The inositol polyphosphate 5-phosphatases: traffic controllers, waistline watchers and tumour suppressors?

Megan V. Astle, Kristy A. Horan, Lisa M. Ooms and Christina A. Mitchell

Department of Biochemistry and Molecular Biology Monash University, Clayton, Victoria, Australia, 3800

Abstract

Phosphoinositide signals regulate cell proliferation, differentiation, cytoskeletal rearrangement and intracellular trafficking. Hydrolysis of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 by inositol polyphosphate 5-phosphatases regulates synaptic vesicle recycling (synaptojanin-1), hematopoietic cell function [SHIP1(SH2-containing inositol polyphosphate 5-phosphatase-1)], renal cell function [OCRL (oculocerebrorenal syndrome of Lowe)] and insulin signalling (SHIP2). We present here a detailed review of the characteristics of the ten mammalian 5-phosphatases. Knockout mouse phenotypes and underexpression studies are associated with significant phenotypic changes, indicating non-redundant roles, despite, in many cases, overlapping substrate specificity and tissue expression. The extraordinary complexity in the control of phosphoinositide signalling continues to be revealed.

Phosphoinositide signalling

Phosphoinositides are ubiquitous components of eukaryotic cell membranes that act as signalling molecules, regulating many cellular processes including actin polymerization, apoptosis and vesicular membrane trafficking [1]. PtdIns(4,5)P_2, the substrate for PI3K (phosphoinositide 3-kinase) is in its own right a signalling molecule regulating actin polymerization, cell

'To whom correspondence should be addressed (email Christina.mitchell@med.monash.edu.au).
migration, membrane trafficking, endocytosis and ion channel activity [2]. PtdIns(4,5)\(P_2\) promotes actin polymerization at the PM (plasma membrane) by activating Cdc42, and together, Cdc42 and PtdIns(4,5)\(P_2\) activate N-WASP (neuronal Wiskott–Aldrich syndrome protein), which stimulates the actin-nucleating activity of the Arp2/3 complex (actin-related protein 2/3 complex). PtdIns(4,5)\(P_2\) promotes the formation of integrin-dependent focal adhesions and regulates cell adhesion [3]. Classical clathrin-mediated endocytosis involves consecutive steps of clathrin recruitment and coating, vesicle budding, fission and later uncoating. PtdIns(4,5)\(P_2\) is an important effector in this process, facilitating the recruitment of endocytic proteins such as dynamin, epsin, AP180 and clathrin adaptor AP (adaptor protein)-2, to the plasma membrane and site of endocytosis [4].

Insulin, or growth factor stimulation activates PI3K which phosphorylates PtdIns(4,5)\(P_2\), transiently generating PtdIns(3,4,5)\(P_3\) at the PM, leading to recruitment of effectors to this site [5]. PtdIns(3,4,5)\(P_3\) stimulates metabolic, proliferative and cell survival signalling pathways resulting in stimulation of glucose uptake, and in mitotic cells, proliferation and inhibition of apoptosis [6]. PtdIns(3,4,5)\(P_3\) binds to the PH (pleckstrin homology) domain of the AGC family protein kinases, including PKB/Akt (protein kinase B) and S6K (p70 ribosomal S6 kinase), localizing and activating these kinases [1]. Following phosphoinositide binding, Akt is activated by phosphorylation on Ser\(^{473}\) and Thr\(^{308}\) by specific kinases. Active Akt phosphorylates both cytosolic and nuclear targets, including BAD (Bcl-2/Bcl-X\(_L\)-antagonist, causing cell death), CREB (cAMP-response-element-binding protein), Forkhead, MDM2 and NF\(\kappa\)B (nuclear factor \(\kappa\)B), leading to decreased cell death and prolonged cell survival [7]. The role of PtdIns(3,4,5)\(P_3\) versus PtdIns(3,4)\(P_2\) in the activation of Akt remains unresolved. In vitro, the Akt PH domain binds to PtdIns(3,4,5)\(P_3\) and PtdIns(3,4)\(P_2\) with equal affinity. The rate of Akt phosphorylation by PDK-1 (protein dependent kinase 1) is greatly increased in vitro by the addition of PtdIns(3,4)\(P_2\) [8].

