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Resource

Specificity and Commonality of the Phosphoinositide-Binding Proteome Analyzed by Quantitative Mass Spectrometry

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SUMMARY

Phosphoinositides (PIPs) play key roles in signaling and disease. Using high-resolution quantitative mass spectrometry, we identified PIP-interacting proteins and profiled their binding specificities toward all seven PIP variants. This analysis revealed 405 PIP-binding proteins, which is greater than the total number of phospho- or ubiquitin-binding domains. Translocation and inhibitor assays of identified PIP-binding proteins confirmed that our methodology targets direct interactors. The PIP interactome encompasses proteins from diverse cellular compartments, prominently including the nucleus. Our data set revealed a consensus motif for PI(3,4,5)P3-interacting pleckstrin homology (PH) domains, which enabled in silico identification of phosphoinositide interactors. Members of the dedicator of cytokinesis family C exhibited specificity toward both PI(3,4,5)P3 and PI(4,5)P2. Structurally, this dual specificity is explained by a decreased number of positively charged residues in the L1 subdomain compared with DOCK1. The presented PIP-binding proteome and its specificity toward individual PIPs should be a valuable resource for the community.

INTRODUCTION

Phosphatidylinositol is a negatively charged phospholipid that represents less than 5% of the total phospholipid pool at the cytosolic side of eukaryotic cell membranes (Nasuhoglu et al., 2002). Phosphatidylinositol can be phosphorylated by a variety of kinases on position 3, 4, or 5 of the inositol ring in seven different combinations. Phosphorylated forms of phosphatidylinositols, known as phosphoinositides (PIPs), play important roles in lipid-mediated cell signaling, membrane trafficking, and diseases involving these processes (Di Paolo and De Camilli, 2006). PIPs can act as precursors for secondary messengers or interact directly with proteins to orchestrate spatiotemporal activation of downstream signaling components (Berridge and Irvine, 1984; Cantley, 2002).

Despite biological interest in the PIP signaling pathways, our knowledge about the proteins that specifically interact with PIPs is limited. This is mainly due to the absence of "unbiased" technologies for detecting PIP interactions on a proteome-wide scale. Affinity matrices carrying tethered PIP variants have been used to isolate PIP-interacting proteins (Painter et al., 2001; Krugmann et al., 2002). Although this constituted an elegant biochemical approach, only a few PIPs could be investigated at a given time, and the specificity of the identified PIP-interacting candidates was unclear. Mass spectrometry (MS)-based proteomics has emerged as a key technology for comprehensive mapping of proteomes (de Godoy et al., 2008; Altelaar et al., 2013) and posttranslational modifications (PTMs) (Jensen, 2006; Witze et al., 2007), and is frequently employed to identify proteins bound to a "bait" such as peptides, RNA, or DNA. A central challenge in these experiments is to distinguish proteins that bind nonspecifically to the bait (background binders) from genuine interactors (specific binders). For example, previous studies performed pull-down experiments with immobilized PIPs to determine PI(3,5)P2, PI(4,5)P2, and PI(3,4,5)P3 interactors (Pasquali et al., 2007; Catimel et al., 2008, 2009; Rowland et al., 2011). Using low-resolution ion traps for data acquisition, these reports identified some of the known PIP binders, but also many additional proteins that are unlikely to be specific PIP binders. One can address this challenge by performing interaction screens in a quantitative format, most accurately by using stable isotope labeling approaches such as *s*table *i*sotope *l*abeling by *a*mino acids in *c*ell culture (SILAC) (Ong and Mann, 2005; Bantscheff et al., 2007). A strategy employing double-encoded quantitation for PIP studies was recently demonstrated, but the analysis was limited to a specific cellular compartment and only protein interactors for a single PIP were probed (Dixon et al., 2011; Lewis et al., 2011).

