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Global approaches for the elucidation of phosphoinositide-binding proteins

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ABSTRACT

Phosphoinositide lipids (PIP_ns) control numerous critical biological pathways, typically through the regulation of protein function driven by non-covalent protein–lipid binding interactions. Despite the importance of these systems, the unraveling of the full scope of protein–PIP_n interactions has represented a significant challenge due to the massive complexity associated with these events, including the large number of diverse proteins that bind to these lipids, variations in the mechanisms by which proteins bind to lipids, and the presence of multiple distinct PIP_n isomers. As a result of this complexity, global methods in which numerous proteins that bind PIP_ns can be identified and characterized simultaneously from complex samples, which have been enabled by key technological advancements, have become popular as an efficient means for tackling this challenge. This review article provides an overview of advancements in large-scale methods for profiling protein–PIP_n binding, including experimental methods, such as affinity enrichment, microarray analysis and activity-based protein profiling, as well as computational methods, and combined computational/experimental efforts.

1. Introduction

The phosphatidylinositol polyphosphate lipids (phosphoinositides, PIP_ns, PtdIns-Ps) have elicited considerable interest among a broad range of researchers since the signaling lipids that comprise this family control numerous critical biological processes. A primary mechanism by which PIP_ns act involves their roles as site-specific ligands that enforce the binding of peripheral proteins to membrane surfaces, which generally regulates the function of the protein target (Lemmon, 2008; Cho and Stahelin, 2005). In these instances, protein activity can either be modulated directly through lipid binding or indirectly by bringing the bound protein into proximity of enzymes or binding partners at the membrane surface (Cho and Stahelin, 2005). PIP_n sub-cellular localization is also tightly controlled, which in turn dictates the location of bound proteins within the cell (Sprong et al., 2001). The PIP_ns additionally play important roles in the biosynthesis of important molecules including diacylglycerol (DAG) and the soluble inositol phosphates (InsPs) via the production of inositol-(1,4,5)-triphosphate ($Ins(1,4,5)P_3$, IP_3) (Streb et al., 1983; Carrasco and Merida, 2007; Gomez-Fernandez and Corbalan-Garcia, 2007; Sakane et al., 2007). As a result of these critical signaling activities, aberrant phosphoinositide activities have been linked to a number of diseases including cancer and diabetes (Di Paolo and De Camilli, 2006; Pendaries et al., 2003; Vicinanza et al., 2008; Wymann and Schneiter, 2008).

Despite the significance of the PIP_n family, the quest for a complete understanding of the activities of these lipids is complicated by the highly complex nature associated with their biological functions. This starts with the PIP_n structures themselves, which contain a conserved *myo*-inositol headgroup that can be phosphorylated at every permutation of the 3-, 4-, and 5-positions yielding seven isomers with independent biological activities (Fig. 1a–g). As a result of the sophisticated nature of these structures, considerable efforts have been invested in the synthesis of these compounds as well as functionalized derivatives for use as probes (Best et al., 2010; Conway and Miller, 2007).

The complications related to studying the protein-binding properties and associated biological functions of PIP_ns also result from the massive scope and intricate details associated with these molecular recognition events. There are numerous proteins that are now known to be bound and regulated by PIP_ns, including proteins that may or may not contain conserved binding units such as the PH, PX, C2, FYVE, ENTH, ANTH, PROPPIN and tubby domains (Lemmon, 2003, 2007, 2008; Cho and Stahelin, 2005; Hurley, 2006; Hurley and Misra, 2000; Lemmon and Ferguson, 2000; McLaughlin et al., 2002). This broad scope is further complicated by details at the molecular level; for example not all members of these domain families exhibit PIP_n-binding, and there is significant variation in PIP_n-binding specificities and affinities, even within individual domain families.

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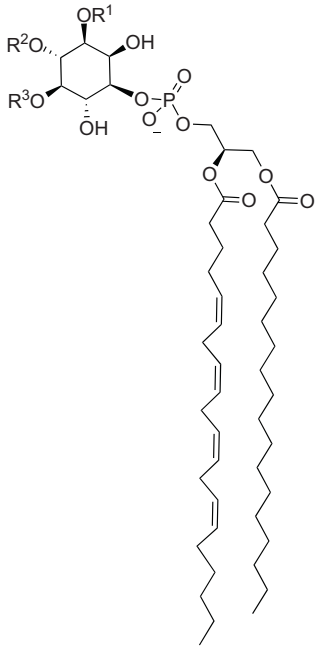

1a: PIP_3, R^1, R^2, $R^3 = PO_3^{-2}$

1b: $PI(3,4)P_2$, R^1, $R^2 = PO_3^{-2}$, $R^3 = H$

1c: $PI(4,5)P_2$, R^2, $R^3 = PO_3^{-2}$, $R^1 = H$

1d: $PI(3,5)P_2$, R^1, $R^3 = PO_3^{-2}$, $R^2 = H$

1e: PI(3)P, $R^1 = PO_3^{-2}$, R^2, $R^3 = H$

1f: PI(4)P, $R^2 = PO_3^{-2}$, R^1, $R^3 = H$

1g: PI(5)P, $R^3 = PO_3^{-2}$, R^1, $R^2 = H$

Fig. 1. Structures of the seven naturally occurring PIP_n isomers, depicted with representative acyl chains.

