THE POLYBASIC REGION THAT Follows THE PLANT HOMEODOMAIN ZINC FINGER 1 OF PF1 IS NECESSARY AND SUFFICIENT FOR SPECIFIC PHOSPHOINOSITIDE BINDING*

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Running Title: PHD zinc fingers

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The plant homeodomain (PHD) zinc finger is one of 14 known zinc-binding domains. PHD domains have been found in more than 400 eukaryotic proteins and are characterized by a Cys4-His-Cys3 zinc-binding motif that spans 50-80 residues. The precise function of PHD domains is currently unknown; however, the PHD domains of the ING1 and ING2 tumor suppressors have been shown recently to bind phosphoinositides (PIs). We have recently identified a novel PHD-containing protein, Pf1, as a binding partner for the abundant and ubiquitous transcriptional corepressor mSin3A. Pf1 contains two PHD zinc fingers, PHD1 and PHD2, and functions to bridge mSin3A to the TLE1 corepressor. Here, we show that PHD1, but not PHD2, binds several monophosphorylated PIs but most strongly to PI(3)P. Surprisingly, a polybasic region that follows the PHD1 is necessary for PI(3)P binding. Furthermore, this polybasic region binds specifically to PI(3)P when fused to maltose binding protein, PHD2, or as an isolated peptide, demonstrating that it is sufficient for specific PI binding. By exchanging the polybasic regions between different PHD fingers we show that this region is a strong determinant of PI binding specificity. These findings establish the Pf1 polybasic region as a phosphoinositide binding module and suggest that the PHD domains function downstream of phosphoinositide signaling triggered by the interaction between polybasic regions and phosphoinositides.

Complex signaling pathways control cellular growth, differentiation and proliferation by precisely orchestrating gene expression programs. Not surprisingly, alterations of these programs underlie many pathological conditions. The transfer of signals from plasma membrane to the cell nucleus is a complex, multi-step process that strongly depends, among other components, on lipid signaling molecules. Phosphoinositides (PIs or phosphatidylinositol phosphates) are involved in cell signaling pathways that control broad aspects of cell physiology including proliferation, death, motility, cytoskeletal regulation, intracellular vesicle trafficking, and metabolism (1,2). PIs exert their pleiotropic effects either as precursors of second messengers such as inositol 1,4,5-triphosphate and diacylglycerol or directly by interacting with signaling effectors. Specific PIs interact with these effectors through highly conserved motifs. Examples include the pleckstrin homology (PH) and phox homology (PX) domains which interact with mono, bi, or tri-phosphorylated PIs; the epsin N-terminal homology (ENTH) domain which interacts with mono- or bi-phosphorylated PIs; and the Fab1p/YOTB/Vac1p/EEA1 (FYVE) domain which interacts with PI(3)P (3). Most characterized PI-binding proteins are principally cytoplasmic or membrane bound; however, recent biochemical and imaging studies have detected PIs in the nucleus, suggesting that they also function in this compartment (4, 6).

Little is known about the function of nuclear PIs; however recent reports suggest a role in chromatin-dependent changes in gene expression. For example, PI(4,5)P2 stabilizes the association of the SWI/SNF-like BAF chromatin remodeling complex with the nucleosomal template and the nuclear matrix in response to antigen stimulation (7). Furthermore, the ING2 tumor suppressor component of the mSin3A corepressor complex binds PI(5)P. This
interaction regulates ING2’s association with chromatin and induction of p53-dependent apoptosis (8). Pf(5)P interacts with a C-terminal Plant homeodomain (PHD) in ING2 providing mechanistic insight into how nuclear PIs modulate cell function. PHD Zinc fingers from other proteins also interact with PIs, demonstrating that this function is not restricted to PHD domains present in the ING family.