Activation of the PI3K pathway is a critical event in tumour development. Many oncogenes such as EGFR (epidermal growth factor receptor), HER2, and K-Ras stimulate tumour growth by enhancing PI3K signals [9]. In addition, the gene encoding the PI3K catalytic subunit (PIK3CA) is amplified and/or mutated in various cancers, and PtdIns(3,4,5)\(P_3\) effectors such as AKT2 are amplified in ovarian and pancreatic cancer [10].

**Regulation of phosphoinositide signals by the inositol polyphosphate 5-phosphatases**

The inositol polyphosphate 5-phosphatases (5-phosphatases) are Mg\(^{2+}\)-dependent phosphoesterases comprising ten mammalian and four yeast family members [11]. The defining feature of the 5-phosphatases is a central catalytic domain of approximately 350 amino acids that exhibits homology to the apurinic/apyrimidinic family of endonucleases [12,13]. The 5-phosphatase enzymes remove the 5-position phosphate from membrane bound PtdIns(3,4,5)\(P_3\) and...
The inositol polyphosphate 5-phosphatases

PtdIns(4,5)_P_\text{2} forming PtdIns(3,4)_P_\text{2} and PtdIns4_P_\text{2} respectively, as depicted in Figure 1. In some instances the 5-phosphatases hydrolyse the 5-position phosphate from PtdIns(3,5)_P_\text{2} forming PtdIns3_P_\text{2}, however, the in vivo significance of this activity has not been demonstrated [14]. They can also hydrolyse soluble inositols, Ins(1,4,5)_P_\text{3} and Ins(1,3,4,5)_P_\text{4}, which are important for intracellular calcium signalling.

Members of the 5-phosphatase family show varying substrate preferences, with most capable of hydrolysing both membrane-bound phosphoinositides and soluble inositols. There are several exceptions to this rule: 5-phosphatase I only hydrolyses the 5-position phosphate from Ins(1,3,4,5)_P_\text{4} and Ins(1,4,5)_P_\text{4}, whereas the 72 kDa 5-phosphatase (also called the Type IV 5-phosphatase) is reported to specifically hydrolyse PtdIns(4,5)_P_\text{2}, PtdIns(3,4,5)_P_\text{3} and PtdIns(3,5)_P_\text{2} and not inositol phosphates [15,16]. In addition, the yeast 5-phosphatases also do not hydrolyse inositol phosphates [17]. Previous classification of 5-phosphatases as Type I–IV, on the basis of in vitro substrate specificity [18], may not necessarily reflect in vivo enzymatic activity.

© 2007 The Biochemical Society
Domain organization of the mammalian 5-phosphatases.

Each of the 5-phosphatase family members contains a central conserved 5-phosphatase (5-ptase) catalytic domain. Synaptojanin-1 and -2 also have a Sac-1 catalytic domain. Protein interaction domains include PRDs, SAM (sterile α motif) and Src-homology-2 (SH2) domains. CAAX motifs or SKICH domains mediate PM localization.

Many of the 5-phosphatases contain N- and C-terminal regions, including PRDs (proline rich domains), SH2 domains and or C-terminal CAAX motifs, as shown in Figure 2, that facilitate their temporal and spatial subcellular localization. The 5-phosphatases also demonstrate developmentally regulated or tissue-specific expression of splice variants which modulate protein partner binding ability or subcellular localization. The 5-phosphatases synaptojanin-1 and synaptojanin-2 contain an additional N-terminal catalytic domain, designated the Sac-1 domain, which contains a CX,R motif that, in vitro, shows broad specificity towards PtdIns3P, PtdIns4P and PtdIns(3,5)P2, hydrolysing these phosphoinositides to PtdIns [19].