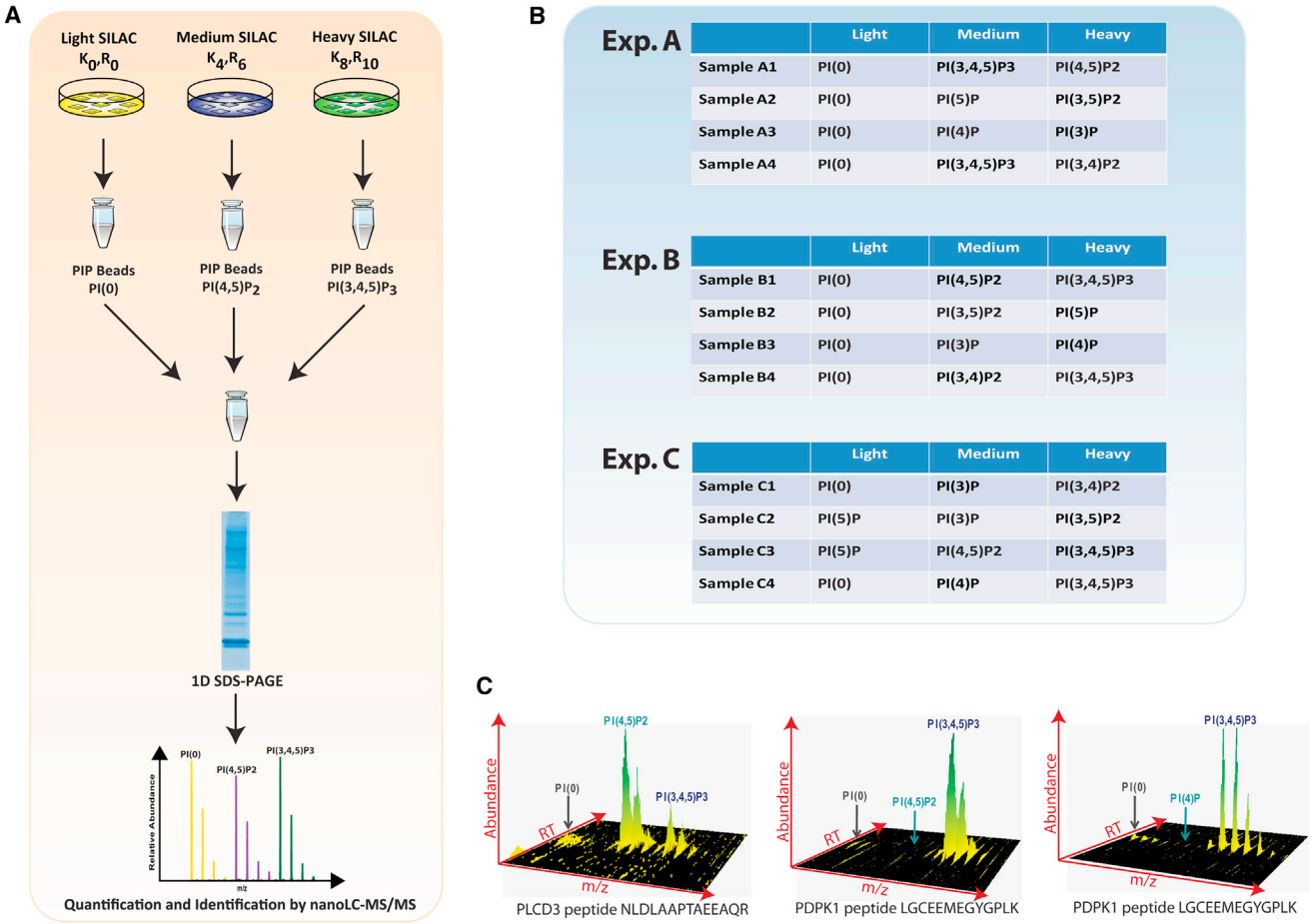

Figure 1. Experimental Setup

(A) Triple-encoded SILAC cell lysates were individually incubated with various PIPs.

(B) Three biological replicate experiments were performed to assess reliability and reproducibility. Each experiment contained four triple-encoded SILAC experiments (a total of 12 samples, labeled A1–C4).

(C) Each triple-encoded SILAC sample yielded quantitative information that was used to establish the PIP specificity for each identified protein. Data for two peptides from sample B1 (PLCD3 and PDPK1 peptide) and one PDPK1 peptide derived from sample C4 are depicted. Combining the quantitative information from all three experiments yielded a relative PIP specificity ratio (PSR) that corresponds to the PIP specificity for each quantified protein.

Here, we used triple-encoded SILAC quantification and high-resolution MS to systematically profile protein interaction specificities for all seven PIP variants. The data reveal an extensive catalog of PIP interactors and a quantitative estimate of their preferences for different PIP isoforms. We identified a large number of specific PIP interactors and validated several candidates by membrane translocation assays, which we discuss further below with regard to known PIP-binding domains (Seet et al., 2006). By probing highly specific PIP interactors in our data set, we established an extended PI(3,4,5)P3 consensus motif, allowing for in silico identification of PI(3,4,5)P3-binding proteins. Moreover, our data reveal insights into known PIP-binding protein families, as demonstrated by the identification of PIP-binding proteins, and extend the known PIP-binding specificity of dedicator of cytokinesis (DOCK) family members. Collectively, the presented data set provides a valuable resource for identifying PIP-binding proteins in human cells.

RESULTS

PIP Interaction Profiling Strategy

To identify PIP-interacting proteins and quantitatively profile the binding specificities for the different PIP isoforms, we used a SILAC-based proteomics approach (Figure 1A). HeLaS3 cells were grown under identical conditions in media containing "light," "medium," or "heavy" isotope-labeled variants of lysine and arginine (Ong et al., 2002). For an unbiased investigation of the human PIP interactome, all PIP isoforms were individually immobilized on agarose beads (PIP beads; Echelon) and incubated separately with light-, medium-, or heavy-labeled SILAC cell lysates. In the triple-SILAC approach, the interaction profile of three conditions can be analyzed simultaneously in a single experiment. However, to distinguish "background binders" from PIP-specific interactors, light-labeled SILAC lysates were incubated with beads coupled to PI(0), whereas phosphorylated