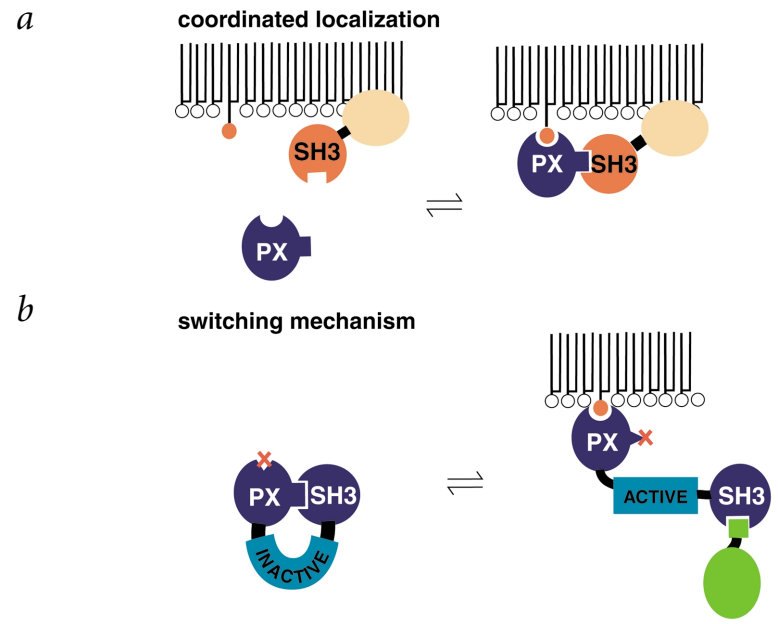

Fig. 2 Possible regulation by multifunctional domains, such as PX domains. ***a***, PX domain localization might only occur in the presence of both phosphoinositide and SH3 domain-based signals. In this scenario, PX domain localization would only occur in the presence of both phosphoinositide and SH3 domain-based signals. This type of mechanism would allow for the precise regulation of protein localization. ***b***, The PX domain as a switching element if binding to SH3 domains and phospholipids is exclusive. Intramolecular interactions between the PX domain and an SH3 domain in a protein could be involved in autoinhibition. Phosphoinositide binding to the PX domain could then disrupt the PX–SH3 domain interaction, owing to linkage between the two binding sites. Such a mechanism could result in activating conformational changes or recruitment of other partners by the free SH3 domain.

significant chemical shift change upon binding of PtdIns(3)P (ref. 3).

Linkage between the SH3 domain and phospholipid binding sites of PX domains could give rise to interesting forms of regulation. For example, PX domains could require coordinated presentation of both phospholipid and SH3 domains for proper cooperative binding and localization (Fig. 2*a*). Whether proper cellular localization of these domains occurs with selective mutation of either the SH3 binding site or the phospholipid binding site will be fascinating to see. Alternatively, the domain could function as a switching element, depending on exclusive or anti-cooperative binding of the two ligand types. For example (Fig. 2*b*), an SH3 domain tied up in an autoinhibitory intramolecular interaction with a PX domain may, in principle, be released upon phospholipid binding to the PX domain. The coupled release of such an autoinhibitory interaction could allow for activating conformational changes or allow for new SH3 mediated intermolecular interactions. This chain of interaction partner switching could allow phospholipid signals to trigger reorganization and assembly of protein complexes.

In the case of the NADPH oxidase, the activation of the enzyme in an *in vitro* reconstituted system has been known to require treatment with agents such as detergent (SDS) or arachidonic acid, which appear to relieve the intramolecular interactions that mask the SH3 domains[7]. The relationship of these treatments to physiologically relevant signals has been unclear. Given the new results, SDS and arachidonic acid may interact with the PX domains in a manner mimicking phospholipids. Much of this model is still extremely speculative, and future work will be required to determine whether PX domains are used to integrate signals in this manner. In any event, the PX domain joins a growing list of protein modules that can carry out several functions, adding even more complexity to Nature's signaling toolkit.

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