In the following pages we will outline some of the salient features of the ten mammalian 5-phosphatases, as listed in Table 1. Some 5-phosphatases, such as SHIP1 and synaptojanin-1, have been extensively characterized, and the reader is directed to recent reviews [20–25]. The 5-phosphatases appear not to be functionally redundant, as knockouts of single 5-phosphatases in mice (SHIP1, SHIP2 and synaptojanin-1) and mutations in humans [OCRL (oculocerebrorenal syndrome of Lowe)] cause significant phenotypes. The 5-phosphatases may act as both signal terminating and generating enzymes dependent on the cellular type and stimulus, which may, in part, explain the diverse cellular functions this family of enzymes regulates.
Table 1 Mammalian inositol polyphosphate 5-phosphatases. Protein and gene names of the ten mammalian inositol polyphosphatase 5-phosphatases and phenotypes of characterized knockout mice are listed. NA, not applicable (at time of publication, knockout mice have not been described). For references see text.

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Alias(es)</th>
<th>Gene name</th>
<th>Mouse knockout phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-phosphatase I</td>
<td>43 kDa inositol polyphosphate 5-phosphatase</td>
<td>INPP5A</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Type I inositol polyphosphate 5-phosphatase</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inositol polyphosphate-5-phosphatase A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-phosphatase II</td>
<td>75 kDa inositol polyphosphate 5-phosphatase</td>
<td>INPP5B</td>
<td>Males - progressive testicular degeneration leading to sterility. Double KO with Ocrl causes embryonic lethality</td>
</tr>
<tr>
<td></td>
<td>Type II inositol polyphosphate 5-phosphatase</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inositol polyphosphate-5-phosphatase B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHIP1</td>
<td>SHIP</td>
<td>INPP5D</td>
<td>Decreased body weight, increased B cell and macrophage activation, lung infiltration by macrophages, CML like, osteoporotic</td>
</tr>
<tr>
<td></td>
<td>inositol polyphosphate-5-phosphatase D</td>
<td>SHIP1</td>
<td></td>
</tr>
<tr>
<td>SHIP2</td>
<td>51C protein</td>
<td>INPP5I</td>
<td>Resistant to weight gain on high-fat diet</td>
</tr>
<tr>
<td></td>
<td>SHIP2</td>
<td>INPP5E</td>
<td>NA</td>
</tr>
<tr>
<td>72 kDa inositol polyphosphate</td>
<td>Pharbin</td>
<td>OCRL</td>
<td>Humans - renal failure, cataracts, growth and mental retardation</td>
</tr>
<tr>
<td>5-phosphatase</td>
<td>Type IV inositol polyphosphate 5-phosphatase</td>
<td>INPP5F</td>
<td>Mice - slight effects on motor coordination Double KO with Inpp5B causes embryonic lethality Neurological defects, die shortly after birth</td>
</tr>
<tr>
<td>OCRL</td>
<td>Inositol polyphosphate-5-phosphatase E</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lowe oculocerebrorenal syndrome protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inositol polyphosphate 5-phosphatase F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synaptojanin-1</td>
<td>None</td>
<td>SYNJ1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>INPP5G</td>
<td></td>
</tr>
<tr>
<td>Synaptojanin-2</td>
<td>None</td>
<td>SYNJ2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>INPP5H</td>
<td>NA</td>
</tr>
<tr>
<td>PIPP</td>
<td>Phosphatidylinositol (4,5) bisphosphate 5-phosphatase A</td>
<td>PIBSPA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INPP5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>INPP</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SKIP</td>
<td>NA</td>
</tr>
<tr>
<td>SKIP</td>
<td>None</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SHIP1

SHIP1 plays a significant role in regulating the immune system, bone maintenance, cancer and HSC (haematopoietic stem cell) function. It is expressed predominantly in haematopoietic cells where it hydrolyses the 5’ position phosphate from PtdIns(3,4,5)P$_3$ and Ins(1,3,4,5)P$_4$, however, changes in the levels of Ins(1,3,4,5)P$_4$ following SHIP1 over/underexpression have not yet been reported [26–28].