As a result of the impressive scale and detail associated with proteins that bind PIP_ns, global methods for elucidating these targets at the genomic scale are of considerable value as they allow for the simultaneous analysis of a large number of proteins (Cho et al., 2012; Scott et al., 2012). In addition, these techniques can circumvent challenges associated with the study of membrane-interacting proteins, such as the difficulties of overexpression and purification. Toward this end, recent advances in both experimental and computational approaches, as well as combinations thereof, have significantly impacted the understanding of these complex recognition events. This review article will provide an overview of advances in such large scale studies aimed at the elucidating PIP_n-binding proteins, and then conclude by discussing the benefits and complementarity of the various approaches as well as the prospects for future studies.

2. Experimental approaches for large scale identification of PIP_n-binding partners

2.1. Affinity enrichment using PIP_n-derivatized solid supports

A prevalent approach to performing global identification of PIP_n-binding proteins from complex samples has involved affinity enrichment. In this area, various moieties containing aspects of the PIP_n structures have been attached onto solid support in order to separate cognate binding proteins based on non-covalent binding interactions with the resin-bound ligand. In this way, non-binding proteins are first eluted off, followed by subsequent release of the solid-support bound target proteins, as depicted in Fig. 2. As

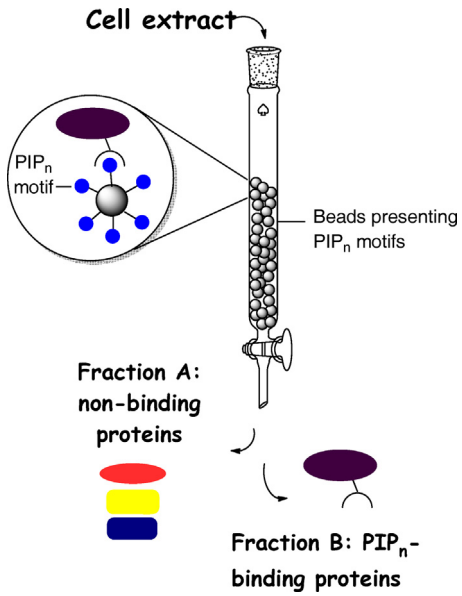

Fig. 2. Schematic for the affinity chromatography approach for selective enrichment of PIP_n-binding proteins.

can be seen in the following cases, the affinity chromatography approach has significantly benefitted from recent advancements in bioanalytical tools, and particularly mass spectrometry (MS)-based proteomic protocols for the large-scale identification of protein components in complex mixtures (Cravatt et al., 2007).

Affinity enrichment was initially reported for the isolation and identification of individual PIP_n-binding proteins. For example, Hammonds-Odie and co-workers used a combination of affinity chromatography and photoaffinity labeling (Dorman and Prestwich, 2000) to identify and characterize centaurin-α as a PIP_n-binding protein from rat brain (Hammonds-Odie et al., 1996). In prior work involving the identification of proteins that bind the soluble $Ins(1,3,4,5)P_4$ via an InsP affinity resin (Theibert et al., 1991, 1992), the authors observed a 46-kDa protein, but this protein was not labeled using an $Ins(1,3,4,5)P_4$ probe bearing a phenylazide photoaffinity tag. Further studies revealed that this protein was successfully labeled with photoaffinity probe **2** (Fig. 3), which was attributed to the enhanced hydrophobicity and increased stability of the benzophenone affinity tag. In addition, this protein was most effectively competed off of affinity resin using PIP_3, after which it was demonstrated that cloned protein could be isolated using the $Ins(1,3,4,5)P_4$ affinity resin. This work illustrates the tricky nature of differentiating PIP_n- and InsP-binding proteins due to their similar phosphorylated *myo*-inositol moieties and since many PIP_n-binding proteins bind to the headgroups of these lipids with reasonable affinities. A similar approach was used to characterize $p42^{IP4}$ (Reiser et al., 1995; Stricker et al., 1995, 1997, 2003).

Tanaka and co-workers synthesized PIP_3 analog **3** bearing an aminophenyl group for conjugation onto affi-gel beads containing succinimidyl ester functionalities (Tanaka et al., 1997). Notably, derivative **3** includes a carbonate linkage at the 1-position of the *myo*-inositol headgroup in place of the natural phosphodiester linkage. The resulting support was employed to purify protein D89940, which was then termed PIP_3 binding protein (PIP3BP), containing a zinc finger and two PH domains. Next, competition experiments for PIP_3-resin binding were used to show that the protein exhibited specificity for PIP_3 over $PI(4,5)P_2$ and $PI(3,4)P_2$, and mutagenesis studies demonstrated that mutations in either PH domain diminished binding. In a follow-up study, PIP_3-amine conjugate **4** was developed, which contained the natural phosphodiester linkage of the 1-position of the *myo*-inositol headgroup as well as a simplified