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## Identification of mitogen-activated protein kinase docking sites in enzymes that metabolize phosphatidylinositols and inositol phosphates

Kevin K Caldwell\*, Marcos Sosa and Colin T Buckley

Address: Department of Neurosciences University of New Mexico Health Sciences Center Albuquerque, NM 87131 USA

Email: Kevin K Caldwell\* - [KCaldwell@salud.unm.edu](mailto:KCaldwell@salud.unm.edu); Marcos Sosa - [MSosa@salud.unm.edu](mailto:MSosa@salud.unm.edu); Colin T Buckley - [CBuckley@salud.unm.edu](mailto:CBuckley@salud.unm.edu)

\* Corresponding author

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### Abstract

**Background:** Reversible interactions between the components of cellular signaling pathways allow for the formation and dissociation of multimolecular complexes with spatial and temporal resolution and, thus, are an important means of integrating multiple signals into a coordinated cellular response. Several mechanisms that underlie these interactions have been identified, including the recognition of specific docking sites, termed a D-domain and FXFP motif, on proteins that bind mitogen-activated protein kinases (MAPKs). We recently found that phosphatidylinositol-specific phospholipase C- $\gamma$ 1 (PLC- $\gamma$ 1) directly binds to extracellular signal-regulated kinase 2 (ERK2), a MAPK, via a D-domain-dependent mechanism. In addition, we identified D-domain sequences in several other PLC isozymes. In the present studies we sought to determine whether MAPK docking sequences could be recognized in other enzymes that metabolize phosphatidylinositols (PIs), as well as in enzymes that metabolize inositol phosphates (IPs).

**Results:** We found that several, but not all, of these enzymes contain identifiable D-domain sequences. Further, we found a high degree of conservation of these sequences and their location in human and mouse proteins; notable exceptions were PI 3-kinase C2- $\gamma$ , PI 4-kinase type II $\beta$ , and inositol polyphosphate 1-phosphatase.

**Conclusion:** The results indicate that there may be extensive crosstalk between MAPK signaling and signaling pathways that are regulated by cellular levels of PIs or IPs.

### Background

MAPKs catalyze the transfer of the  $\gamma$ -phosphate of adenosine triphosphate (ATP) to serine (S) or threonine (T) residues that precede proline (P) [1,2]; thus, these enzymes are termed proline-directed serine/threonine kinases. Although the sequences ST and TP are sufficient for phosphorylation to occur, the optimal sequence for phosphorylation by a MAPK is PX(S/T)P [1,3]. The majority of cellular proteins contain an SP or a TP sequence, yet, many of these proteins are not MAPK substrates [4], indi-

cating that a mechanism exists for achieving substrate specificity for the MAPKs. This specificity is conferred by the substrate through a docking domain. In addition to underlying specificity, these docking interactions increase the catalytic efficiency of substrate phosphorylation [5-7].

### MAPK docking sites

A MAPK docking site, distinct from the phosphoacceptor site, was first identified in c-Jun [8,9], a c-Jun N-terminal kinase (JNK) substrate; this site was designated the " $\delta$

domain". Subsequently, a JNK binding site in the transcription factor ATF-2 [10,11] and a motif termed the "d-box" of Elk-1 that binds ERK2 [4,12] were noted to be similar in sequence to the JNK binding site in c-Jun. Related motifs have been identified in a number of other proteins and have been given various names, including DEJL (docking sites for ERK and JNK, LXL) domain [4], kinase interaction motif (KIM) [13,14], MAPK-docking site [15,16], D box [5,12], D-site [17] and D-domain [6,18-20]. It is important to note that, although these domains were identified based on the ability to bind one or more MAPK, there are differences in the consensus sequences used to identify each of them. For example, MacKenzie et al. [14] proposed a consensus KIM sequence of (V/L) $X_2$ (R/K)(R/K) $X_{(3-6)}$ L, with V, L, R, and K representing the amino acids valine, leucine, arginine and lysine, respectively; Bardwell et al. [16] define a consensus MAPK-binding site sequence of (R/K) $X_{(2-6)}$ (L/I)X(L/I), with I representing the amino acid isoleucine; and Kornfeld and colleagues [4] reported two consensus sequences for the DEJL domain: (K/R)X(X/K/R)(K/R) $X_{(1-4)}$ (L/I)X(L/I) and (K/R)(K/R)(K/R) $X_{(1-5)}$ (L/I)X(L/I). In the present studies we use the term D-domain and the consensus sequences reported by Kornfeld and colleagues [4].

Sharrocks and colleagues [21] report that D-domains are characterized by a cluster of basic residues positioned amino-terminal to an (L/I)X(L/I) motif followed by a triplet of hydrophobic amino acids that precedes a series of proline residues [17,21]. These investigators assessed the role of each of these regions in the binding of ERK2 and p38 to transcription factors, MEF2A, SAP-1, and Elk-1. They determined that mutation of the basic region of the transcription factors reduced their phosphorylation by both phospho-ERK2 and phospho-p38 [21]. This suggests that the basic residues are important for both ERK2 and p38 targeting of MAPK substrates. Mutation of the (L/I)X(L/I) motif (also called the LXL motif) diminished phosphorylation of phospho-ERK substrates, whereas it is not required for phosphorylation of substrates by the MAPK, phospho-p38 [21]. It was also determined that the hydrophobic patch plays an important role in phosphorylation of the substrates by both phospho-ERK and phospho-p38; however, this patch is more important for p38 binding than ERK2 binding. Barsyte-Lovejoy et al. [21] concluded that the proline residues were not important in specificity determination of MAPK substrates. Therefore, the authors hypothesize that the proline residues may play a structural role within the motif.

D-domains can show specificity for families of MAPKs; for example, the Elk-1 D-domain binds JNK and ERK, but not p38 [5,22]; both the SAP-1 and Elk-1 D-domains bind ERK2, whereas the SAP-1, but not Elk-1, D-domain binds p38 $\alpha$  [22]. Other D-domains show specificity within a

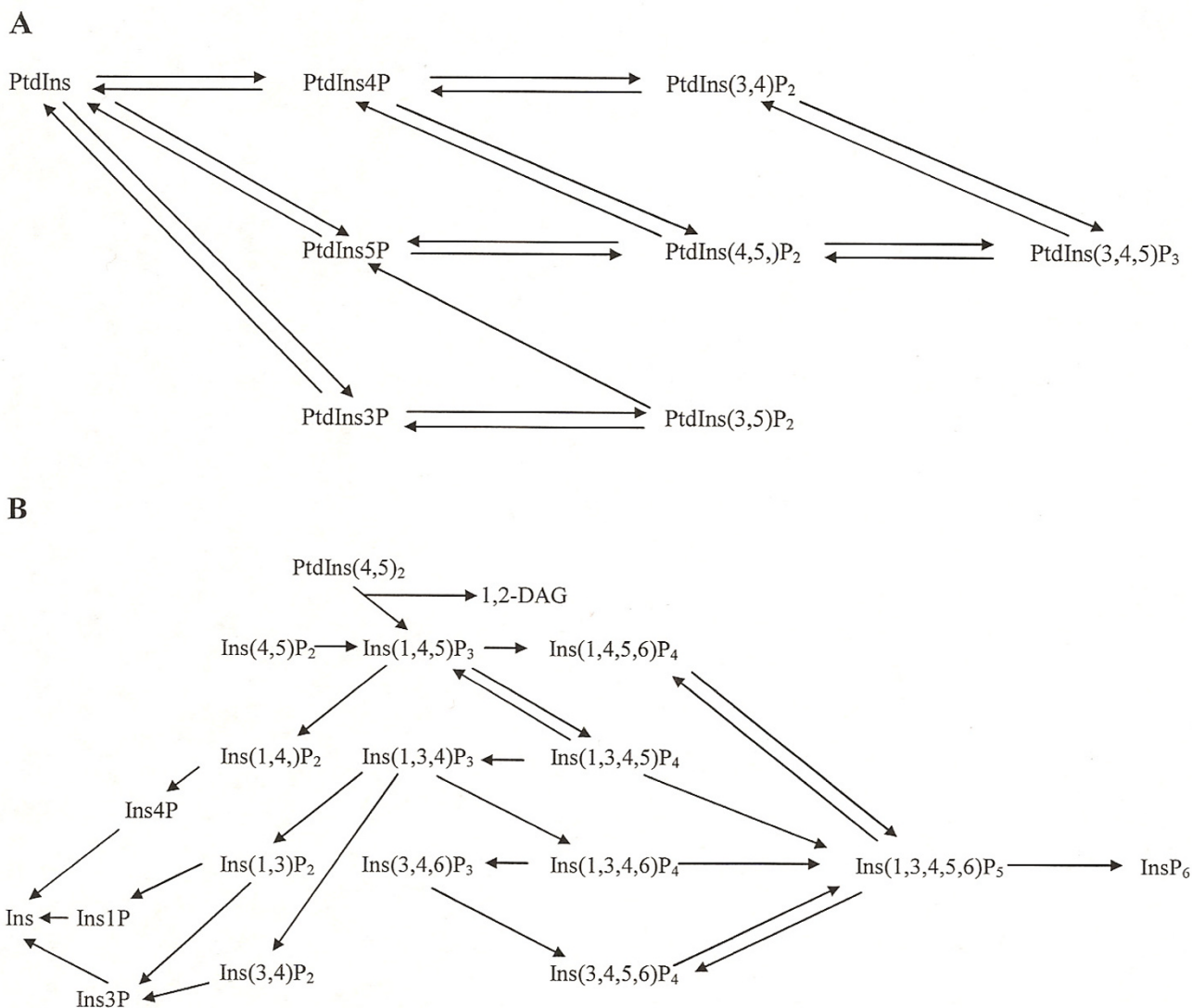
MAPK family; for example, the SAP-1 D-domain binds p38 $\alpha$  and p38 $\beta$ , but not p38 $\delta$  [22]. The D-domain can be positioned either N- or C-terminal to the phosphorylation site [7,12,16,19,23].

A second MAPK docking motif has also been identified: the FXFP motif, or DEF (docking site for ERK, FXFP) motif [4,18,22], where F and P represent the amino acids phenylalanine and proline, respectively. Binding and substrate phosphorylation can occur in the absence of the proline residue [14,18]; however, its presence does increase the effectiveness of the motif [18]. Thus, we chose to include the proline in our searches. The identity of the second (X) residue is highly variable [4,18]. In most, if not all, proteins, the FXFP motif is C-terminal to the phosphorylation site [4,18,19,24]. In general, it appears that the FXFP motif occurs more proximal to a phosphorylation site than is often the case for a D-domain [4,18,20,22,24]. The FXFP motif binds ERK2 and p38 $\alpha$  [18,22], but not JNK3 [4] and p38 $\beta$  [4,22]. The FXFP motif and D-domain are each sufficient for MAPK docking; however, when both are present in a protein, they function additively [18,22].

We recently identified a D-domain sequence in PLC- $\gamma$ 1 and provided strong evidence that this sequence mediated an observed interaction between PLC- $\gamma$ 1 and phospho-ERK2 [25]. We have also reported that PLC- $\gamma$ 2, - $\beta$ 1, - $\beta$ 2, and - $\beta$ 4 each have at least one identifiable D-domain, as well co-immunoprecipitate with ERK2 [26]. Based on these observations, we have proposed that MAPK signaling and the metabolism of PIs are integrated. In order to substantiate this hypothesis, we sought to determine whether MAPK docking sites could be recognized in other enzymes that metabolize PIs; additionally, we sought support for extending this hypothesis to include the metabolism of IPs.

#### **Overview of phosphatidylinositol and inositol phosphate metabolism and signaling**

Eight PIs and more than 20 IPs have been identified [27-30]. Several reviews of the metabolism and cellular roles of these molecules have appeared [27-35]. As the physiologic functions of the PIs and IPs were not a primary focus of the present studies, we will not summarize this information here; instead, the interested reader is directed to the sources cited above; we acknowledge that this is only a partial listing of the reviews that have been written on these subjects. PIs are substrates for a variety of phospholipases, acyl transferases, kinases and phosphatases, while IPs are metabolized by a series of kinases and phosphatases. Of these enzymes, we have limited the scope of the present studies to kinases and phosphatases. In order to assist the reader in understanding the reactions catalyzed by the enzymes which we analyzed, the pathways for the metabolism of PIs and IPs by various kinases and



**Figure 1**

**A: Pathways of metabolism of phosphatidylinositols in animals.** This figure is based on similar figures in Toker [28] and Parker [29] and information provided in the Results and Discussion section. We are not aware of evidence that PtdIns5P is a substrate for a PI 3-kinase, producing PtdIns(3,5)P<sub>2</sub>; therefore, an arrow has not been included for this reaction.