SHIP1 was first isolated as a 145 kDa tyrosine phosphorylated protein in complex with Shc and Grb2 following stimulation by erythropoietin, IL (interleukin)-3, IL-2, GM-CSF (granulocyte/macrophage colony-stimulating factor), Steel Factor and/or activation of the Bcr (B-cell receptor complex) [29–33]. Binding to Shc requires both the SHIP1 N-terminal SH2 domain and C-terminal PRD [34–37]. It has been proposed that upon co-ligation of FcγRIIB with the Bcr, SHIP1 competes with Grb2 to bind Shc, reducing the recruitment of the Grb2-Sos complex to the PM and resulting in decreased Ras and ERK (extracellular-signal-regulated kinase) 1/2 activity [38]. However, other studies have demonstrated that SHIP1, Shc and Grb2 form a multi-protein complex in response to ligation of CD22 in B cells and erythropoietin binding the EPO-R (erythropoietin receptor) [39–41].

SHIP1 is recruited to the cytoplasmic tail of the ITIM (immunoreceptor tyrosine-based inhibition motif) containing receptors FcγRIIB and CD22, upon their co-ligation with the Bcr [41–44]. SHIP1 can also associate with activating receptors such as the immunoreceptor tyrosine-based activation motif (ITAM) containing receptors FcεRIa and FcεRI in macrophages and mast cells respectively [45–47] and 2B4 (CD244) in natural killer cells [48]. Shc-dependent or -independent interactions of SHIP1 with immunoreceptors are predicted to bring SHIP1 into close proximity to its substrate PtdIns(3,4,5)P$_3$ at the PM. Hydrolysis of PtdIns(3,4,5)P$_3$ to form PtdIns(3,4)P$_2$ regulates cell survival and proliferation via the Akt (protein kinase B)/mTor (target of rapamycin) pathway, as well as governing actin dynamics [49,50].

The importance of SHIP1 in regulating immune cell function is demonstrated by SHIP1$^{-/-}$ knockout mice which fail to thrive, exhibit splenomegaly and massive infiltration of the lungs by macrophages. The thymus and lymph nodes are largely unaffected, with normal proportions of cells of the T cell lineage [51]. Analysis of splenic SHIP$^{-/-}$ B cell populations has revealed a decrease in transitional B cells, but an over representation of mature B cells, [34,52,53]. In addition, SHIP1$^{-/-}$ B cells have increased migratory ability [52,53]. These findings may account for the decreased number of B cells detected in the bone marrow and blood of SHIP1$^{-/-}$ mice. SHIP1$^{-/-}$ B cells also demonstrate hyper-responsiveness to antigen [27] and increased antibody production [52].

SHIP1$^{-/-}$ BMM (bone marrow-derived macrophages) demonstrate an enhanced response to FcR clustering and enhanced phagocytic ability, correlating with increased levels of PtdIns(3,4,5)P$_3$ [54,55]. SHIP1$^{-/-}$ BMDCs (bone marrow-derived mast cells) are hyper-responsive to antigen-, Steel Factor- and IgE alone-induced degranulation [56]. SHIP1 is a key regulator in the inhibitory phenomenon observed when BMDCs are stimulated with supraoptimal antigen.
concentrations [56]. SHIP1 deficiency in HSCs leads to enhanced proliferation, but defects in the ability of HSCs to repopulate the bone marrow, presumably due to a defect in homing [57]. A role for SHIP1 has also been indicated in natural killer cell activation in response to antibody, IL-12 [58] and platelet signalling [59]. Interestingly, SHIP1−/− mice are also osteoporotic, due to increased number and activity of osteoclasts [60].