While one function of PHD zinc fingers seems to be PI binding, current evidence suggests that this is not the sole function of the domain. For example, the domain is highly conserved across phyla and present in more than 400 eukaryotic proteins, suggesting a diversity of functions. Furthermore, mutations in PHD zinc fingers or PHD-containing proteins have been observed in different tumors and a variety of genetic disorders indicating divergent rather than common functions in normal cellular physiology (9,10). In spite of this likely functional diversity, most PHD zinc finger-containing proteins are present in the nucleus as components of large multi-protein transcriptional regulatory complexes that also contain other conserved domains implicated in gene regulation. Together, this collective evidence suggests broad common function for PHD zinc finger-containing proteins in the chromatin-based regulation of gene expression.

Recently, we identified a novel protein called Pf1 (PHF12) that contains two conserved PHD zinc fingers (PHD1 and PHD2) as an interacting partner for the mSin3A corepressor (11). The mSin3A corepressor complex is abundant, widely expressed and requires, in part, interactions with histone deacetylases to drive transcriptional repression (12,13). Pf1 has two independent binding sites for mSin3A and appears to function as a bridging protein to mediate interactions with another abundant corepressor called TLE1 (11). Pf1 also tethers MRG15, which is a member of the MORF family of transcriptional regulators, to mSin3A (14). None of the protein-protein interactions made by Pf1 require its PHD zinc fingers, suggesting that they contribute to some other aspect of Pf1 function (11,14). We show here that one PHD zinc finger of Pf1 interacts specifically with monophosphorylated PIs, but surprisingly a polybasic region located just C-terminal of the PHD zinc finger per se is both necessary and sufficient for specific PI binding. Furthermore, we show that PI binding to a number of previously characterized PHD zinc fingers can be attributed to a polybasic region located at their C-termini. Together, our data suggest that the PHD zinc finger and the polybasic region have separable functions that cooperate to regulate gene expression in response to extracellular signals.

Experimental procedures

Plasmids and protein expression- ING1, ING2 and ACF1 constructs were provided by Or Gozani. The PHD zinc fingers, with or without the polybasic region (PBR), of Pf1, ING1, ING2, and ACF1 were PCR amplified and cloned in-frame with maltose binding protein (MBP) in pMAL-C2 vector (New England Biolabs). To exchange the polybasic regions among the PHD zinc fingers, reverse primers that include the PBR DNA sequence were used in PCR amplifications. The products were then cloned into pMAL-C2 vector. Similarly, to generate the MBP-PBR fusions of Pf1 and ING2, primers encompassing the PBR DNA sequence were used in PCR amplifications. All constructs were verified by sequencing. The recombinant protein for each construct was expressed and purified from E.coli using previously published techniques. Briefly, induced cells were lysed in 1XPBS (137mM NaCl, 2.7mM KCl, 1.4mM KH2PO4, 4.3mM Na2HPO4, 7H2O) containing 0.25% NP40 and further solubilized in 1% triton-X100. The solubilized extract was passed over a column containing amylose resin (New England Biolabs) and bound protein eluted with 10 mM maltose. Purified protein was dialyzed overnight against 1X PBS containing 5% glycerol and protease inhibitors (1.9µg/ml aprotinin, 1µg/ml leupeptin, 1µg/ml pepstatin and 50nM PMSF). The polybasic region peptide with or without biotin (Biotin-Aminohexanoine acid-TVRRKKREQKK) was synthesized at the peptide core facility at the University of Utah.

Protein-lipid blot assays- PIP strips and arrays were purchased from Echelon Biosciences. Protein-lipid blot assays were performed as described (8). Briefly, membrane was blocked for
1 hour in TBST (10 mM Tris (pH 8.0), 150 mM NaCl, and 0.1% Tween-20) containing 3% fatty acid-free BSA (Sigma). Recombinant protein from 5-10nM was added to the block solution and the membrane incubated overnight at 4°C. Following binding, membranes were washed three times in TBST and bound proteins detected using anti-MBP as the primary antibody (1:5000 dilution; New England Biolabs) and anti-mouse Horse Radish Peroxidase as the secondary antibody (1:5000; Amersham). Blots were developed using ECL reagent (Amersham).