**B: Pathways of inositol phosphate metabolism.** The pathways of the metabolism of inositol phosphates in animal cells are shown; additional pathways are present in plants and slime mold [30]. The PLC-catalyzed synthesis of 1,2-diaclyglycerol (1,2-DAG) and Ins(1,4,5)P<sub>3</sub> is also shown. The figure is based on similar figures found in Irvine and Schell [30], Shears [36], and Irvine [37], as well as information provided in the Results and Discussion section.

phosphatases are shown in Figures 1A and 1B, respectively. We use the IUPAC-IUB nomenclature for the identification of phosphatidylinositol (PtdIns) and inositol (Ins) phosphates (P): numbers are used to designate the carbon atoms to which phosphate groups are bound and the total number of phosphate groups is designated by a subscript, with the exception that no subscript is employed to designate the presence of a single phosphate group. For example, phosphatidylinositol 4-phosphate is

designated PtdIns4P and inositol 1,4,5-trisphosphate is designated Ins(1,4,5)P<sub>3</sub>. For a review of inositol phosphate chemistry the reader is referred to recent articles by Shears [36] and Irvine [37].

**Results and discussion**

**Search strategy**

We obtained the primary sequences of human and mouse kinases and phosphatases that control the phosphoryla-

**Table 1: Kinases (human) that have an FXFP motif and/or D-domain sequence. The number of potential MAPK phosphorylation sites, sequences fitting the optimal MAPK phosphorylation consensus sequence of PX(S/T)P, and FXFP motifs and D-domain sequences in kinases that use phosphatidylinositols or inositols as substrates are listed in the table; all sequences are for human proteins.**

Isozyme	GenBank Accession #	# amino acids	number of (S/T)P sites	Site(s) with the consensus PX(S/T)P sequence	FXFP motif	D-domain motif
<i>PI 3-kinase</i>						
<i>Class I</i>						
PI 3-kinase $\alpha$	<a href="#">P42336</a>	1068	6	none	none	271KYIRSCIML 398RAARLCLSI
PI 3-kinase $\gamma$	<a href="#">P48736</a>	1101	13	none	none	806KKKPLWL
PI 3-kinase $\delta$	<a href="#">NP_005017</a>	1044	4	none	585FSFP	none
<i>Class II</i>						
PI 3-kinase C2- $\alpha$	<a href="#">NP_002636</a>	1686	16	118PVTP 199PLTP 202PATP	none	238KNGKARTDLEI
PI 3-kinase, C2- $\gamma$	<a href="#">CAA03853</a>	1448	9	none	none	400KVSROCLLTL 830KEQKLIKI 1327KKPKVQLVI 1347KHMKNIHL
<i>Class III</i>						
PI 3-kinase Vps34-type	<a href="#">S57219</a>	887	6	none	none	656KLLRKENLDL
<i>PI 4-kinase</i>						
PI 4-kinase $\alpha$ (PI4K230)	<a href="#">P42356</a>	2044	16	215PSSP 590PPSP	1822FVF P	15RRRDAVIAL 15RRRDAVIALGI 1340KRLREDISI 1340KRLREDISIMI 1429KRRRTLLL 1429KRRTLLLSL 244RGTKLRKLLIL 360KTQRLISELSL 608KPYKILVI
PI 4-kinase $\beta$ (PI4K92)	<a href="#">NP_002642</a>	828	7	none	none	435KDNKSPLHL 239KVGGRKFHRIGL
PI 4-kinase type II (PI4K55)	<a href="#">NP_060895</a>	479	5	49PGSP	none	
PI 4-kinase type II- $\beta$ (PI4K55)	<a href="#">NP_060793</a>	481	3	none	none	
<i>PIP kinase</i>						
<i>Type I PIPK (PI4P 5-kinase)</i>						
PI4P 5-kinase, type I $\alpha$	<a href="#">NO_003548</a>	549	3	501PQTP	none	376RNSKGERLLL 379KGERLLLYI 379KGERLLLYIGI
PI4P 5-kinase, type I $\beta$	<a href="#">NP_003549</a>	540	4	464PSTP	none	335KSHRGEKLLL
PI4P 5-kinase type I $\gamma$	<a href="#">NP_036530</a>	668	6	510PCTP	none	386RGERLLLHI 386RGERLLLHIGI
<i>Type III PIPK (PIKfyve; PI3P 5-kinase)</i>						
PIKfyve	<a href="#">Q9Y217</a>	2098	32	18PRSP 21PTSP 32PLTP 251PRTP 581PFTP 1524PPSP	none	259KASRNIFL 700KNPKILLL 1981KMVRDNPLYI
<i>Ins(1,4,5)P<sub>3</sub> 3-kinase</i>						
Ins(1,4,5)P <sub>3</sub> 3-kinase B	<a href="#">P27987</a>	946	24	69PRSP 164PRSP 262PASP	56FLFP	665KKKYPWIIQL
<i>Ins(1,3,4,5,6)<sub>5</sub> 2-kinase</i>						
Ins(1,3,4,5,6) <sub>5</sub> 2-kinase	<a href="#">AAM75353</a>	491	5	none	240FFFFP	none

**Table 2: Kinases (human) that do not have an FXFP motif and/or D-domain sequence. Kinases that use phosphatidylinositols or inositols as substrates and do not contain an FXFP or D-domain sequence are listed in the table; all sequences are for human proteins. The number of potential MAPK phosphorylation sites and sequences fitting the optimal MAPK phosphorylation in these kinases are also identified.**

Isozyme	GenBank Accession #	# amino acids	number of (S/T)P sites	Site(s) with the consensus PX(S/T)P sequence
PI 3-kinase $\beta$	<a href="#">NP_006210</a>	1070	3	none
PI 3-kinase, C2- $\beta$	<a href="#">O00750</a>	1634	17	none
PIP kinase Type II $\alpha$	<a href="#">NP_005019</a>	406	5	<sup>314</sup> PDSP
PIP kinase Type II $\beta$	<a href="#">P78356</a>	416	4	<sup>324</sup> PDSP
PIP kinase Type II $\gamma$	<a href="#">NP_079055</a>	421	1	none
Ins(1,4,5)P <sub>3</sub> 3-kinase A	<a href="#">P23677</a>	461	4	<sup>18</sup> PCSP <sup>93</sup> PTSP
Ins(1,4,5)P <sub>3</sub> 3-kinase C	<a href="#">NP_079470</a>	683	8	<sup>334</sup> PETP
Ins(1,3,4)P <sub>3</sub> 5/6-kinase/Ins(3,4,5,6)P <sub>4</sub> 1-kinase	<a href="#">NP_055031</a>	414	3	none
Inositol polyphosphate multikinase/Ins(1,3,4,6)P <sub>4</sub> 5-kinase	<a href="#">Q8NFU5</a>	416	5	none

tion state of the inositol ring in PIs and IPs from GenBank at NCBI and searched these sequences for an FXFP motif and the two consensus sequences for a D-domain reported by Kornfeld and colleagues [4]: (K/R)X(X/K/R)(K/R)X<sub>(1-4)</sub>(L/I)X(L/I) or (K/R)(K/R)(K/R)X<sub>(1-5)</sub>(L/I)X(L/I). We note that this strategy most likely failed to identify all possible sequences that may function as MAPK docking sites. For example, both the human and mouse Class I PI 3-kinase  $\beta$  include the sequence LILRRHGNIIFI, which contains a KIM, as defined by MacKenzie et al [16], and a MAPK-docking site, as defined by Bardwell et al. [16], but not a D-domain according to the criteria that we employed. Similarly, the human and mouse Ins(1,3,4)P<sub>3</sub> 5/6-kinase/Ins(3,4,5,6) 1-kinase contain the sequence LCRKRGXEVVQLNL (X is M in human and I in mouse), which fits the consensus sequences for a KIM and a MAPK-binding site, but not a D-domain. We chose to use the criteria of Kornfeld and colleagues based on its successful application in the identification of D-domains in PLC isozymes [25,26]. Although the list that we have compiled is likely to be incomplete, it does serve as a useful first approximation. We also searched each of the enzyme sequences for potential MAPK phosphorylation sites (i.e., (S/T)P sequences) and MAPK optimal phosphorylation sequences, PX(S/T)P. Finally, we note that we did not analyze the sequences of enzymes that control the metabolism of the diphosphorylated IPs.

#### Presentation of the data

In order to facilitate the presentation of the data, we separated human from mouse enzymes, kinases from phosphatases, and enzymes in which we identified a D-domain and/or FXFP motif from those in which we did not. Human and mouse kinases having a D-domain and/or FXFP motif are contained in Tables 1 and 3, respectively, whereas human and mouse kinases that do not

have either of these sequences are presented in Tables 2 and 4, respectively. Similarly, human and mouse phosphatases containing a D-domain and/or FXFP motif are listed in Tables 6 and 8, respectively, while human and mouse phosphatases that are devoid of these sequences are contained in Tables 7 and 9, respectively.

In the following discussion we use the terms "alternative pair" and "overlapping" in referring to relationships of D-domains. An alternative pair of D-domains has the same amino-terminus and two possible carboxyl-termini: e.g., 15RRRDAVIAL and 15RRRDAVIALGI in human PI 4-kinase  $\alpha$  (Table 1). We use the term overlapping to identify D-domains that have distinct amino-termini and carboxyl-termini, but share a region of sequence: e.g., 376RNSKGERLLL and 379KGERLLLYI found in human PI4P 5-kinase type I $\alpha$  (Table 1).

#### PI 3-kinase

Phosphatidylinositol 3-kinase (also called phosphoinositide 3-kinase, PtdIns 3-kinase, PI 3-kinase, and PI3K) isozymes catalyze the phosphorylation of the 3-position of the inositol ring of phosphatidylinositols [32]. Three classes (I, II, and III) of PI 3-kinase have been identified [38,39]. These classes are differentiated on the basis of their subunit composition, substrate specificity and mechanisms of regulation. The Class I enzymes are heterodimers of a regulatory subunit, of various sizes, and a catalytic subunit of approximately 110 kDa. Three distinct forms of the catalytic subunit (p110 $\alpha$ , p110 $\beta$ , and p110 $\delta$ ) and five forms of the regulatory subunit (p85 $\alpha$ , p85 $\beta$ , p55 $\alpha$ , p55 $\gamma$ , and p50 $\alpha$ ) have been identified. The class IB isoform consists of a p101 regulatory subunit coupled to a p100 $\gamma$  catalytic subunit. Class I enzymes catalyze the synthesis of PtdIns3P, PtdIns(3,4)P<sub>2</sub>, and PtdIns(3,4,5)P<sub>3</sub> [40,41]; PtdIns(4,5)P<sub>2</sub> may be the preferred substrate *in vivo* [42].

**Table 3: Kinases (mouse) that have an FXFP motif and/or D-domain sequence. The number of potential MAPK phosphorylation sites, sequences fitting the optimal MAPK phosphorylation consensus sequence of PX(S/T)P, and FXFP motifs and D-domain sequences in kinases that use phosphatidylinositols or inositols as substrates are listed in the table; all sequences are for mouse proteins.**

Isozyme	GenBank Accession #	# amino acids	# (S/T)P sites	Site(s) with the consensus PX(S/T)P sequence	FXFP motif	D-domain motif
<i>PI 3-kinase</i>						
<i>Class I</i>						
PI 3-kinase $\alpha$	<a href="#">NP_032865</a>	1068	6	none	none	271KYIRSCIML 398RLARLCLSI
PI 3-kinase $\gamma$	<a href="#">NP_064668</a>	1102	12	none	none	807KKKPLWL
<i>Class II</i>						
PI 3-kinase, C2- $\alpha$	<a href="#">NP_035213</a>	1509	13	24PLTP 27PATP 1374PFSP	none	63KNGKARTDLEI
PI 3-kinase, C2- $\gamma$ 1	<a href="#">NP_997566</a>	1506	12	none	none	930KDIKTCHLPL 1409KHLKNIHL
PI 3-kinase, C2- $\gamma$ 2	<a href="#">NP_035214</a>	653	3	none	none	77KDIKTCHLPL 556KHLKNIHL
<i>Class III</i>						
PI 3-kinase Vps34-type	<a href="#">NP_852079</a>	887	4	none	none	656KLLRKENLDL
<i>PI 4-kinase</i>						
PI 4-kinase $\alpha$ (PI4K230)	<a href="#">NP_001001983</a>	2044	16	215PSSP 590PPSP	1822FVFP	15RRRDAVIAL 15RRRDAVIALGI 1340KRLREDISI 1340KRLREDISIMI 1429KRRLLLL 1429KRRLLLLSL
PI 4-kinase $\beta$ (PI4K92)	<a href="#">NP_780565</a>	801	8	none	none	232RGTKLRKLIL 333KTQRLISELSL 581KPYKILVI
PI 4-kinase type II (PI4K55)	<a href="#">NP_663476</a>	479	5	49PCSP	none	435KDNKSPLHL
<i>PIP kinase</i>						
<i>Type I PIPK (PI4P 5-kinase)</i>						
PI4P 5-kinase, type I $\alpha$	<a href="#">NP_032872</a>	539	3	464PSTP	none	335KSHRGEKLLL
PI4P 5-kinase type I $\beta$	<a href="#">NP_032873</a>	546	5	498PQTP	none	374RNNKGERLLL 377KGERLLLYI 377KGERLLLYIGI
PI4P 5-kinase type I $\gamma$	<a href="#">NP_032870</a>	661	5	509PCTP	none	386RGERLLLHI 386RGERLLLHIGI
<i>Type III PIPK (PIKfyve; PI3P 5-kinase)</i>						
PIKfyve	<a href="#">NP_035216</a>	2052	31	18PRSP 21PASP 32PLTP 262PRTP 536PFTP 1479PPSP	none	270KASRNIFL 655KNPKILLL 1935KMVRDNPLYI

Class II isozymes are monomeric catalytic subunits ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) containing a carboxyl-terminal C2 domain; these enzymes may be referred to as PI3K-C2. PtdIns and PtdIns4P, and under certain conditions PtdIns(4,5)P<sub>2</sub>, are substrates for the Class II PI 3-kinase [39,43]. A single

Class III isozyme, which is specific for PtdIns, has been identified [44].