Perturbations in SHIP1 regulation may cause or enhance tumourigenesis. SHIP1−/− mice demonstrate a myeloproliferative disorder of the blood, similar to CML (chronic myelogenous leukaemia) [51]. CML is associated with the oncogenic transformation by BCR/ABL [61]. SHIP1 is down-regulated in BCR/ABL-transformed cells, and reconstitution of SHIP1 in these leukemic cells inhibits migration [62]. BCR/ABL oncogene activation is sufficient to lead to CML, and the aberrant regulation of SHIP1 may be a consequence of the disease, leading to further deregulation. Interestingly, a recent study has proposed [63] SHIP1 may affect tumour progression in multiple ways, through direct regulation of cell growth and also through immuno-surveillance, providing multiple potential opportunities for development of new treatment strategies.

**SHIP2**

The 142 kDa SHIP2 plays an important role in insulin signalling and obesity regulation. SHIP2 substrate specificity was originally predicted to be limited to PtdIns(3,4,5)P_3 and Ins(1,3,4,5)P_5, generating PtdIns(3,4)P_2 and Ins(1,3,4)P_3, respectively. However, recent in vitro studies have suggested that SHIP2 shows the following order of preference for specific substrates Ins(1,2,3,4,5)P_5 > Ins(1,3,4,5)P_5 > PtdIns(3,4,5)P_3 = PtdIns(3,5)P_3 = Ins(1,4,5,6)P_5 = Ins(2,4,5,6)P_4 [64]. The functional significance of the hydrolysis of the preferred inositol phosphates by SHIP2 remains unexplored.

SHIP2 contains an N-terminal SH2 domain, a central catalytic domain and a C-terminal PRD. The C-terminal PRD carries a number of protein interaction motifs including two WW binding motifs, an NPXY motif and a SAM (sterile alpha motif) domain. SHIP2 is widely expressed, most highly in brain, skeletal muscle and heart. Unlike its close homologue SHIP1, which differs in amino acid sequence in its C-terminal region, SHIP2 is expressed in both haematopoietic and non-haematopoietic tissues [65]. Interestingly, SHIP1 and SHIP2 are both expressed, and show non-redundant functions in some haematopoietic cells including platelets and macrophages [66,67]. SHIP2 localizes to the cytosol in resting cells, relocating to membrane ruffles during cell attachment or following growth factor stimulation [66,68,69]. SHIP2 has also been detected in nuclear speckles of vascular smooth muscle cells, although its function in the nucleus remains unknown [70].

SHIP2 forms complexes with proteins that regulate actin cytoskeletal dynamics including the actin-binding protein filamin, p30Cas and Shc, regulating membrane ruffling [68,71], with the hepatocyte growth factor (HGF/SF) receptor c-Met to regulate cell scattering and spreading [72] and with
vinexin [73] to regulate cell attachment. SHIP2 also complexes with both the c-Cbl, CAP (c-Cbl-associated protein) and the insulin receptor, although the functional effects of this interaction are unknown [23].

Studies in a variety of experimental systems have supported to varying degrees a role for SHIP2 in the negative regulation of insulin signalling. Based on results of over- and under-expression studies in insulin-dependent cell lines, such as 3T3-L1 adipocytes, CHO-IR cells (CHO cells overexpressing the insulin receptor) and L6 myotubes, SHIP2 may negatively regulate PI3K-dependent Akt phosphorylation and the translocation of the glucose reporter GLUT4 (glucose transporter 4) to the plasma membrane [24]. However, RNAi (interference RNA)-mediated knockdown of SHIP2 in adipocytes does not support a role for this 5-phosphatase in negatively regulating insulin signalling in 3T3-L1 adipocytes [74].

The phenotype of the SHIP2 knockout is contentious, and was originally reported to show insulin hypersensitivity, resulting from enhanced insulin-stimulated GLUT4 translocation to the plasma membrane [75]. However, the targeting construct also inadvertently deleted a second gene, Phox2a [76]. Recently another SHIP2 knockout (Inppl−/−) mouse, using a different targeting construct was reported [77]. These mice are viable, have normal glucose and insulin levels, however, are highly resistant to weight gain when placed on a high-fat diet [77]. The molecular basis for this phenotype is unclear. SHIP2 negatively regulates PI3K signals and the Inppl1−/− mice show increased Akt and p70S6K activation and this may promote a feedback loop, attenuating insulin signalling and fat metabolism [78].