RESULTS

Previous reports examining PI binding by PHD fingers utilized constructs expressing the PHD finger itself and approximately 25 C-terminal residues (8). For each PHD finger that bound PIs, the following C-terminal region had a tendency towards positively charged amino acids (Fig. 1A). Further, mutation of six lysines to alanine in the polybasic region that follows the PHD domain of ING2 eliminated ING2 binding to PI(5)P (8). Together these data suggest that the polybasic region that follows some PHD domains contributes to specific PI binding. PHD1 of Pf1 is followed by a polybasic stretch with 10 of 26 residues being lysine or arginine. By contrast, the 26 residues that follow PHD2 of Pf1 only contain three basic residues (Fig. 1A). These two observations suggest that PHD1, but not PHD2, may bind PIs. We refer to the combination of the PHD domain and the C-terminal 25 amino acids as PHD(C). To determine the lipid binding properties of PHD1(C) and PHD2(C), both were expressed as Maltose Binding Protein (MBP) fusion proteins and tested for binding to a variety of immobilized lipids in an overlay format (Fig. 1B). MBP-PHD1(C) bound mono-phosphorylated PIs with a preference for PI(3)P. It also bound weakly to PI(3,5)P2. By contrast, no lipid binding was detected for the control MBP or MBP-PHD2(C) fusion protein (Fig. 1C). The binding of MBP-PHD1(C) to different amounts of immobilized PIs revealed that PHD1(C) binds PI(3)P with the highest affinity, followed by PI(4)P, PI(5)P and PI(3,5)P2 (Fig. 1D).

The lack of PI binding by PHD2(C) suggests that PHD2 cannot bind PIs or implicates the following polybasic region as a determinant of PI binding. We tried two additional approaches to determine which features of PHD1(C) contributed to PI binding. To disrupt the structure of the PHD domain of PHD1(C) we added the coordinated Zn molecules were chelated with phenanthroline or a Zn-coordinating cysteine was mutated to arginine. In neither case was binding to mono-phosphorylated PIs disrupted (Fig. 2A and B), suggesting that the structure of the PHD domain itself was not required for PI binding and that binding might be attributed to the following polybasic region. Accordingly, deletion of the polybasic region from PHD1(C) resulted in a complete loss of PI binding (Fig. 2C). We also tested the contribution of the PHD1 polybasic region to PI binding in the context of a fusion protein that contains both PHD1(C) and PHD2(C) separated by their normal spacing. The PHD1(C)-PHD2(C) fusion bound mono-phosphorylated PIs with the same specificity as PHD1(C) alone, but surprisingly the fusion also bound phosphatidylserine. Like PI binding by PHD1(C), lipid binding by the double PHD domain fusion required the polybasic region C-terminal to PHD1 (Fig. 2D). Together these data demonstrate that the polybasic region C-terminal to PHD1 is necessary for specific lipid binding and further suggests that the PHD domain per se of PHD1(C) may be dispensable for interaction.

We next tested whether the polybasic region was sufficient for PI binding. We first replaced the 23 residues that follow the PHD domain of the non-binding PHD2(C) protein with the polybasic residues from the PI binding PHD1(C). We called this construct PHD2(1C). As before, PHD2(C) was unable to bind PIs; however, PHD2(1C) bound monophosphorylated PIs with identical specificity to PHD1(C) and showed similar weak binding to PI(3,5)P2 (Fig. 3A). Therefore, the polybasic region that follows PHD1 is sufficient for PI binding but does not rule out the possibility that both the PHD finger and the following polybasic region are required for PI binding. To address the PI-binding capacity of the polybasic region (PBR) in isolation, we fused only the polybasic region from PHD1(C) directly to MBP. This fusion protein, MBP-PBR(Pf1), bound PIs with near identical specificity to PHD1(C), suggesting that the polybasic region was sufficient for PI binding (Fig. 3B). Furthermore, the similar binding specificity of the two constructs suggests
that the polybasic region is a strong determinant of PI binding specificity. To test the generality of our finding, we made a MBP fusion to the polybasic region that follows the PHD finger of ING2. This protein, MBP-PBR(ING2), bound PIs but showed preferential binding to PI(4)P instead of the broader binding specificity of MBP-PBR(Pf1) (Fig. 3B). Together these findings suggest that the polybasic region is sufficient for PI binding and is a determinant of PI-binding specificity.