The Class I p110 $\alpha$  and p110 $\gamma$  human (Table 1) and mouse (Table 3) proteins contain D-domains, whereas the p110 $\beta$

**Table 4: Kinases (mouse) that do not have an FXFP motif and/or D-domain sequence. Kinases that use phosphatidylinositols or inositols as substrates and do not contain an FXFP or D-domain sequence are listed in the table; all sequences are for mouse proteins. The number of potential MAPK phosphorylation sites and sequences fitting the optimal MAPK phosphorylation in these kinases are also identified.**

Isozyme	GenBank Accession #	# amino acids	# (S/T)P sites	Site(s) with the consensus PX(S/T)P sequence
PI 3-kinase $\beta$	<a href="#">NP_083370</a>	1064	4	none
PI 3-kinase $\delta$	<a href="#">NP_032866</a>	1043	3	none
PI 4-kinase type II- $\beta$ (PI4K55)	<a href="#">NP_083020</a>	445	2	none
PIP kinase Type I $\alpha$	<a href="#">NP_032871</a>	405	4	<sup>314</sup> PDSP
PIP kinase Type I $\beta$	<a href="#">Q80X14</a>	416	3	<sup>324</sup> PDSP
PIP kinase Type I $\gamma$	<a href="#">NP_473438</a>	421	2	none
Ins(1,4,5)P <sub>3</sub> 3-kinase A	<a href="#">Q8R071</a>	459	5	<sup>18</sup> PCSP <sup>92</sup> PASP
Ins(1,4,5)P <sub>3</sub> 3-kinase C	<a href="#">NP_853624</a>	678	8	<sup>328</sup> PETP
Ins(1,3,4)P <sub>3</sub> 5/6-kinase/Ins(3,4,5,6)P <sub>4</sub> I-kinase	<a href="#">NP_766172</a>	419	4	none
Inositol polyphosphate multikinase/Ins(1,3,4,6)P <sub>4</sub> 5-kinase	<a href="#">QZTT16</a>	396	2	none

(Tables 2 and 4) and p110 $\delta$  (Tables 1 and 4) isozymes do not. Of all the PI 3-kinase sequences that we analyzed, only the human PI 3-kinase  $\delta$  (Table 1) contains an FXFP motif (<sup>585</sup>FSFP).

The Class II PI3-kinase C2- $\alpha$  contains a D-domain that is conserved in the human (Table 1) and mouse (Table 3) isozymes. PI 3-kinase C2- $\alpha$  was the only PI 3-kinase isozyme in which we found an optimal MAPK phosphorylation sequence (Tables 1, 2, 3, 4). The human and mouse PI 3-kinase C2- $\alpha$  proteins each contain three such sequences; a sequence alignment revealed that two of these (PLTP and PATP) are conserved, while the third, <sup>118</sup>PVTP and <sup>1374</sup>PFSP in human and mouse, respectively, is unique. Both the human (Table 1) and mouse (Table 3) PI 3-kinase C2- $\gamma$  contain D-domains. A sequence align-

ment of these two proteins revealed that only the last D-domain (<sup>1347</sup>KHMKNIHL in human and <sup>1409</sup>KHLKNIHL in mouse) aligns; all of the other identified D-domains are unique to one or the other protein. These results indicate the MAPK-dependent regulation of the human and mouse Class II PI 3-kinases may be significantly different and, thus, caution should be exercised when comparing studies on these two proteins.

The human (Table 1) and mouse (Table 3) Class III PI 3-kinase contains a conserved D-domain. Neither of these proteins contains an FXFP motif or an optimal phosphorylation sequence for MAPKs.

#### PI 4-kinase

Phosphatidylinositol 4-kinase (PtdIns 4-kinase, PI 4-kinase) catalyzes the phosphorylation of PtdIns to produce PtdIns4P. Subfamilies (Types II and III; or PI4K230, PI4K92 and PI4K55 using the nomenclature of Heilmeyer et al. [45]) of PI 4-kinase have been identified; these possess a conserved C-terminal catalytic domain and diverse N-terminal regulatory domains [45]. Differing physiologic functions have been ascribed to each of the three subfamilies [45-49].

PI4K $\alpha$  (also called PI 4-kinase 230) contains three alternative pairs of D-domains that are conserved in human (Table 1) and mouse (Table 3) sequences. PI4K $\alpha$  also contains an FXFP sequence and two optimal MAPK phosphorylation sequences that are conserved in human and mouse proteins. PI 4-kinase  $\beta$  (also called PI 4-kinase 92) contains three D-domains that are conserved in the human (Table 1) and mouse (Table 3) isozymes. The first of these (<sup>232</sup>RGTKLRKLIL) overlaps with an identifiable bipartite nuclear localization sequence motif [45]. The human and mouse PI 4-kinase- $\beta$  do not contain an FXFP motif or optimal MAPK phosphorylation sequence. The

**Table 5: Reactions catalyzed by PIP kinases (based on [50] and [51])**

<i>Type I PIP kinase</i>
PI $\rightarrow$ PI5P
PI3P $\rightarrow$ PI(3,4)P <sub>2</sub>
PI4P $\rightarrow$ PI(4,5)P <sub>2</sub>
PI3P $\rightarrow$ PI(3,4,5)P <sub>3</sub>
PI(3,4)P <sub>2</sub> $\rightarrow$ PI(3,4,5)P <sub>3</sub>
<i>Type II PIP kinase</i>
PI3P $\rightarrow$ PI(3,4)P <sub>2</sub>
PI5P $\rightarrow$ PI(4,5)P <sub>2</sub>
PI3P $\rightarrow$ PI(3,4,5)P <sub>3</sub>
<i>Type III</i>
PI $\rightarrow$ PI5P
PI3P $\rightarrow$ PI(3,5)P <sub>2</sub>

**Table 6: Phosphatases (human) that have an FXFP motif and/or D-domain sequence. The number of potential MAPK phosphorylation sites, sequences fitting the optimal MAPK phosphorylation consensus sequence of PX(S/T)P, and FXFP motifs and D-domain sequences in phosphatases that use phosphatidylinositols or inositols as substrates are listed in the table; all sequences are for human proteins.**

Isozyme	GenBank Accession #	# amino acids	# (S/T)P sites	Site(s) with the consensus PX(S/T)P sequence	FXFP motif	D-domain motif
<i>monophosphatase</i>						
inositol monophosphatase A1	<a href="#">NP_005527</a>	277	2	none	none	<sup>261</sup> RIAKEIQVIPL
<i>1-phosphatase</i>						
Inositol polyphosphate 1-phosphatase	<a href="#">P49441</a>	399	1	none	none	<sup>378</sup> RKRLETFLSL
<i>3-phosphatase</i>						
Myotubularin-related protein 1 isoform 1; MTMR1-1	<a href="#">NP_003819</a>	665	3	none	none	<sup>177</sup> KDMRNLR
Myotubularin-related protein 1 isoform 2; MTMR1-2	<a href="#">NP_789746</a>	568	2	none	none	<sup>177</sup> KDMRNLR
PI(3,4,5)P <sub>3</sub> 3-phosphatase, PTEN	<a href="#">P60484</a>	403	2	none	<sup>241</sup> FEFP	none
<i>4-phosphatase</i>						
Inositol polyphosphate 4-phosphatase, Type Ia	<a href="#">NP_004018</a>	938	7	none	none	<sup>147</sup> RHHRLHLTL
Inositol polyphosphate 4-phosphatase, Type Ib	<a href="#">NP_001557</a>	954	8	none	none	<sup>147</sup> RHHRLHLTL
Inositol polyphosphate 4-phosphatase, Type Iα3	<a href="#">AAK58870</a>	977	7	none	none	<sup>147</sup> RHHRLHLTL
SAC1 poly-phosphatidylinositol phosphatase	<a href="#">AAQH16559</a>	587	3	none	none	<sup>345</sup> KNMRWDRLSI <sup>348</sup> RWDRLSILL <sup>461</sup> RTGKRTHLGL <sup>517</sup> RDWKFLAL <sup>517</sup> RDWKFLALPI
<i>5-phosphatase</i>						
<i>Group II</i>						
Inositol polyphosphate 5-phosphatase OCRL-1	<a href="#">Q01968</a>	901	3	none	none	<sup>446</sup> RLKFDQLNI
Inositol polyphosphate 5-phosphatase OCRL-2	<a href="#">NP_001578</a>	893	3	none	none	<sup>446</sup> RLKFDQLNI
Synaptojanin 2 (synaptic inositol 1,4,5-trisphosphate 5-phosphatase 2)	<a href="#">Q15056</a>	1496	17	<sup>987</sup> PVSP <sup>1218</sup> PETP <sup>1249</sup> PLSP	none	<sup>617</sup> RSHRYILL
Sac-domain-containing inositol phosphatase 2 isoform 1; inositol polyphosphate 5-phosphatase F isoform 1	<a href="#">NP_055752</a>	1132	12	<sup>259</sup> PETP	none	<sup>85</sup> KVTKIAVLSL
Sac-domain-containing inositol phosphatase 3	<a href="#">NP_055660</a>	907	5	none	none	<sup>663</sup> KFHKYEIEIDI
Inositol polyphosphate 5-phosphatase, 75 kDa	<a href="#">NP_005531</a>	748	5	none	none	<sup>31</sup> RQSRLGL <sup>335</sup> KYAKVKLIRL <sup>340</sup> KLIRLVGIML
Inositol polyphosphate 5-phosphatase, 75 kDa; Inositol polyphosphate 5-phosphatase B	<a href="#">NP_032411</a>	993	5	none	none	<sup>31</sup> RQSRLGL <sup>420</sup> KFVRLVGIML
<i>Group III</i>						
Inositol polyphosphate 5-phosphatase, 145 kDa; PI(3,4,5)P <sub>3</sub> 5-phosphatase; SH2-containing inositol 5'-phosphatase 1; SHIP1	<a href="#">NP_005532</a>	1188	18	<sup>286</sup> PESP <sup>960</sup> PPTP <sup>968</sup> PISP <sup>1133</sup> PPTP	none	<sup>348</sup> KSQKFLNKLVI
<i>Group IV</i>						
PI polyphosphate 5-phosphatase type IV; Inositol polyphosphate 5-phosphatase E	<a href="#">AAF81404</a>	644	10	<sup>55</sup> PATP <sup>239</sup> PRSP	none	<sup>79</sup> RLERALS <sup>596</sup> RPGRDNIPL <sup>608</sup> KFDRELYL <sup>608</sup> KFDRELYLLGI



human (Table 1) and mouse (Table 3) PI 4-kinase type II (also called PI 4-kinase 55) contain a conserved D-domain and optimal phosphorylation sequence for a MAPK. Neither protein contains an FXFP motif. The human (Table 1), but not the mouse (Table 4), PI 4-kinase type II- $\beta$  contains a single D-domain, while neither protein contains a sequence fitting the MAPK optimal phosphorylation sequence or the FXFP motif.

### PIP kinase

Phosphatidylinositol phosphate kinases (PIP kinases, PIPKs) utilize PtdIns3P, PtdIns4P, and PtdIns5P as substrates, catalyzing the synthesis of PtdIns(3,4)P<sub>2</sub>, PtdIns(3,5)P<sub>2</sub>, and PtdIns(4,5)P<sub>2</sub> (Table 5). These enzymes are also able to catalyze the formation of PtdIns5P and PtdIns(3,4,5)P<sub>3</sub> (Table 5). Three types (I, II and III) of PIP kinase are defined based on primary sequence, substrate specificity, and subcellular localization [51]. These families are often designated by the reaction that they catalyze most efficiently. Thus, the Type I PIP kinases are also termed PI4P 5-kinase, the Type II PIP kinases are termed PI5P 4-kinases, and the Type III PIP kinases are called PI3P 5-kinases. Three forms (termed  $\alpha$ ,  $\beta$ , and  $\gamma$ ), each with multiple splice variants, of Type I and Type II PIP kinase have been identified [52-55]. It should be noted that the nomenclature for the  $\alpha$ - and  $\beta$ -forms of the human and mouse Type I PIP kinase are reversed: that is, the human Type I $\alpha$  enzyme corresponds to the mouse Type I $\beta$  enzyme, and vice versa.