Interestingly, SHIP2 knockdown studies in macrophages have revealed that SHIP2, like SHIP1, plays a significant role in regulating macrophage phagocytosis [79]. Therefore FcγR-mediated phagocytosis of IgG-coated particles is regulated by at least three lipid phosphatases including PTEN (phosphatase and tensin homologue deleted on chromosome 10), SHIP1 and SHIP2 [55,79]. SHIP2, like SHIP1, translocates to the phagocytic cup and downregulates FcγR-mediated phagocytosis. In addition, SHIP2 is tyrosine-phosphorylated in M-CSF-stimulated human alveolar macrophages, human THP-1 cells, murine macrophages and the murine macrophage cell line RAW264; associating with the M-CSF receptor after M-CSF stimulation [80]. In macrophages SHIP2 functions to regulate both phagocytosis and Akt-dependent activation of NF-κB-mediated gene transcription [80].

Human genotype analysis has revealed that the SHIP2 gene may represent a candidate gene predisposing for Type II diabetes [81]. A 16 bp deletion in the SHIP2 gene that alters its expression has been detected in 2.0% of Type II diabetics versus 0.7% of control subjects. In addition, genotyping of 12 polymorphisms in diabetic families has revealed an association of specific single nucleotide polymorphisms and SHIP2 gene haplotypes with individual or combined features of the metabolic syndrome, including hypertension, Type II diabetes, central obesity and dyslipidaemia [82]. SHIP2 gene variants correlate with the presence or absence of hypertension in Type II diabetic patients [82]. Interestingly, genotyping identified 10 polymorphisms including 4 missense mutations, including one in the 5-phosphatase catalytic coding sequence, in
a Japanese diabetic cohort [45]. Therefore SHIP2 gene polymorphisms may contribute to Type II diabetes and insulin resistance.

**SKIP**

The skeletal muscle and kidney enriched inositol polyphosphate 5-phosphatase, SKIP, is a ubiquitously expressed 51 kDa protein which contains a central 5-phosphatase domain and a novel C-terminal domain designated the ‘SKICH domain’. SKIP hydrolyses the 5' position phosphate from PtdIns(3,4,5)P$_3$, PtdIns(4,5)P$_2$, Ins(1,3,4,5)P$_4$ and Ins(1,4,5)P$_3$, displaying some preference for PtdIns(4,5)P$_2$ over PtdIns(3,4,5)P$_3$ in purified component enzymatic assays [14,83]. However, overexpression studies have revealed that SKIP decreases the total cellular levels of insulin-stimulated PtdIns(3,4,5)P$_3$, as well as decreasing PtdIns(4,5)P$_2$ cellular levels [84]. In non-stimulated cells SKIP localizes in a perinuclear distribution, co-localizing with markers of the endoplasmic reticulum. Following growth factor- or serum-stimulation, SKIP translocates to plasma membrane ruffles [85]. The SKICH domain mediates the plasma membrane localization of SKIP via an unknown mechanism, and is also present in the 107 kDa 5-phosphatase PIPP (proline-rich inositol polyphosphatase 5-phosphatase), and members of the TRAF6-binding protein family [85]. Unlike SHIP2, overexpression of either SKIP or PIPP does not affect the extent of membrane ruffling upon growth factor stimulation; however, SKIP expression decreases actin stress fibres [83].

SKIP is highly expressed in skeletal muscle and is implicated in the negative regulation of insulin-stimulated PI3K-dependent insulin signalling. SKIP inhibits insulin-stimulated PtdIns(3,4,5)P$_3$ signalling and thereby the phosphorylation and activation of Akt and p70S6K. Downregulation of SKIP in muscle cells dramatically enhances insulin-stimulated Akt phosphorylation [84], promoting GLUT4 translocation and glucose uptake.