The above data suggest that the polybasic region is sufficient for binding, but it was possible that the PHD finger or MBP have ancillary functions in PI binding; perhaps a folded domain is required for the functional presentation of the polybasic region. We therefore tested a biotinylated polybasic synthetic peptide representing the 11 amino acids immediately C-terminal to the final cysteine of PHD1. This polybasic peptide bound monophosphorylated PIs similarly to PHD1(C) and MBP-PBR(Pf1), showing a slight preference for PI(3)P over PI(4)P and PI(5)P. The basic residue-rich tail of histone H2A did not bind immobilized lipids, suggesting that the binding of the PHD1 polybasic region was specific (Fig. 4A and B). Furthermore, the polybasic region did not bind all lipids, suggesting that interaction is not simply mediated by charge-charge interactions between the positively charged amino-acids in the peptide and negatively charged phosphate groups on the lipids. Therefore, the polybasic region that follows PHD1 is sufficient for specific PI binding.

To further address the PI binding specificity of the polybasic region we created a number of chimeric proteins that encoded a PHD finger from one protein followed by the polybasic region from a different protein. In contrast to the previously published report (8), MBP-ING2(C) bound most strongly to PI(4)P followed by PI(3)P and only weakly to PI(5)P (Fig. 5A). Consistent with the polybasic region dictating PI binding specificity, a chimera between the PHD1 domain of Pf1 and the polybasic region of ING2, MBP-PHD1(PBR-ING2), bound PIs with similar affinity and identical specificity to MBP-ING2(C) (Fig. 5A). Finally, fusion of the polybasic region of PHD1 to the PHD fingers of ING1, ING2 and ACF1 resulted in proteins that bound PIs with similar, but not identical, specificity. MBP-ING1(PBR-Pf1) bound identically to MBP-PHD1(C), whereas MBP-ING2(PBR-Pf1) also bound PI(3)P and PI(4)P but did not bind PI(5)P or PI(3,5)P2 (Fig. 5B). By contrast, a chimera containing the PHD1 polybasic region and PHD finger of ACF1, MBP-ACF1(PBR-Pf1) had a much relaxed lip binding specificity. This fusion protein bound to each of the mono and bis-phosphorylated PIs and also showed weak binding to lysophosphatidic acid (LPA), phosphatidylethanolamine (PE), phosphatidic acid (PA) and PI(3,4,5)P3 (Fig. 5B). Together these data suggest that even though the polybasic region is a determinant of lipid binding specificity, the PHD finger also contributes.

DISCUSSION

The PHD zinc finger is a broadly expressed domain conserved across phyla that appears to have a predominant role in transcriptional regulation (9). One reported function of the PHD zinc finger is the binding of PIs, suggesting that PHD-containing proteins are responsive to PI-dependent signaling pathways (8,15). We show here that a polybasic region that follows some, but not all PHD domains is necessary and sufficient for PI binding. Furthermore, PI binding specificity tracked primarily with the polybasic region and was relatively independent of the linked PHD finger (Fig. 3 and 4). As such, we propose that the polybasic regions that follow the PHD1 finger of Pf1 or the single PHD finger of ING2 are the prime determinants of PI binding specificity and PHD domains themselves make relatively minor contributions. Finally, the polybasic regions of PHD1 of Pf1 or ING2 are sufficient for specific PI binding in the complete absence of their cognate PHD finger (Fig. 4), strongly suggesting the PHD finger per se in these proteins plays, at most, only an ancillary role in interactions with PIs. While we have only reexamined the PI binding of the PHD finger of ING2 in detail, a polybasic region follows each of the PHD domains previously shown to interact with PIs (8). As such, the relative contribution of these PHD domains and their cognate polybasic regions to PI interaction should be reevaluated in the light of our present findings.