Each of the Type I isozymes contain at least one identifiable D-domain that was conserved in humans and in mice. In human PI4P 5-kinase Type I $\alpha$  (Table 1) an alternative pair of D-domains (<sup>379</sup>KGERLLLYI and <sup>379</sup>KGERLLLYIGI) overlaps with a different D-domain (<sup>376</sup>RNSKGERLLL). This observation raises the intriguing possibility that these sequences are specific for binding of a particular MAPK, or MAPK family, and that there is competition among MAPKs for binding, with the likelihood that a mechanism(s) exists for the regulation of this binding. Further, the MAPK that is bound may affect the kinetics of the signal that is generated and, thus, the downstream response. The identified alternative pair of D-domains in PI4P 5-kinase type I $\gamma$  (PIPKI $\gamma$ ) (Tables 1 and 3) may be responsible for binding phospho-ERK1, which has been shown to catalyze the phosphorylation of serine 650 in PIPKI $\gamma$  [56]. Phosphorylation of serine 650 inhibits the binding of talin to PIPKI $\gamma$  and may play a role in synaptic neurotransmission and focal adhesion disassembly during mitosis. None of the Type I PIP kinases that we analyzed contains an FXFP motif (Tables 1 and 3). Each of the Type I PIPKs that we analyzed contains an optimal MAPK phosphorylation site that is conserved in human and mouse proteins. In the case of PIPKI $\gamma$ , this is not serine-650. However, it is possible that other MAPKs are capable of

phosphorylating threonine-512 (human)/threonine-511 (mouse).

The Type II isozymes (Tables 2 and 4) were found to be devoid of D-domain sequences and FXFP motifs. However, the Type II $\alpha$  and Type II $\beta$  isozymes do contain an optimal MAPK phosphorylation sequence that is conserved in human (Table 2) and mouse (Table 4) protein sequences.

The PIKfyve human (Table 1) and mouse (Table 3) sequences each contain six MAPK optimal phosphorylation sequences. This is the most that we found in any of the sequences that we analyzed. These enzymes contain three conserved D-domain sequences, but are devoid of an FXFP motif.

### Ins(1,4,5)P<sub>3</sub> 3-kinase

Ins(1,4,5)P<sub>3</sub> 3-kinase catalyzes the formation of Ins(1,3,4,5)P<sub>4</sub> from Ins(1,4,5)P<sub>3</sub>. Three forms of Ins(1,4,5)P<sub>3</sub> 3-kinase have been cloned; these forms differ in their molecular mass, regulation by Ca<sup>2+</sup>/calmodulin, tissue distribution and intracellular localization [57-64]. All three forms of human (Tables 1 and 2) and both forms of mouse (Table 4) Ins(1,4,5)P<sub>3</sub> 3-kinase contain one or more optimal sequence for MAPK phosphorylation. Human Ins(1,4,5)P<sub>3</sub> 3-kinase B contains both an FXFP motif and a D-domain, whereas the A and C isoforms do not contain either of these two MAPK docking sequences (Tables 1 and 2). Ins(1,4,5)P<sub>3</sub> 3-kinase B, which plays a critical role in T-cell development [65,66], is associated with the endoplasmic reticulum via its N-terminus [57,67]. Thus, it is possible that the MAPK binding to the <sup>56</sup>FLFP sequence motif and/or phosphorylation of serine-71 within the optimal phosphorylation sequences (<sup>69</sup>PRSP) regulates Ins(1,4,5)P<sub>3</sub> 3-kinase B interaction with the endoplasmic reticulum.

### Ins(1,3,4)P<sub>3</sub> 5/6-kinase/Ins(3,4,5,6) 1-kinase

Wilson and Majerus [68] cloned an Ins(1,3,4)P<sub>3</sub> 5/6-kinase, which was subsequently shown by Yang and Shears [69] to be the same as Ins(3,4,5,6)P<sub>4</sub> 1-kinase. Interestingly, this enzyme also possesses Ins(1,3,4,5,6)P<sub>5</sub> 1-phosphatase activity [70]. Regulation of these latter two reciprocal activities provides a mechanism for tight control of Ins(3,4,5,6)P<sub>4</sub> levels in cells. The production of Ins(1,3,4,6)P<sub>4</sub> by Ins(1,3,4)P<sub>3</sub> 5/6-kinase is the rate-limiting step in the synthesis of inositol hexakisphosphate from Ins(1,3,4)P<sub>3</sub> [71]. We were unable to identify an optimal sequence for MAPK phosphorylation, an FXFP motif or a D-domain in human (Table 2) or mouse (Table 4) Ins(1,3,4)P<sub>3</sub> 5/6-kinase/Ins(3,4,5,6) 1-kinase.

**Table 7: Phosphatases (human) that do not have an FXFP motif and/or D-domain sequence. Phosphatases that use phosphatidylinositols or inositols as substrates and do not contain an FXFP or D-domain sequence are listed in the table; all sequences are for human proteins. The number of potential MAPK phosphorylation sites and sequences fitting the optimal MAPK phosphorylation in these kinases are also identified.**

Isozyme	GenBank Accession #	# amino acids	# (S/T)P sites	Site(s) with the consensus PX(S/T)P sequence
inositol monophosphatase A2	<a href="#">NP_055029</a>	288	1	none
inositol monophosphatase A3	<a href="#">NP_060283</a>	359	3	none
Myotubularin; MTM1	<a href="#">Q13496</a>	603	3	586PTSP 589PSSP
Myotubularin-related protein 2; MTMR2	<a href="#">Q13614</a>	643	4	none
Myotubularin-related protein 3; MTMR3	<a href="#">Q13615</a>	1198	9	582PTTP
Myotubularin-related protein 6; MTMR6	<a href="#">Q9Y217</a>	621	3	559PESP
Inositol polyphosphate 4-phosphatase, Type II	<a href="#">NP_003857</a>	924	7	485PPSP
Inositol 1,4,5-trisphosphate 5-phosphatase, Type I	<a href="#">Q14642</a>	412	3	none
Synaptojanin 1 (synaptic inositol 1,4,5-trisphosphate 5-phosphatase 1)	<a href="#">Q43426</a>	1575	23	1090PATP 1148PPSP 1161PKSP
Sac-domain-containing inositol phosphatase 2 isoform 2; inositol polyphosphate 5-phosphatase F isoform 2	<a href="#">NP_938144</a>	500	8	none
Sac-domain-containing inositol phosphatase 2 isoform 3; inositol polyphosphate 5-phosphatase F isoform 3	<a href="#">NP_938145</a>	219	1	none
PI(4,5)P <sub>2</sub> 5-phosphatase A; PIB5PA; PIPP	<a href="#">Q15735</a>	1006	26	148PRSP 199PSTP 345PRSP
Skeletal muscle and kidney enriched inositol phosphatase isoform 1; SKIP 1	<a href="#">NP_057616</a>	448	4	283PDTP 356PSSP
Skeletal muscle and kidney enriched inositol phosphatase isoform 2; SKIP 2	<a href="#">NP_570122</a>	372	4	207PDTP 280PSSP
Phospholipids-inositol phosphatase; PTEN-like phosphatase; PLIP; PTPM1	<a href="#">AAH20242</a>	201	1	none
Inositol polyphosphate 5-phosphatase 2; SH2-containing inositol 5'-phosphatase 2; SHIP2	<a href="#">JCS765</a>	1258	7	156PSSP 163PETP 956PLTP 1001PPSP

### **Inositol polyphosphate multikinase/Ins(1,3,4,6)P<sub>4</sub> 5-kinase**

Inositol polyphosphate multikinase catalyzes the formation of Ins(1,3,4,5,6)P<sub>5</sub> from Ins(1,4,5)P<sub>3</sub> by phosphorylation of both the 3- and 6-position of the inositol ring [30,36], with phosphorylation of the 3-position possibly preceding that of the 6-position [72]. The enzyme also phosphorylates the 1-position of Ins(4,5)P<sub>2</sub> to form Ins(1,4,5)P<sub>3</sub> [72]. Majerus and colleagues [73] reported that *in vitro* the enzyme displays specificity as an Ins(1,3,4,6) 5-kinase. Neither the human (Table 2) nor mouse (Table 4) protein contains an FXFP motif, a D-domain or optimal sequence for MAPK phosphorylation.

### **Ins(1,3,4,5,6) 2-kinase**

Ins(1,3,4,5,6)P<sub>2</sub> 2-kinase catalyzes the final step in the synthesis of inositol hexakisphosphate from Ins(1,3,4)P<sub>3</sub>, and ultimately from Ins(1,4,5)P<sub>3</sub> [71]. The human 2-kinase contains an FXFP motif, but does not contain a D-domain (Table 1). We did not identify an optimal sequence for MAPK phosphorylation.

### **Inositol monophosphatase**

Inositol monophosphatase is a Mg<sup>2+</sup>-dependent enzyme that catalyzes the hydrolysis of Ins1P, Ins3P and Ins4P, as well as several related compounds, but does not hydrolyze Ins2P [74-78]. Inositol monophosphatase has received significant attention as a potential site of action for lithium in the treatment of bipolar disorder. However, recent studies have identified a number of other lithium targets, as well [79].

Three forms of inositol monophosphatase have been identified: A1 (IMPA1), A2 (IMPA2) and A3 (IMPA3). Of these three forms, only inositol monophosphatase A1 contains an identifiable D-domain (Tables 6 and 8). The human and mouse sequences differ by two amino acids in this region resulting in an alternative pair of D-domains in the mouse isoform (and <sup>261</sup>RIAKEIEI and <sup>261</sup>RIAKEIEIIPL) and a single D-domain in the human isoform (<sup>261</sup>RIAKEIQVIPL). We did not find an optimal sequence for MAPK-catalyzed phosphorylation or an FXFP motif in

any of the human or mouse inositol monophosphatase isozymes.

### 1-phosphatase

Inositol polyphosphate 1-phosphatase is a  $Mg^{2+}$ -dependent enzyme that hydrolyzes  $Ins(1,4)P_2$  and  $Ins(1,3,4)P_3$ , and is inhibited by lithium [76,80-82]. Similar to inositol monophosphatase, the 1-phosphatase has received attention as a site of therapeutic action for lithium in the treatment of bipolar disorder [83]. Notably, the human (Table 6), but not the mouse (Table 9), 1-phosphatase contains a D-domain. Neither the human nor mouse enzyme contains an FXFP motif or an optimal sequence for MAPK phosphorylation.

### 3-phosphatase

Myotubularin, which was originally identified as a candidate gene mutated in X-linked myotubular myopathy [84], has been shown to possess PtdIns3P 3-phosphatase activity [85,86]. A number of myotubularin-related (MTMR) proteins have also been identified [87,88]. Myotubularin (also called MTM1), MTMR1, MTMR2, MTMR3, and MTMR6 possess PtdIns(3)P 3-phosphatase activity [89]. In addition to their PtdIns3P 3-phosphatase activity, myotubularin, [90], MTMR2 [91], MTMR3 [92], and MTMR6 [90] also possess PtdIns(3,5)P<sub>2</sub> 3-phosphatase activity; however, it should be noted that Kim et al. [89] reported that myotubularin and MTMR2 do not hydrolyze PtdIns(3,5)<sub>2</sub>. Additionally, myotubularin and MTMR2 have been shown to hydrolyze  $Ins(1,3)P_2$  [89].

When we analyzed the human (Table 7) and mouse (Table 9) myotubularin protein sequences, we did not find either an FXFP or D-domain sequence. However, the human sequence does contain two optimal MAPK phosphorylation sequences not present in the mouse sequence. We identified a conserved D-domain in the human (Table 6) and mouse (Table 8) MTMR1 protein, but did not find an optimal sequence for MAPK phosphorylation or FXFP motif in either protein. Neither the human (Table 7) nor the mouse (Table 9) MTMR2 has an optimal sequence for MAPK phosphorylation, a D-domain, or an FXFP motif. Although the human (Table 7) and mouse (Table 9) MTMR3 and MTMR6 each contain a sequence fitting the optimal sequence for MAPK phosphorylation, they do not contain an identifiable D-domain or FXFP motif.

PTEN (phosphatase and tensin homologue deleted on chromosome 10) is a PtdIns(3,4,5)P<sub>3</sub> 3-phosphatase [93], as well as  $Ins(1,3,4,5)P_4$  3-phosphatase [93] and  $Ins(1,3,4,5,6)P_5$  3-phosphatase [94]. The human (Table 6) and mouse (Table 8) PTEN sequences contain a conserved FXFP sequence, but no identifiable D-domain or optimal MAPK phosphorylation sequence.

### 4-phosphatase

Two forms (Types I and II) of inositol polyphosphate 4-phosphatase have been identified [95-97]. These enzymes cleave the 4-phosphate from  $Ins(1,3,4)P_2$ ,  $Ins(3,4)P_2$ , and PtdIns(3,4)P<sub>2</sub>. The Type I 4-phosphatase has been reported to localize to endosomes, where it plays an important role in the generation of PtdIns3P [98]. In growth factor-stimulated cells the Type I 4-phosphatase also localizes to plasma membrane ruffles, where it hydrolyzes PtdIns(3,4)P<sub>2</sub>, thereby regulating the association of PtdIns(3,4)P<sub>2</sub>-binding proteins with the plasma membrane [98]. We identified a conserved D-domain in human (Table 6) and mouse (Table 8) Type I 4-phosphatase. However, we did not identify an optimal phosphorylation sequence or an FXFP motif in this isoform. In contrast, we did not identify a D-domain in the human Type II 4-phosphatase (Table 7), although it does contain a consensus MAPK phosphorylation site. Human Type II 4-phosphatase also does not contain an FXFP motif.