SKIP is also implicated in a contiguous-gene syndrome, MDS (the Miller-Dieker syndrome), a severe form of lissencephaly (smooth brain) caused by defects in neuronal migration [86]. Although isolated lissencephaly is caused by point mutations or deletions in the LIS1 gene, the severe cortical phenotype and craniofacial and limb defects seen in MDS result from heterozygous deletions of eight genes (PRP8, RILP, SREC, PITPNa, **SKIP**, MYO1C, CRK, and 14-3-3ζ) within a 400 kb region on 17p13.3 [87]. Heterozygous deletion of these genes may mediate facial dysmorphisms and neuronal migration defects. SKIP may affect craniofacial development by regulating neuronal migration, however, this has yet to be shown. SKIP is located on chromosome 17p13.3, a region frequently deleted or hypermethylated in human cancers, distal to the p53 locus at 17p13.1 [88]. Allelic loss at 17p13.3 independent of the p53 locus frequently occurs in human malignancies including breast, ovarian, and neural tumours [89–91]. The role SKIP plays in regulating cellular proliferation or cell survival has not been reported. Finally, the discrete role SKIP plays in regulating PI3K or PtdIns(4,5)P$_2$-dependent signalling awaits the development and characterization of SKIP knockout mice.
PIPP

PIPP is one of the least characterized members of the polyphosphate 5-phosphatase family [92]. Based on Northern analysis, PIPP is highly expressed in brain, heart, kidney, small intestine and lung. This 107 kDa 5-phosphatase was initially reported to hydrolyse Ins(1,3,4,5)\(_4\), Ins(1,4,5)\(_3\), and PtdIns(4,5)\(_2\) and localizes to membrane ruffles in stimulated cells [92]. More recently, PIPP has been described as a novel PtdIns(3,4,5)\(_3\) 5-phosphatase [93]. PIPP contains PRDs at its N- and C-terminus, a central 5-phosphatase domain, and a recently described SKICH domain that mediates its constitutive plasma membrane association [85]. As yet, no PIPP binding partners have been described, despite bioinformatic analysis predicting putative binding partners such as 14-3-3ζ.

In the rat pheochromocytoma PC12 cell line PIPP negatively regulates PI3K-dependent neurite elongation [93]. PC12 cells exhibit NGF (nerve growth factor)-dependent neuronal differentiation, forming and extending neurites. PI3K is activated specifically at the neurite growth cone, thereby localizing Akt activation and down-stream inactivation of GSK3\(\beta\), to promote microtubule polymerization and neurite extension [94,95]. In differentiating PC12 cells, PIPP is specifically enriched in the neurite growth cone. RNAi-mediated targeted depletion of PIPP in PC12 cells results in hyper-elongated neurites upon NGF stimulation. The growth cones of these neurites show enhanced PtdIns(3,4,5)\(_3\) accumulation and increased Akt and GSK3\(\beta\) phosphorylation, associated with increased polymerized microtubules in the neurite shaft [93]. Therefore PIPP is likely to have an important role in spatially regulating PtdIns(3,4,5)\(_3\) signalling during neuronal differentiation. The molecular mechanisms by which PIPP subcellular localization and its 5-phosphatase activity are regulated at the growth cone have yet to be elucidated.

A large scale RNAi screening of all phosphatases and kinases in the human genome has identified PIPP (PIB5PA) as a putative regulator of cell survival [96]. Independently, a screen of approx. 5000 genes identified PIB5PA as one of 231 genes significantly associated with breast cancer disease outcome [97]. PIPP expression correlates with oestrogen receptor status and better prognosis. However, the role PIPP plays in regulating PI3K-dependent cell proliferation and cell survival remains largely unexplored. To date no PIPP knockout mouse has been reported so the distinct role this 5-phosphatase plays remains to be delineated.