Several experiments from the previous study examining PI binding by ING2 are
consistent with the polybasic region that follows the ING2 PHD domain being necessary for PI interaction. For example, deletion of this polybasic region completely eliminated PI binding as did mutation of 6 lysines or arginines, located in the polybasic region, to alanine (8). In contrast to our findings, these authors did not observe that the polybasic region of ING2 was sufficient for PI binding. This difference may be attributed to the different purification tags used in each experiment. Our studies utilized a MBP tag whereas a GST tag was used previously; perhaps the GST moiety, which is known to dimerize (16), makes the short C-terminal polybasic extension unavailable for PI binding. In addition, we observed that ING2 PHD(C) had a preference for PI(4)P rather than the previously observed PI(5)P (Fig. 3 and 5) (8). As we observed the same preference for PI(4)P using our MBP-ING2 as well as GST-ING2 construct used in the original publication (data not shown), the difference is likely due to differences in the commercial lipid blots used in the two experiments, rather than attributable to differences in the purification tags.

Interactions between PIs and a variety of polybasic motifs have precedence in the literature. Most recently, PI(4,5)P2 binding to a polybasic region of N-WASP activates its actin-filament function (17). Similarly, polybasic motifs found in myristoylated alanine-rich C kinase substrate (MARCKs) or MARCKs related protein (MRP) mediate actin-polymerization in response to PI(4,5)P2 (18). Interestingly, phosphorylation of key serine residues in the polybasic motif by protein kinase C (PKC) blocks the effect of PI(4,5)P2 (19). Therefore, one possibility for proteins such as Pf1 and ING2 that have PHD fingers with PI-binding polybasic regions is to function as mediators of lipid-dependent signaling and transmit extracellular signaling events to multi-protein complexes, ultimately regulating gene expression. The precise mechanistic details of how nuclear PI levels regulate gene expression remain to be determined. Pf1 and ING family proteins (11,20) all interact with mSin3A, yet have different PI-binding specificity, thus raising the exciting possibility that the mSin3A-corepressor integrates signals from different PI-dependent signaling pathways.

Nuclear levels of most PIs are nearly undetectable; however, multiple experiments suggest nuclear functions for this important class of signaling molecules. For example, while low in quiescent cells, nuclear PIs increase in response to signaling (1-3,21,22). Furthermore, PIs and PI kinases are present in distinct subnuclear domains called nuclear speckles; association with these structures is dynamic suggesting association is regulated (23,24). Finally, subnuclear localization of ING2 appears to be dictated by PI binding (15). Based on these findings we have begun to address how PI-binding effects Pf1’s nuclear function. Altering interactions between Pf1 and PIs by deleting its polybasic region or lowering PI(3)P levels with MTMR7, a PI(3)P phosphatase (25), had no affect on Pf1’s known protein-protein interactions, transcription repression function or nuclear or subnuclear localization (data not shown). Therefore, PI-binding may affect Pf1 function in ways not assayed here or, perhaps, Pf1 has a unique PI-dependent function distinct from transcriptional repression.

Finally, our experiments suggest that the PHD finger motif only plays an ancillary role in PI binding, suggesting that the primary function is still unknown. Given the preponderance of this domain in chromatin modifying complexes, a function in recognizing features of the nucleosomal template seems most likely based on the current evidence. For example, the PHD domains of ACF1 bind to all four core histones (26), and the PHD domain of ING family members bind the tail of histone H3 that is trimethylated on lysine 4 (27). In preliminary experiments we observe that PHD2, but not PHD1, of Pf1 interacts with histone H3 that is trimethylated on lysine 36 (data not shown). Therefore, some PHD fingers recognize key features of the histone code. In the case of ING2, histone H3 K4-trimethyl binding and PI binding are independent suggesting a possible integration between nuclear PI signaling and the histone code. How broadly these concepts can be applied to other PHD finger remains to be determined.