The Sac phosphatase domain, a region of sequence homology found in several yeast, plant and animal proteins, can hydrolyze the 3-, 4-, or 5-position phosphate from PIs, although vicinal phosphate groups are resistant to hydrolysis [99,100]; thus, PtdIns3P, PtdIns4P and PtdIns(3,5)P<sub>2</sub> are substrates, whereas PtdIns(4,5)P<sub>2</sub> is not. The PtdIns4P phosphatase activity, but not PtdIns3P or PtdIns(3,5)P<sub>2</sub> phosphatase activities, of mammalian Sac1 complements phenotypic defects observed in yeast having deletions of Sac1p [101], indicating that the 4-phosphatase activity of these proteins is the most important *in vivo*. The human (Table 6) and mouse (Table 8) Sac1 proteins each contain five identifiable D-domains. Two of these D-domains are overlapping sequences (<sup>345</sup>KNMRWDRLSI and <sup>348</sup>RWDRLSILL) and two are an alternative pair of D-domains (<sup>517</sup>RDWKFLAL and <sup>517</sup>RDWKFLALPI). Neither the human nor mouse protein contains an optimal phosphorylation site for MAPKs or an FXFP motif.

### 5-phosphatase

The inositol polyphosphate 5-phosphatases are commonly classified on the basis of their substrate specificities [27,50]. In this system of classification, the Group I enzymes hydrolyze the water-soluble compounds  $Ins(1,4,5)P_3$  and  $Ins(1,3,4,5)P_4$ ; the Group II enzymes hydrolyze both water-soluble and lipid substrates (e.g., PtdIns(4,5)P<sub>2</sub> and PtdIns(3,4,5)P<sub>3</sub>); the Group III enzymes hydrolyze the 3-phosphate-containing compounds,  $Ins(1,3,4,5)P_4$  and PtdIns(3,4,5)P<sub>3</sub>; and, the single Group IV enzyme hydrolyzes only the lipid substrates PtdIns(3,4,5)P<sub>3</sub> and PtdIns(4,5)P<sub>2</sub> [27,102]. Although we have used this classification system, we note that recent studies have demonstrated that the substrate specificities of several of the identified 5-phosphatases do not fit into

**Table 8: Phosphatases (mouse) that have an FXFP motif and/or D-domain sequence. The number of potential MAPK phosphorylation sites, sequences fitting the optimal MAPK phosphorylation consensus sequence of PX(S/T)P, and FXFP motifs and D-domain sequences in phosphatases that use phosphatidylinositols or inositols as substrates are listed in the table; all sequences are for mouse proteins.**

Isozyme	GenBank Accession #	# amino acids	# (S/T)P sites	Site(s) with the consensus PX(S/T)P sequence	FXFP motif	D-domain motif
<i>monophosphatase</i>						
inositol monophosphatase A1	<a href="#">Q55023</a>	277	2	none	none	<sup>261</sup> RIAKEIEI <sup>261</sup> RIAKEIEIPL
<i>3-phosphatase</i>						
Myotubularin-related protein I; MTMR1	<a href="#">NP_058681</a>	669	4	none	none	<sup>181</sup> KDMRNLRL
PI(3,4,5)P <sub>3</sub> 3-phosphatase, PTEN	<a href="#">Q08586</a>	403	2	none	<sup>241</sup> FEFP	none
<i>4-phosphatase</i>						
Inositol polyphosphate 4-phosphatase, Type Ia	<a href="#">NP_084542</a>	939	7	none	none	<sup>147</sup> RHRLHRTL
SAC1 poly-phosphatidylinositol phosphatase	<a href="#">CAC20672</a>	587	3	none	none	<sup>345</sup> KNMRWDRLSI <sup>348</sup> RWDRLSILL <sup>461</sup> RTGKRTQLGL <sup>517</sup> RDWKFLAL <sup>517</sup> RDWKFLALPI
<i>5-phosphatase</i>						
<i>Group II</i>						
Inositol polyphosphate 5-phosphatase OCRL	<a href="#">NP_796189</a>	900	2	none	none	<sup>445</sup> KLLKFDGLNI
Synaptojanin 2 (synaptic inositol 1,4,5-trisphosphate 5-phosphatase 2)	<a href="#">Q9D2G5</a>	1434	15	<sup>987</sup> PVSP	none	<sup>617</sup> RSHRYILL
Sac-domain-containing inositol phosphatase 3	<a href="#">NP_598760</a>	907	5	none	none	<sup>663</sup> KFHRWEEIIDI
Inositol polyphosphate 5-phosphatase, 75 kDa; Inositol polyphosphate 5-phosphatase B	<a href="#">NP_032411</a>	993	5	none	none	<sup>31</sup> RQSRLGL <sup>420</sup> KFVRLVGIML
<i>Group III</i>						
Inositol polyphosphate 5-phosphatase, 145 kDa; inositol polyphosphate 5-phosphatase D; SH2-containing inositol 5'-phosphatase I; SHIP1	<a href="#">NP_034696</a>	1191	12	<sup>962</sup> PPTP <sup>970</sup> PLSP	none	<sup>352</sup> KSQKFLNKLVI
<i>Group IV</i>						
PI polyphosphate 5-phosphatase type IV; Inositol polyphosphate 5-phosphatase E	<a href="#">Q9JIII</a>	647	7	<sup>243</sup> PRSP	none	<sup>83</sup> KLERTLSL <sup>599</sup> RPGRDNIPL <sup>611</sup> KFDRELYL <sup>611</sup> KFDRELYLIGI

this simple system [103,104]. For example, Schmid et al [103] have shown that there are significant differences in the substrate specificities of several "Type II" 5-phosphatases: synaptojanin 1, synaptojanin 2, the gene product responsible for Lowe's oculocerebrorenal syndrome (OCRL), skeletal muscle and kidney enriched phosphatase (SKIP), and INPP5B. Additionally, it should be noted that the *in vitro* and *in vivo* specificities of these enzymes may differ.

We did not identify a D-domain, FXFP motif or optimal MAPK phosphorylation sequence in either the human (Table 7) or mouse (Table 9) Type I 5-phosphatase. Sev-

eral of the Group II 5-phosphatases (Tables 6 and 8) contain an identifiable D-domain. OCRL is the gene responsible for oculocerebrorenal dystrophy or Lowe's syndrome, when mutated [27]. Both the human (Table 6) and mouse (Table 8) OCRL proteins contain a D-domain, but do not contain an FXFP motif or an optimal sequence for MAPK phosphorylation. Synaptojanin 1 and synaptojanin 2 are neuronal proteins that play a role in synaptic vesicle trafficking. They contain both a 5-phosphatase domain and a Sac phosphatase domain [99]. The 5-phosphatase domain is responsible for the reported PtdIns(4,5)P<sub>2</sub>-hydrolyzing activity of synaptojanins, while the Sac domain of synaptojanins accounts for their

ability to also hydrolyze other PIs, such as the PtdIns4P product generated by the action of its 5-phosphatase domain [105]. Both synaptojanin 1 and 2 contain optimal phosphorylation sites for MAPKs. However, only synaptojanin 2 contains a D-domain, which is conserved in the human (Table 6) and mouse (Table 8) proteins. The synaptojanin sequences that we searched do not contain an FXFP motif. The human (Table 6) and mouse (Table 8) 75-kDa inositol polyphosphate 5-phosphatases (inositol polyphosphate 5-phosphatase B) contain two conserved D-domains, but are devoid of an FXFP motif and an optimal sequence for MAPK phosphorylation.

The Group III SH2-containing inositol 5'-phosphatase 1 (SHIP1) is a hematopoietic-specific enzyme that hydrolyzes both PtdIns(3,4,5)<sub>3</sub> and Ins(1,3,4,5)P<sub>4</sub> [106]. In addition, SHIP1 is able to hydrolyze the 4-phosphate from PtdIns(4,5) *in vitro*, thereby generating PtdIns5P [107]. The human (Table 6) and mouse (Table 8) SHIP1 contain an identifiable D-domain. The human SHIP1 contains four optimal MAPK phosphorylation sequences; corresponding sequences for two of these are present in the mouse protein, whereas two are unique to the human protein. SHIP1 does not contain a sequence conforming to an FXFP motif. The distribution of SHIP2 is more ubiquitous than is that of SHIP1 [106]. The human SHIP2 is devoid of a D-domain or an FXFP motif (Table 7). It does contain four sequences that fit the optimal phosphorylation sequence for a MAPK. The human (Table 6) and mouse (Table 8) Group IV 5-phosphatase contain a conserved optimal sequence (PRSP) for MAPK phosphorylation; the human protein contains an additional optimal sequence of MAPK phosphorylation (<sup>55</sup>PATP). Both the human and mouse Group IV 5-phosphatase contain an alternative pair of D-domains, as well as two other D-domains; neither contains an FXFP motif.

#### Additional sequence analyses

We examined each of the D-domains that we identified to determine if it overlaps with a KIM [14] or fits the MAPK-docking site consensus sequence defined by Bardwell and colleagues [15,16]. We found only one instance of overlap with a KIM: in the human PI 4-kinase  $\alpha$ , the sequence <sup>12</sup>LDERRRDAVIALGI not only contains the alternative pair of D-domains that we identified (Table 1), but also contains a KIM. Examination of the sequences found in Tables 1, 3, 6, and 8 revealed that, in several instances, the D-domain sequence fits the MAPK-docking sequence of Bardwell and colleagues. It should be noted that in several proteins we identified, but did not catalog, one or more sequence that conformed to the MAPK-docking motif of Bardwell and colleagues but did not conform to the more restrictive sequence that we used for a D-domain.

Finally, we also analyzed the sequences of PtdIns synthase (CDP-1,2-diacyl-*sn*-glycerol:*myo*-inositol 3-phosphatidyltransferase) isozymes, which catalyze the production of PtdIns from cytidine diphosphodiacylglycerol and *myo*-inositol. We did not identify either an FXFP or a D-domain sequence in human or mouse PtdIns synthase sequences (GenBank:NP\_006310, NP\_665695, and NP\_620093; data not shown). Further, we identified a MAPK phosphorylation site only in the human isoform 2. These results indicate that the isozymes that catalyze PtdIns synthesis are not likely to be directly regulated by MAPK; however, they do not rule out the possibility that MAPKs may control PtdIns synthesis via an effect on *myo*-inositol (e.g., via an effect on inositol monophosphatase A1) or cytidine diphosphodiacylglycerol levels.

#### Conclusion

We found a high degree of conservation of D-domain sequence and location in human and mouse proteins. Notable exceptions were PI 3-kinase C2- $\gamma$ , PI 4-kinase type II $\beta$ , and inositol polyphosphate 1-phosphatase. For each of these proteins, either the human or mouse protein contained one or more D-domains that were not found in the other, with the differences in PI 3-kinase C2- $\gamma$  being the most striking.

Other than within subtypes of an isozyme, we found no evidence of sequence conservation of D-domains in enzymes that metabolize PIs and IPs. That is, the primary sequences of the D-domains that we found were quite variable. This indicates that, both within a family and across families of these enzymes, there may be specificity for binding interactions with MAPKs. Detailed studies examining the relative specificities of each of these proteins for individual MAPKs, if they indeed bind MAPKs, and the contribution of individual amino acids within these sequences to MAPK binding, will provide important information for the development of tools aimed at modifying the integration of MAPK and signaling via PIs and IPs.

When D-domains were found in more than one location in a protein, each D-domain had a unique sequence. This is noted because it is not a universal property of protein-protein interaction domains when present in multiples in a protein- e.g., an SH<sub>2</sub>-binding motif is commonly present in multiple copies in docking proteins [108]. Within a protein, it is possible that each D-domain binds a unique MAPK, or set of MAPKs, allowing for interaction with more than one MAPK signaling pathway, and, thus, the regulation of the metabolism of a particular PI/IP by various combinations of stimuli, or the formation of differing combinations of signaling complexes allowing for the generation of various downstream signals. Further, it is also possible that each interaction is independently regulated. Therefore, it is reasonable to think that these D-

**Table 9: Phosphatases (mouse) that do not have an FXFP motif and/or D-domain sequence. Phosphatases that use phosphatidylinositols or inositols as substrates and do not contain an FXFP or D-domain sequence are listed in the table; all sequences are for mouse proteins. The number of potential MAPK phosphorylation sites and sequences fitting the optimal MAPK phosphorylation in these kinases are also identified.**

Isozyme	GenBank Accession #	# amino acids	# (S/T)P sites	Site(s) with the consensus PX(S/T)P sequence
inositol monophosphatase A2	<a href="#">NP_444491</a>	290	1	none
inositol monophosphatase A3	<a href="#">NP_808398</a>	356	2	none
Inositol polyphosphate 1-phosphatase	<a href="#">P49442</a>	396	2	none
Myotubularin; MTM1	<a href="#">Q9Z2C5</a>	603	1	none
Myotubularin-related protein 2; MTMR2;	<a href="#">Q9Z2D1 (AAH63050)</a>	643	4	none
Myotubularin-related protein 3; MTMR3	<a href="#">CAI35186</a>	1159	7	582PSTP
Myotubularin-related protein 6; MTMR6	<a href="#">NP_659092</a>	617	3	555PETP
Inositol polyphosphate 4-phosphatase, Type Ib (variant)	<a href="#">NP_766559</a>	679	3	none
Inositol 1,4,5-trisphosphate 5-phosphatase, Type I	<a href="#">AAH56341</a>	412	2	none
Synaptojanin 1 (synaptic inositol 1,4,5-trisphosphate 5-phosphatase I)	<a href="#">Q8CHC4</a>	1574	19	1145PPSP 1158PKSP 1443PNSP
PI(4,5)P <sub>2</sub> 5-phosphatase A; PIB5PA; PIPP	<a href="#">NP_766027</a>	1003	31	197PQSP 200PSSP 346PRSP
Skeletal muscle and kidney enriched inositol phosphatase isoform I; SKIP 1	<a href="#">Q8C5L6</a>	468	5	none
Phospholipid-inositol phosphatase; PTEN-like phosphatase; PLIP	<a href="#">NP_079852</a>	261	3	none

domains do not simply serve to amplify signaling through a single signaling pathway, but, instead, allow for the integration of multiple MAPK pathways with the metabolism of a specific PI or IP.