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FOOTNOTES

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The abbreviations used are: PIs, Phosphoinositides or phosphatidylinositol phosphates; MBP, maltose binding protein; aa, amino acids; PCR, polymerase chain reaction; PBS, phosphate buffered saline; TBS, tris buffered saline.

**FIGURE LEGENDS**

Fig. 1. PHD1(C), but not PHD2(C), of Pf1 binds mono-phosphorylated phosphoinositides. A, alignment of the PHD fingers of ING2, ING1, Pf1, and ACF1. Basic residues are bolded and an asterisk indicates the last two conserved zinc-coordinating residues. B, schematic of lipid blot membrane containing the following samples in 20-pmol spots: lysophosphatidic acid (LPA), lysophosphocholine (LPC), Phosphatidylinositol (PI), PI(3)P, PI(4)P, PI(5)P, phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphingosine 1-phosphate (S1P), PI(3,4)P2, PI(3,5)P2, PI(4,5)P2, PI(3,4,5)P3, phosphatidic acid (PA), phosphatidylserine (PS) and blank. C & D, 0.1µg/ml of the indicated MBP fusion proteins: PHD1(C), (aa 44-128); PHD2(C), (aa 259-343); and MBP alone as negative control were incubated with PIP-strips or with PIP array as described in the materials and methods. Lipids bound most strongly are indicated.

Fig. 2. A polybasic region that follows PHD1 is necessary for phosphoinositide binding. A, MBP-PHD1(C) was bound to immobilized lipids in the presence of 10mM phenanthroline. B, MBP-PHD1(C)C59R with a mutation in a zinc coordinating residue was bound to immobilized lipids. C, PHD1(C) or MBP-PHD1(C)ΔPBR, (aa 44-113) were tested for lipid binding. D, MBP-PHD1-2 (aa 44-343) and MBP-PHD1-2ΔPBR (aa 44-343 but lacks aa 105-113) were tested for lipid binding. Lipids bound most strongly are indicated.

Fig. 3. The polybasic region is sufficient for specific phosphoinositide binding. A, MBP-PHD2(1C) with polybasic region (aa 105-113) of PHD1 following aa 320 of PHD2, or PHD2(C) were tested for lipid binding. B, The polybasic regions of PHD1 of Pf1 (aa 102-113) or ING2 (aa 264-281) were cloned in frame with MBP to make MBP-PBR(Pf1) and MBP-PBR(ING2), respectively. These chimeras were tested for lipid binding. Lipids bound most strongly are indicated.

Fig. 4. The polybasic region dictates PI binding specificity. A, Schematic of the peptides used in this experiment. B, The indicated peptides were tested for binding to immobilized lipid blots at 100nM. Streptavidin Horseradish Peroxidase conjugate was used to detect bound biotinylated peptide. Lipids bound most strongly are indicated.

Fig. 5. The polybasic region is a determinant of phosphoinositide binding specificity. A, MBP-ING2(C) (aa 201-281) or. MBP-PHD1(PBR-ING2), containing the PHD1 zinc finger (aa 44-104) of Pf1 fused to the polybasic region of ING2 (aa 264-281) were tested for lipid binding. B, The polybasic region of PHD1 from Pf1 (aa 105-113) was fused to the PHD domains of ING1, MBP-ING1(PBR-Pf1), ING2, MBP-ING2(PBR-Pf1), or ACF1, MBP-ACF1(PBR-Pf1), and tested for lipid binding. The PHD regions for ING1, ING2 and ACF1 spanned aa 200-258, aa 203-261, aa 1131-1197, respectively. Lipids bound most strongly are indicated.
**Figure 1**

A

\[
\begin{align*}
P_{1-3} & : \text{PGEWMCRTVRRKKREEQKKEGLHVNGLVDKSRVR} \\
P_{1-4} & : \text{PRGRMCPNHIEHVVLNCNMTLSNRCQVEDRFQDD} \\
P_{1-5} & : \text{PKGKWYCPKCSMNEKTMDKALEKPSKKEERTYNR} \\
P_{2-3} & : \text{PGEWFCPECRCQRSRRLSRQ} \\
P_{2-4} & : \text{PBR-PF1} \\
P_{2-5} & : \text{PBR-PF1-PHD1} \\
P_{3-4} & : \text{PF1-PHD2} \\
\end{align*}
\]