The frequency of occurrence of an identifiable FXFP motif in the enzymes that we analyzed was significantly less than that of a D-domain. There were only five enzymes in which we found a motif conforming to the sequence FXFP; four of these were in kinases (PI 3-kinase  $\delta$ , PI 4-kinase  $\alpha$ , Ins(1,4,5)P<sub>3</sub> 3-kinase B, and Ins(1,3,4,5,6) 2-kinase) and only a single one was found in a phosphatase (PTEN). It is noteworthy that of all the enzymes that we analyzed, only PI 4-kinase  $\alpha$  and Ins(1,4,5)P<sub>3</sub> 3-kinase B contain both a D-domain and an FXFP sequence. Finally, we found several sequences fitting an FXP motif (data not shown). As noted in the Introduction, the FXP sequence has been reported to be sufficient for MAPK binding, indicating that these sites may also bind MAPKs.

At this time, we can only speculate on the physiologic significance of the presence of MAPK binding domains in enzymes that control the metabolism of PIs and IPs. We have previously shown that phospho-ERK2-dependent phosphorylation of PLC- $\gamma$ 1 opposes tyrosine kinase-dependent activation of PLC- $\gamma$ 1 [25]. Similarly, MAPKs may regulate (either stimulating or inhibiting) the catalytic activity, or specificity, of kinases and phosphatases that are involved in the metabolism of PIs or IPs, and thereby exert regulatory actions on PI- and/or IP-depend-

ent signaling pathways. Intriguing possibilities exist when a kinase and phosphatase are present in the same complex and one or both of them bind a MAPK. For example, the p85 subunit of Class I PI 3-kinase has been reported to form a complex with Type I inositol polyphosphate 4-phosphatase [109], SHIP1 5-phosphatase [110], and Type IV 5-phosphatase [111]. In these complexes, MAPKs may regulate the relative level or turnover of the substrates and products; for example, by enhancing PI3-kinase activity and associated SHIP1 (or Type IV) 5-phosphatase activity, it would be possible to increase PtdIns(3,4)P<sub>2</sub> levels without increasing PtdIns(3,4,5)P<sub>3</sub> levels. Other scenarios (e.g., delayed activation kinetics of the associated 5-phosphatase) are also imaginable, resulting in a transient rise in PtdIns(3,4,5)P<sub>3</sub> with a delayed elevation in PtdIns(3,4)P<sub>2</sub> levels. Similarly, complexes of the Type I inositol polyphosphate 4-phosphatase and PI 3-kinase could produce locally elevated levels of PtdIns3P, without elevating PtdIns(3,4)P<sub>2</sub> levels, or elevate PtdIns(3,4)P<sub>2</sub> and, with a delay, PtdIns3P. It is also possible that the interaction between a MAPK and a PI/IP kinase or phosphatase may recruit the MAPK to a multimolecular signal transduction complex containing components of pathways that regulate the activity of the MAPK (e.g., binding of a MAPK to PI 3-kinase may act to recruit the MAPK to a growth factor signaling complex containing Ras and a MAPK kinase) or target the MAPK to a particular subcellular localization (e.g., binding to Type I inositol polyphosphate 4-phosphatase may act to target the MAPK to endosomes).

It should be noted that a protein can serve as a MAPK substrate without directly binding the MAPK: c-Jun-bound proteins that lack a JNK binding site can be phosphorylated by JNK [112]. Thus, our inability to identify a MAPK binding site in a protein does not preclude it from being a MAPK substrate. For example, the MAPK optimal phosphorylation site identified in Type II $\alpha$  PIPK could be phosphorylated by a MAPK bound to Type I $\alpha$  PIPK, which has been shown to co-immunoprecipitate with Type II $\alpha$  PIPK [113]. Similarly, although we did not identify a D-domain or FXFP motif in the 3-phosphatase myotubularin, it may be a MAPK substrate when bound to the 3-phosphatase adaptor protein (3-PAP) subunit [114], which has four recognizable D-domains (data not shown). In fact, human myotubularin does contain two optimal MAPK phosphorylation sequences (Table 7), indicating that it may be a MAPK substrate.

PI 3-kinase, Ins(1,4,5)P<sub>3</sub> 3-kinase B, SHIP1 and PTEN have each been proposed to regulate MAPK signaling. In the case of PI 3-kinase, several studies have been published showing that wortmannin and/or LY-294002, which are inhibitors of PI 3-kinase catalytic activity, block the activation of MAPKs by various stimuli. However, to our knowledge, a direct interaction between a PI 3-kinase and a MAPK has not been demonstrated, and the mechanisms underlying the apparent PI 3-kinase-dependent regulation of MAPKs remain speculative. In the case of PI 3-kinase  $\gamma$ , the effect could be mediated by MEK-1, a MAPK kinase which has been shown to be an *in vitro* substrate of PI 3-kinase  $\gamma$  [115]. Wen et al [65] have shown that ERK1/2 activation in response to suboptimal stimulation of thymocytes is dependent on Ins(1,4,5)P<sub>3</sub> 3-kinase B; they propose a model in which Ins(1,4,5)P<sub>3</sub> 3-kinase B-dependent production of Ins(1,3,4,5)P<sub>4</sub> acts to sequester an Ins(1,3,4,5)P<sub>4</sub>-binding GTPase-activating protein 1 [116], promoting Ras-dependent activation of ERK1/2. SHIP1, which contains an identifiable D-domain, but no FXFP motif, has been shown to be a negative regulator of JNK activation in B cells [117], ERK1/2 activation in the erythropoietin-dependent cell line AS-E2 [118], and MAPK (ERK1/2, JNK and p38) activation in RAW264.7 macrophages [119]. In the latter case, the action was shown to be independent of the SHIP1 5-phosphatase activity [119].

Interestingly, SHIP2, which does not have an identifiable D-domain or FXFP motif, has been reported to not exert an effect on cellular MAPKs [120,121]. PTEN, which does not contain a D-domain sequence but does contain an FXFP motif, has been reported to inhibit insulin-stimulated ERK1/2 activation in MCF-7 epithelial breast cancer cells [122,123]. Weng et al. [122] concluded that the effect of PTEN is the result of PTEN-dependent dephosphorylation of the insulin receptor substrate 1, and consequent

coupling to ERK1/2 activation. In contrast to the studies of Eng and colleagues, Tang et al. [121] reported that short interfering RNA-induced reductions of PTEN expression in 3T3-L1 adipocytes did not affect insulin-dependent signaling to ERK1/2.

In conclusion, there appear to be a plethora of potential sites of crosstalk between MAPK signaling pathways and the enzymes controlling cellular PIs and IPs, and, thus, the signaling pathways that are regulated by the levels of these intracellular signals. We hope that the identification of these sites of signal integration will initiate a series of studies aimed at determining whether these interactions occur *in vivo* and the physiologic relevance of each to cellular responding.

### Competing interests

The author(s) declare that they have no competing interests

### Authors' contributions

Kevin Caldwell was involved in the conception of these studies, collecting and analyzing sequence data, and drafting and revising the manuscript. Marcos Sosa was responsible for collecting and analyzing sequence data, as well as reviewing the manuscript. Colin Buckley was involved in the conception of these studies, collecting and analyzing sequence data, and reviewing the manuscript. All authors have given final approval of the manuscript.

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### References

- Clark-Lewis I, Sanghera JS, Pelech SL: **Definition of a consensus sequence for peptide substrate recognition by p44<sup>mpk</sup>, the meiosis-activated myelin basic protein kinase.** *J Biol Chem* 1991, **266**:15180-15184.
- Songyang Z, Lu KP, Kwon YT, Tsai L-H, Filhol O, Cochet C, Brickey DA, Soderling TR, Bartleson C, Graves DJ, DeMaggio AJ, Hoekstra MF, Blenis J, Hunter T, Cantley LC: **A structural basis for substrate specificities of protein Ser/Thr kinases: primary sequence preference of casein kinases I and II, NIMA, phosphorylase kinase, calmodulin-dependent kinase II, CDK5, and Erk1.** *Mol Cell Biol* 1996, **16**:6486-6493.
- Gonzalez FA, Raden DL, Davis RJ: **Identification of substrate recognition determinants for human ERK1 and ERK2 protein kinases.** *J Biol Chem* 1991, **266**:22159-22163.
- Jacobs D, Glossip D, Xing H, Muslin AJ, Kornfeld K: **Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase.** *Genes Dev* 1999, **13**:163-175.
- Yang S-H, Whitmarsh AJ, Davis RJ, Sharrocks AD: **Differential targeting of MAP kinases to the ETS-domain transcription factor Elk-1.** *EMBO J* 1998, **17**:1740-1749.
- Yang S-H, Galanis A, Sharrocks AD: **The Elk-1 ETS-Domain Transcription Factor Contains a Mitogen-Activated Protein Kinase Targeting Motif.** *Mol Cell Biol* 1999, **19**:4028-4038.
- Seidel JJ, Graves BJ: **An ERK2 docking site in the Pointed domain distinguishes a subset of ETS transcription factors.** *Genes Dev* 2002, **16**:127-137.

8. Adler V, Franklin CC, Kraft AS: **Phorbol esters stimulate the phosphorylation of c-Jun but not v-Jun: regulation by the N-terminal  $\delta$  domain.** *Proc Natl Acad Sci USA* 1992, **89**:5341-5345.
9. Hibi M, Lin A, Smeal T, Minden A, Karin M: **Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain.** *Genes Dev* 1993, **7**:2135-2148.
10. Gupta S, Campbell D, Derijard B, Davis RJ: **Transcription factor ATF2 regulation by the JNK signal transduction pathway.** *Science* 1995, **267**:389-393.
11. Livingstone C, Patel G, Jones N: **ATF-2 contains a phosphorylation-dependent transcriptional activation domain.** *EMBO J* 1995, **14**:1785-1797.
12. Yang S-H, Yates PR, Whitmarsh AJ, Davis RJ, Sharrocks AD: **The Elk-1 ETS-Domain Transcription Factor Contains a Mitogen-Activated Protein Kinase Targeting Motif.** *Mol Cell Biol* 1998, **18**:710-720.
13. Pulido R, Zúñiga A, Ullrich A: **PTP-SL and STEP protein tyrosine phosphatases regulate the activation of the extracellular signal-regulated kinases ERK1 and ERK2 by association through a kinase interaction motif.** *EMBO J* 1998, **17**:7337-7350.
14. MacKenzie SJ, Baillie GS, McPhee I, Bolger GB, Houslay MD: **ERK2 mitogen-activated protein kinase binding, phosphorylation, and regulation of the PDE4D cAMP-specific phosphodiesterases. The involvement of COOH-terminal docking sites and NH<sub>2</sub>-terminal UCR regions.** *J Biol Chem* 2000, **275**:16609-16617.
15. Bardwell AJ, Flatauer LJ, Matsukuma K, Thorne J, Bardwell L: **A conserved docking site in MEKs mediates high-affinity binding to MAP kinases and cooperates with a scaffold protein to enhance signal transmission.** *J Biol Chem* 2001, **276**:10374-10386.
16. Bardwell AJ, Abdollahi M, Bardwell L: **Docking sites on mitogen-activated protein kinase (MAPK) kinases, MPK phosphatases and the Elk-1 transcription factor compete for MAPK binding and are crucial for enzymatic activity.** *Biochem J* 2003, **370**:1077-1085.
17. Ho DT, Bardwell J, Abdollahi M, Bardwell L: **A docking site in MKK4 mediates high affinity binding to JNK MAPKs and competes with similar docking sites in nJNK substrates.** *J Biol Chem* 2003, **278**:32662-32672.
18. Fantz DA, Jacobs D, Glossip D, Kornfeld K: **Docking sites on substrate proteins direct extracellular signal-regulated kinase to phosphorylate specific residues.** *J Biol Chem* 2001, **276**:27256-27265.
19. Tanoue T, Nishida E: **Molecular recognitions in the MAP kinase cascade.** *Cell Signal* 2003, **15**:455-462.
20. Biondi RM, Nebreda AR: **Signaling specificity of Ser/Thr protein kinases through docking-site-mediated interactions.** *Biochem J* 2003, **372**:1-13.
21. Barsyte-Lovejoy D, Galanis A, Sharrocks AD: **Specificity determinants in MAPK signaling to transcription factors.** *J Biol Chem* 2002, **277**:9896-9903.
22. Galanis A, Yang S-H, Sharrocks AD: **Selective targeting of MAPKs to the ETS domain transcription factor SAP-1.** *J Biol Chem* 2001, **276**:965-973.
23. Smith JA, Potet-Smith CE, Malarkey K, Sturgill T: **Identification of an extracellular signal-regulated kinase (ERK) docking site in ribosomal S6 kinase, a sequence critical for activation by ERK in vivo.** *J Biol Chem* 1999, **274**:2893-2898.
24. Vinciguerra M, Vivacqua A, Fasanella G, Gallo A, Cuzzo C, Morano A, Maggolini M, Musti AM: **Differential phosphorylation of c-Jun and JunD in response to the epidermal growth factor is determined by the structure of MAPK targeting sequences.** *J Biol Chem* 2004, **279**:9634-9641.
25. Buckley CT, Sekiya F, Kim YJ, Rhee SG, Caldwell KK: **Identification of phospholipase C- $\gamma$ 1 as a mitogen-activated protein kinase substrate.** *J Biol Chem* 2004, **279**:41807-41814.
26. Buckley CT, Caldwell KK: **Fear conditioning is associated with altered integration of PLC and ERK signaling in the hippocampus.** *Pharmacol Biochem Behav* 2004, **79**:633-640.
27. Majerus PV, Kisseleva MV, Norris FA: **The role of phosphatases in inositol signaling reactions.** *J Biol Chem* 1999, **274**:10669-10672.
28. Toker A: **Phosphoinositides and signal transduction.** *Cell Mol Life Sci* 2002, **59**:761-779.
29. Parker PJ: **The ubiquitous phosphoinositides.** *Biochem Soc Trans* 2004, **32**:893-898.
30. Irvine RF, Schell MJ: **Back in the water: the return of the inositol phosphates.** *Nat Rev Mol Cell Biol* 2001, **2**:327-338.
31. Rhee SG: **of phosphoinositide-specific phospholipase C.** *Annu Rev Biochem* 2001, **70**:281-312.
32. Cantley LC: **The phosphoinositide 3-kinase pathway.** *Science* 2002, **296**:1655-1657.
33. De Matteis MA, Godi A, Corda D: **Phosphoinositides and the Golgi complex.** *Curr Opin Cell Biol* 2002, **14**:434-447.
34. Janmey PA, Lindberg U: **Cytoskeletal regulation: rich in lipids.** *Nat Rev Mol Cell Biol* 2004, **5**:658-666.
35. Niggli V: **Regulation of protein activities by phosphoinositide phosphates.** *Annu Rev Cell Dev Biol* 2005, **21**:57-79.
36. Shears SB: **How versatile are inositol phosphate kinases?** *Biochem J* 2004, **377**:265-280.
37. Irvine RF: **Inositide evolution- toward turtle domination?** *J Physiol* 2005, **566**:295-300.
38. Chan TO, Rittenhouse SE, Tsichlis PN: **AKT/PKB and other D3 phosphoinositide-regulated kinases: Kinase activation by phosphoinositide-dependent phosphorylation.** *Annu Rev Biochem* 1999, **68**:965-1014.
39. Deane JA, Fruman DA: **Phosphoinositide 3-kinase: Diverse roles in immune cell activation.** *Annu Rev Immunol* 2004, **22**:563-598.
40. Whitman M, Downes CP, Keeler M, Keller T, Cantley L: **Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate.** *Nature* 1988, **332**:644-646.
41. Carpenter CL, Duckworth BC, Auger KR, Cohen B, Schaffhausen BS, Cantley LC: **Purification and characterization of phosphoinositide 3-kinase from rat liver.** *J Biol Chem* 1990, **265**:19704-19711.
42. Stephens LR, Hughes KT, Irvine RF: **Pathway of phosphatidylinositol(3,4,5)-trisphosphate synthesis in activated neutrophils.** *Nature* 1991, **351**:33-39.
43. Domin J, Pages F, Volinia S, Rittenhouse SE, Zvelebil MJ, Stein RC, Waterfield MD: **Cloning of a human phosphoinositide 3-kinase with a C2 domain that displays reduced sensitivity to the inhibitor wortmannin.** *Biochem J* 1997, **326**:139-147.
44. Volinia S, Dhand R, Vanhaesebroeck B, MacDougall LK, Stein R, Zvelebil MJ, Domin J, Panaretou C, Waterfield MD: **A human phosphatidylinositol 3-kinase complex related to the yeast Vps34p-Vps15p protein sorting system.** *EMBO J* 1995, **14**:3339-3348.
45. Heilmeyer LMG Jr, Verb G Jr, Verb G, Kakuk A, Szivák I: **Mammalian phosphatidylinositol 4-kinases.** *IUBMB Life* 2003, **55**:59-65.
46. Berditchevski F, Tolias KF, Wong K, Carpenter CL, Hemler ME: **A novel link between integrins, transmembrane-4 superfamily proteins (CD63 and CD81), and phosphatidylinositol 4-kinase.** *J Biol Chem* 1997, **272**:2595-2598.
47. Balla A, Tuymetova G, Barshishat M, Geiszt M, Balla T: **Characterization of type II phosphatidylinositol 4-kinase isoforms reveals association of the enzymes with endosomal vesicular compartments.** *J Biol Chem* 2002, **277**:20041-20050.
48. Godi A, Pertile P, Meyers R, Marra P, Di Tullio G, Iurisci C, Luini A, Corda D, De Matteis MA: **ARF mediates recruitment of PtdIns-4-OH kinase- $\beta$  and stimulates synthesis of PtdIns(4,5)P<sub>2</sub> on the Golgi complex.** *Nat Cell Biol* 1999, **1**:280-287.
49. Gehrman T, Heilmeyer LM Jr: **Phosphatidylinositol 4-kinases.** *Eur J Biochem* 1998, **253**:357-370.
50. Zhang X, Majerus PV: **Phosphatidylinositol signaling reactions.** *Semin Cell Dev Biol* 1998, **9**:153-160.
51. Doughman RL, Firestone AJ, Anderson RA: **Phosphatidylinositol phosphate kinases put PI4,5P<sub>2</sub> in its place.** *J Membrane Biol* 2003, **194**:77-89.
52. Ishihara H, Shibasaki Y, Kizuki N, Katagiri H, Yazaki Y, Asano T, Oka Y: **Cloning of cDNAs encoding two isoforms of 68-kDa type I phosphatidylinositol-4-phosphate 5-kinase.** *J Biol Chem* 1996, **271**:23611-23614.
53. Loijens JC, Anderson RA: **Type I phosphatidylinositol-4-phosphate 5-kinases are distinct members of this novel lipid kinase family.** *J Biol Chem* 1996, **271**:32937-32943.
54. Ishihara H, Shibasaki Y, Kizuki N, Wada T, Yazaki Y, Asano T, Oka Y: **Type I phosphatidylinositol-4-phosphate 5-kinases. Cloning of the third isoform and deletion/substitution analysis of members of this novel lipid kinase family.** *J Biol Chem* 1998, **273**:8741-8748.



55. Itoh T, Ijuin T, Takenawa T: **A novel phosphatidylinositol-5-phosphate 4-kinase (phosphatidylinositol-phosphate kinase II) is phosphorylated in the endoplasmic reticulum in response to mitogenic signals.** *J Biol Chem* 1998, **273**:20292-20299.
56. Lee SY, Voronov S, Letinic K, Nairn AC, Di Paolo G, De Camilli P: **Regulation of the interaction between PIPK $\gamma$  and talin by proline-directed protein kinases.** *J Cell Biol* 2005, **168**:789-799.
57. Dewaste V, Moreau C, De Smedt F, Bex F, De Smedt H, Wuytack F, Missiaen L, Erneux C: **The three isoenzymes of human inositol 1,4,5-trisphosphate 3-kinase show specific intracellular localization but comparable Ca<sup>2+</sup> responses on transfection in COS-7 cells.** *Biochem J* 2003, **374**:41-49.
58. Choi KY, Kim HK, Lee SY, Moon KH, Sim SS, Kim JW, Chung HK, Rhee SG: **Molecular cloning and expression of a complementary DNA for inositol 1,4,5-trisphosphate 3-kinase.** *Science* 1990, **248**:64-66.
59. Vanweyenberg V, Communi D, D'Santos CS, Erneux C: **Tissue- and cell-specific expression of Ins(1,4,5)P<sub>3</sub> 3-kinase isoenzymes.** *Biochem J* 1995, **306**:429-435.
60. Dewaste V, Pouillon V, Moreau C, Shears S, Takazawa K, Erneux C: **Cloning and expression of a cDNA encoding human inositol 1,4,5-trisphosphate 3-kinase C.** *Biochem J* 2000, **352**:343-351.
61. Dewaste V, Roymans D, Moreau C, Erneux C: **Cloning and expression of a full-length cDNA encoding human inositol 1,4,5-trisphosphate 3-kinase B.** *Biochem Biophys Res Commun* 2002, **291**:400-405.
62. Nalaskowski MM, Bertsch U, Fanick W, Stockebrand MC, Schmale H, Mayr GW: **Rat inositol 1,4,5-trisphosphate 3-kinase C is enzymatically specialized for basal cellular inositol trisphosphate phosphorylation and shuttles actively between nucleus and cytoplasm.** *J Biol Chem* 2003, **278**:19765-19776.
63. Hascakova-Bartova R, Pouillon V, Dewaste V, Moreau C, Jacques C, Banting G, Schurmans S, Erneux C: **Identification and subcellular distribution of endogenous Ins(1,4,5)P<sub>3</sub> 3-kinase B in mouse tissues.** *Biochem Biophys Res Commun* 2004, **323**:920-925.
64. Chamberlain PP, Sandberg ML, Sauer K, Cooke MP, Lesley SA, Spraggon G: **Structural insights into enzyme regulation for inositol 1,4,5-trisphosphate 3-kinase B.** *Biochemistry* 2005, **44**:14486-14493.
65. Wen BG, Pletcher MT, Warashina M, Choe SH, Ziaee N, Wiltshire T, Sauer K, Cooke MP: **Inositol (1,4,5) trisphosphate 3 kinase B controls positive selection of T cells and modulates Erk activity.** *Proc Natl Acad Sci U S A* 2004, **101**:5604-5609.
66. Pouillon V, Hascakova-Bartova R, Pajak B, Adam E, Bex F, Dewaste V, Van Lint C, Leo O, Erneux C, Schurmans S: **Inositol 1,3,4,5-tetrakisphosphate is essential for T lymphocyte development.** *Nat Immunol* 2003, **4**:1136-1143.
67. Soriano S, Thomas S, High S, Griffiths G, D'santos C, Cullen P, Banting G: **Membrane association, localization and topology of rat inositol 1,4,5-trisphosphate 3-kinase B: implications for membrane traffic and Ca<sup>2+</sup> homeostasis.** *Biochem J* 1997, **324**:579-589.
68. Wilson MP, Majerus PW: **Isolation of inositol 1,3,4-trisphosphate 5/6-kinase, cDNA cloning and expression of the recombinant enzyme.** *J Biol Chem* 1996, **271**:11904-11910.
69. Yang X, Shears SB: **Multitasking in signal transduction by a promiscuous human Ins(3,4,5,6)P<sub>4</sub> 1-kinase/Ins(1,3,4)P<sub>3</sub> 5/6-kinase.** *Biochem J* 2000, **351**:551-555.
70. Ho MWY, Yang X, Carew MA, Zhang T, Hua L, Kwon Y-U, Chung S-K, Adelt S, Vogel G, Riley AM, Potter BVL, Shears SB: **Regulation of Ins(3,4,5,6)P<sub>4</sub> signaling by a reversible kinase/phosphatase.** *Curr Biol* 2002, **12**:477-482.
71. Verbsky JW, Chang S-C, Wilson MP, Mochizuki Y, Majerus PW: **The pathway for the production of inositol hexakisphosphate in human cells.** *J Biol Chem* 2005, **280**:1911-1920.
72. Saiardi A, Nagata E, Luo HR, Sawa A, Luo X, Snowman AM, Snyder SH: **Mammalian inositol polyphosphate multikinase synthesizes inositol 1,4,5-trisphosphate and an inositol pyrophosphate.** *Proc Natl Acad Sci USA* 2001, **98**:2306-2311.
73. Chang S-C, Miller AL, Feng Y, Wenthe SR, Majerus PW: **The human homolog of the rat inositol phosphate multikinase is an inositol 1,3,4,6-tetrakisphosphate 5-kinase.** *J Biol Chem* 2002, **277**:43836-43843.
74. Eisenberg F Jr: **D-Myoinositol 1-phosphate as product of cyclization of glucose 6-phosphate and substrate for a specific phosphatase in rat testis.** *J Biol Chem* 1967, **242**:1375-1382.
75. Takimoto K, Okada M, Matsuda Y, Nakagawa H: **Purification and properties of myo-inositol-1-phosphatase from rat brain.** *J Biochem (Tokyo)* 1985, **98**:363-370.
76. Ragan CI, Watling KJ, Gee NS, Aspley S, Jackson RG, Reid GG, Baker R, Billington DC, Barnaby RJ, Leeson PD: **The dephosphorylation of inositol 1,4-bisphosphate to inositol in liver and brain involves two distinct Li<sup>+</sup>-sensitive enzymes and proceeds via inositol 4-phosphate.** *Biochem J* 1988, **249**:143-148.
77. Gee NS, Ragan CI, Watling KJ, Aspley S, Jackson RG, Reid GG, Gani D, Shute JK: **The purification and properties of myo-inositol monophosphatase from bovine brain.** *Biochem J* 1988, **249**:883-889.
78. McAllister G, Whiting P, Hammond EA, Knowles MR, Atack JR, Bailey FJ, Maigetter R, Ragan CI: **cDNA cloning of human and rat brain myo-inositol monophosphatase. Expression and characterization of the human recombinant enzyme.** *Biochem J* 1992, **284**:749-754.
79. Quiroz JA, Gould TD, Manji HK: **Molecular effects of lithium.** *Mol Interv* 2004, **4**:259-272.
80. Inhorn RC, Majerus PW: **Inositol polyphosphate 1-phosphatase from calf brain. Purification and inhibition by Li<sup>+</sup>, Ca<sup>2+</sup>, and Mn<sup>2+</sup>.** *J Biol Chem* 1987, **262**:15946-15952.
81. Inhorn RC, Majerus PW: **Properties of inositol polyphosphate 1-phosphatase.** *J Biol Chem* 1988, **263**:14559-14565.
82. York JD, Majerus PW: **Isolation and heterologous expression of a cDNA encoding bovine inositol polyphosphate 1-phosphatase.** *Proc Natl Acad Sci U S A* 1990, **87**:9548-9552.
83. Steen VM, Lovlie R, Osher Y, Belmaker RH, Berle JO, Gulbrandsen AK: **The polymorphic inositol 1-phosphatase gene as a candidate for pharmacogenetic prediction of lithium-responsive manic-depressive illness.** *Pharmacogenetics* 1998, **8**:259-268.
84. Laporte J, Hu LJ, Kretz C, Mandel JL, Kioschis P, Coy JF, Klauk SM, Poustka A, Dahl N: **A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast.** *Nat Genet* 1996, **13**:175-182.
85. Blondeau F, Laporte J, Bodin S, Superti-Furga G, Payrastre B, Mandel JL: **Myotubularin, a phosphatase deficient in myotubular myopathy, acts on phosphatidylinositol 3-kinase and phosphatidylinositol 3-phosphate pathway.** *Hum Mol Genet* 2000, **9**:2223-2229.
86. Taylor GS, Maehama T, Dixon JE: **Myotubularin, a protein tyrosine phosphatase mutated in myotubular myopathy, dephosphorylates the lipid second messenger, phosphatidylinositol 3-phosphate.** *Proc Natl Acad Sci USA* 2000, **97**:8910-8915.
87. Laporte J, Bedez F, Bolino A, Mandel J-L: **Myotubularin, a large disease-associated family of cooperating catalytically active and inactive phosphoinositides phosphatases.** *Hum Mol Genet* 2003, **12**(Spec No 2):R285-292.
88. Tronchère H, Buj-Bello A, Mandel J-L, Payrastre B: **Implication of phosphoinositide phosphatases in genetic diseases: the case of myotubularin.** *Cell Mol Life Sci* 2003, **60**:2084-2099.
89. Kim S-A, Taylor GS, Torgersen KM, Dixon JE: **Myotubularin and MTMR2, phosphatidylinositol 3-phosphatases mutated in myotubular myopathy and type 4B Charcot-Marie-Tooth disease.** *J Biol Chem* 2002, **277**:4526-4531.
90. Schaletzky J, Dove SK, Short B, Lorenzo O, Clague MJ, Barr FA: **Phosphatidylinositol-5-phosphate activation and conserved substrate specificity of the myotubularin phosphatidylinositol 3-phosphatases.** *Curr Biol* 2003, **13**:504-509.
91. Berger P, Bonneick S, Willi S, Wymann M, Suter U: **Loss of phosphatase activity in myotubularin-related protein 2 is associated with Charcot-Marie-Tooth disease type 4B1.** *Hum Mol Genet* 2002, **11**:1569-1579.
92. Walker DM, Urbé S, Dove SK, Tenza D, Raposo G, Clague MJ: **Characterization of MTMR3, an inositol lipid 3-phosphatase with novel substrate specificity.** *Curr Biol* 2001, **11**:1600-1605.
93. Maehama T, Dixon JE: **The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate.** *J Biol Chem* 1998, **273**:13375-13378.
94. Caffrey JJ, Darden T, Wenk MR, Shears SB: **Expanding coincident signaling by PTEN through its inositol 1,3,4,5,6-pentakisphosphate 3-phosphatase activity.** *FEBS Lett* 2001, **499**:6-10.