B

| LPA | LPC | PI | PI(3) | PI(4) | PI(5) | PE | PC |
|-----|-----|----|-------|-------|-------|----|----|
| 20pmol/spot | S1P | PI(3,4)P2 | PI(3,5)P2 | PI(4,5)P2 | PI(3,4,5)P3 | PA | PS |
| 0.5pmol/spot | S2P | PI(3,4)P2 | PI(3,5)P2 | PI(4,5)P2 | PI(3,4,5)P3 | PA | PS |
| 0.1pmol/spot | S3P | PI(3,4)P2 | PI(3,5)P2 | PI(4,5)P2 | PI(3,4,5)P3 | PA | PS |

C

**Figure 1 continued**

D

![Image](image_url)

\[
\begin{align*}
P_{1-3} & : \text{PGEWMCRTVRRKKREEQKKEGLHVNGLVDKSRVR} \\
P_{1-4} & : \text{PRGRMCPNHIEHVVLNCNMTLSNRCQVEDRFQDD} \\
P_{1-5} & : \text{PKGKWYCPKCSMNEKTMDKALEKPSKKEERTYNR} \\
P_{2-3} & : \text{PGEWFCPECRCQRSRRLSRQ} \\
P_{2-4} & : \text{PBR-PF1} \\
P_{2-5} & : \text{PBR-PF1-PHD1} \\
P_{3-4} & : \text{PF1-PHD2} \\
\end{align*}
\]
Figure 2

A

MBP-PhD1(C) Phenanthroline

B

MBP-PhD1(C) C59R

C

MBP \rightarrow PHI PHD1 \rightarrow PBR-PHI

MBP \rightarrow PHI PHD1

D

MBP \rightarrow PHI PHD1 \rightarrow PBR-PHI \rightarrow PHI PHD2

MBP \rightarrow PHI PHD1 \rightarrow PHI PHD2

PS
Figure 3

A

MBP

PI(3)P
PI(4)P
PI(5)P
PI(3,4)P2
PI(3,5)P2

MBP-

PHD2(C)

MBP-

PHD2(1C)

B

MBP

PI(3)P
PI(4)P
PI(5)P
PI(3,4)P2
PI(3,5)P2

MBP-

PBR(Pf1)

MBP-

PBR(ING2)
A

Pfl PHD1 PBR peptide: $^{103}_{113}$TVRRKREQKK

Pfl PHD1 Biotin PBR peptide: Biotin-$^{103}_{113}$TVRRKREQKK

Biotin H2A peptide: Biotin-$^{25}_{113}$SGGKGGKAGSAKASQSRADAGL

B

PBR(Pf1) Peptide

Biotin-PBR (Pf1) Peptide

Biotin-H2A Peptide
Figure 5

A

MBP → ING2 PHD → PBR-ING2
MBP → PI1 PHD → PBR-ING2

PI(3)P
PI(4)P
PI(5)P
PI(3,4)P2
PI(3,5)P2
PI(4,5)P2
PI(3,4,5)P3
PA
PE
LPA

MBP-ING2(C) → MBP-PHD1 (PBR-ING2)

B

MBP → ING1 PHD → PBR-ING2
MBP → ING2 PHD → PBR-ING2
MBP → ACF1 PHD → PBR-PHDI

PI(3)P
PI(4)P
PI(5)P

MBP-ING1 (PBR-PHDI) → MBP-ING2 (PBR-PHDI)

PI(3,4)P2
PI(3,5)P2
PI(4,5)P2
PI(3,4,5)P3
PA

MBP-ACF1 (PBR-PHDI)
The polybasic region that follows the plant homeodomain zinc finger 1 of PF1 is necessary and sufficient for specific phosphoinositide binding
Mohan R. Kaadige and Donald E. Ayer

J. Biol. Chem. published online August 7, 2006

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