95. Bansal VS, Caldwell KK, Majerus PW: **The isolation and characterization of inositol polyphosphate 4-phosphatase.** *J Biol Chem* 1990, **265**:1806-1811.
96. Norris FA, Auethavekiat V, Majerus PW: **The isolation and characterization of cDNA encoding human and rat brain inositol polyphosphate 4-phosphatase.** *J Biol Chem* 1995, **270**:16128-16133.
97. Norris FA, Atkins RC, Majerus PW: **The cDNA cloning and characterization of inositol polyphosphate 4-phosphatase type II. Evidence for conserved alternative splicing in the 4-phosphatase family.** *J Biol Chem* 1997, **272**:23859-23864.
98. Ivetac I, Munday AD, Kisseleva MV, Zhang X-M, Luff S, Tiganis T, Whisstock JC, Rowe T, Majerus PW, Mitchell CA: **The Type Ia inositol polyphosphate 4-phosphatase generates and terminates phosphoinositide 3-kinase signals on endosomes and the plasma membrane.** *Mol Biol Cell* 2005, **16**:2218-2233.
99. Guo S, Stolz LE, Lemrow SM, York JD: **SAC1-like domains of yeast SAC1, INP52, and INP53 and of human synaptojanin encode polyphosphoinositide phosphatases.** *J Biol Chem* 1999, **274**:12990-12995.
100. Hughes WE, Woscholski R, Cooke FT, Patrick RS, Dove SK, McDonald NQ, Parker PJ: **SAC1 encodes a regulated lipid phosphoinositide phosphatase, defects in which can be suppressed by the homologous Inp52p and Inp53p phosphatases.** *J Biol Chem* 2000, **275**:801-808.
101. Nemoto Y, Kearns BG, Wenk MR, Chen H, Mori K, Alb JG Jr, De Camilli P, Bankaitis VA: **Functional characterization of a mammalian Sac1 and mutants exhibiting substrate-specific defects in phosphoinositide phosphatase activity.** *J Biol Chem* 2000, **275**:34293-343005.
102. Kisseleva MV, Wilson MP, Majerus PW: **The isolation and characterization of a cDNA encoding phospholipid-specific inositol polyphosphate 5-phosphatase.** *J Biol Chem* 2000, **275**:20110-20116.
103. Schmid AC, Wise HM, Mitchell CA, Nussbaum R, Woscholski R: **Type II phosphoinositide 5-phosphatases have unique sensitivities towards fatty acid composition and head group phosphorylation.** *FEBS Lett* 2004, **576**:9-13.
104. Chi Y, Zhou B, Wang W-Q, Chung S-K, Kwon Y-U, Ahn Y-H, Chang Y-T, Tsujishita Y, Hurley JH, Zhang Z-Y: **Comparative mechanistic and substrate specificity study of inositol polyphosphate 5-phosphatase *Schizosaccharomyces pombe* synaptojanin and SHIP2.** *J Biol Chem* 2004, **279**:44987-44995.
105. Chung J-K, Sekiya F, Kang H-S, Lee C, Han J-S, Kim SR, Bae YS, Morris AJ, Rhee SG: **Synaptojanin inhibition of phospholipase D activity by hydrolysis of phosphatidylinositol 4,5-bisphosphate.** *J Biol Chem* 1997, **272**:15980-15985.
106. Rauh MJ, Sly LM, Kalesnikoff J, Hughes MR, Cao LP, Lam V, Krystal G: **The role of SHIP1 in macrophage programming and activation.** *Biochem Soc Trans* 2004, **32**:785-788.
107. Rameh LE, Toliaf KF, Duckworth BC, Cantley LC: **A new pathway for synthesis of phosphatidylinositol-4,5-bisphosphate.** *Nature* 1997, **390**:192-196.
108. Pawson T, Scott JD: **Signaling through scaffold, anchoring, and adaptor proteins.** *Science* 1997, **278**:2075-2080.
109. Munday AD, Norris FA, Caldwell KK, Brown S, Majerus PW, Mitchell CA: **The inositol polyphosphate 4-phosphatase forms a complex with phosphatidylinositol 3-kinase in human platelet cytosol.** *Proc Natl Acad Sci USA* 1999, **96**:3640-3645.
110. Gupta N, Scharenberg AM, Fruman DA, Cantley LC, Kinet J-P, Long EO: **The SH2 domain-containing inositol 5'-phosphatase (SHIP) recruits the p85 subunit of phosphoinositide 3-kinase during FcγRIIb1-mediated inhibition of B cell receptor signaling.** *J Biol Chem* 1999, **274**:7489-7494.
111. Jackson SP, Schoenwaelder SM, Matzaris M, Brown S, Mitchell CA: **Phosphatidylinositol 3,4,5-trisphosphate is a substrate for the 75 kDa inositol polyphosphate 5-phosphatase and a novel 5-phosphatase which forms a complex with the p85/p110 form of phosphoinositide 3-kinase.** *EMBO J* 1995, **14**:4490-4500.
112. Kallunki T, Deng T, Hibi M, Karin M: **c-Jun can recruit JNK to phosphorylate dimerization partners via specific docking interactions.** *Cell* 1996, **87**:929-939.
113. Hinchliffe KA, Giudici ML, Letcher AJ, Irvine RF: **Type IIα phosphatidylinositol phosphate kinase associates with the plasma membrane via interaction with type I isoforms.** *Biochem J* 2002, **363**:563-570.
114. Nandurkar HH, Layton M, Laporte J, Selan C, Corcoran L, Caldwell KK, Mochizuki Y, Majerus PW, Mitchell CA: **Identification of myotubularin as the lipid phosphatase catalytic subunit associated with the 3-phosphatase adapter protein, 3-PAP.** *Proc Natl Acad Sci USA* 2003, **100**:8660-8665.
115. Bondev A, Rubio I, Wetzker R: **Differential regulation of lipid and protein kinase activities of phosphoinositides 3-kinase γ in vitro.** *Biol Chem* **380**:1337-1340.
116. Cullen PJ, Hsuan JJ, Truong O, Letcher AJ, Jackson TR, Dawson AP, Irvine RF: **Identification of a specific Ins(1,3,4,5)P<sub>4</sub>-binding protein as a member of the GAPI family.** *Nature* 1995, **376**:527-530.
117. Robson JD, Davidson D, Veillette A: **Inhibition of the Jun N-terminal protein kinase pathway by SHIP-1, a lipid phosphatase that interacts with the adaptor molecule Dok-3.** *Mol Cell Biol* 2004, **24**:2332-2343.
118. Boer A-K, Drayer AL, Vellenga E: **Effects of overexpression of the SH2-containing inositol phosphatase SHIP on proliferation and apoptosis of erythroid AS-E2 cells.** *Leukemia* 2001, **15**:1750-1757.
119. An H, Xu H, Zhang M, Zhou J, Feng T, Qian C, Qi R, Cao X: **Src homology 2 domain-containing inositol-5-phosphatase I (SHIP1) negatively regulates TLR4-mediated LPS response primarily through a phosphatase activity- and PI-3K-independent mechanism.** *Blood* 2005, **105**:4685-4692.
120. Blero D, Zhang J, Pesesse X, Payrastré B, Dumont JE, Schurmans S, Erneux C: **Phosphatidylinositol 3,4,5-trisphosphate modulation in SHIP2-deficient mouse embryonic fibroblasts.** *FEBS J* 2005, **272**:2512-2522.
121. Tang X, Powelka AM, Soriano NA, Czech MP, Guilherme A: **PTEN, but not SHIP2, suppresses insulin signaling through the phosphatidylinositol 3-kinase/Akt pathway in 3T3-L1 adipocytes.** *J Biol Chem* 2005, **280**:22523-22529.
122. Weng L-P, Smith WM, Brown JL, Eng C: **PTEN inhibits insulin-stimulated MEK/MAPK activation and cell growth by blocking IRS-1 phosphorylation and IRS-1/Grb-2/Sos complex formation in a breast cancer model.** *Hum Mol Genet* 2001, **10**:605-616.
123. Weng L-P, Brown JL, Baker KM, Ostrowski MC, Eng C: **PTEN blocks insulin-mediated ETS-2 phosphorylation through MAP kinase, independently of the phosphoinositide 3-kinase pathway.** *Hum Mol Genet* 2002, **11**:1687-1696.

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