72 kDa 5-phosphatase

We and others have cloned and characterized a novel 72 kDa inositol polyphosphate 5-phosphatase (72 kDa 5-phosphatase in mouse), also called pharbin (in rat), or the type IV 5-phosphatase (in human; gene name INPP5E) [15,16,98] that all share 74% amino acid sequence identity. This 5-phosphatase contains an N-terminal PRD, a central catalytic 5-phosphatase domain and a C-terminal CAAX motif. The 72 kDa 5-phosphatase is the most active of all 5-phosphatases in the hydrolysis of PtdIns(3,4,5)\(_3\), forming PtdIns(3,4)\(_2\).
with a $K_m = 0.65 \, \mu M$ that is approx. 10-fold greater than other inositol polyphosphate 5-phosphatases, including SHIP, OCRL and 5-phosphatase II [15]. Overexpression of this 5-phosphatase in HEK-293 (human embryonic kidney 293) cells is associated with a rapid depletion of both PtdIns(4,5)$P_2$ and PtdIns(3,4,5)$P_3$, resulting in attenuation of Akt phosphorylation and inhibition of Fas-induced apoptosis [99]. In vitro enzyme studies have suggested that the 72 kDa 5-phosphatase also hydrolyses PtdIns(3,5)$P_2$ forming PtdIns3$P$, however the functional consequences of this activity are unclear. This enzyme is widely expressed in tissues including the brain, testis, breast, and haematopoietic cells and localizes to the cytosol and peri-Golgi area [16].

Although the 72 kDa 5-phosphatase is the most active 5-phosphatase in the hydrolysis of PtdIns(3,4,5)$P_3$ [15], its role in regulating cell proliferation and cancer has not been well characterized. Gene expression profiling has revealed that the 72 kDa 5-phosphatase exhibits a 50-fold change in expression in human cervical cancer, one of the top five out of 74 genes with altered expression [100]. In addition, gene profiling has identified that the 72 kDa 5-phosphatase is downregulated in stomach cancer [101]. The role the 72 kDa 5-phosphatase plays in regulating cellular proliferation remains largely unexplored and there are few functional studies of this 5-phosphatase including transgenic or knockout mouse models.

Synaptojanin-1 and synaptojanin-2

The 145 kDa isoform of synaptojanin-1 was first isolated as a binding protein of the SH3 domain of Grb2 [102]. Initial studies co-localized the protein with dynamin, enriched in synaptic terminals, and later identified it as a presynaptic inositol 5-phosphatase and the major constitutively active PtdIns(3,4,5)$P_3$ 5-phosphatase in rat brain [103–105]. Synaptojanin-2 was identified via its homology to synaptojanin-1, with sequence analysis indicating that the two 5-phosphatases share an N-terminal Sac-1 domain and central 5-phosphatase domain, but are divergent in their C-terminal sequences [106]. The C-terminus of synaptojanin-1 contains a PRD, an AP-2 binding motif and three NPF repeats which mediate protein–protein interactions.

The substrate specificity of synaptojanin-1 was first reported as 5’ position phosphate hydrolysis of PtdIns(4,5)$P_2$, Ins(1,4,5)$P_3$ and Ins(1,3,4,5)$P_4$ [104], and later studies revealed a lower activity toward PtdIns(3,4,5)$P_3$ [14]. Enzymatic activity of the synaptojanin proteins is not isolated to the central 5-phosphatase domain, with the N-terminal Sac-1 domain also hydrolysing PtdIns3$P$, PtdIns4$P$ and PtdIns(3,5)$P_2$ [19]. Numerous isoforms have been described for both synaptojanin-1 and synaptojanin-2, with developmentally regulated and tissue-specific alternate splicing producing three isoforms for synaptojanin-1 [107–109] and up to six isoforms for synaptojanin-2 [106,110–112].

Synaptojanin-1 forms complexes with many proteins that cooperate in synaptic vesicle recycling. These include dynamin, amphiphysin I and II [104], endophilin [113,114], syndapin [115], Eps15 [116], AP-2 [117] and Dap160/
intersectin [118]. Phosphorylation events control both catalytic activity and protein complex formation. Synaptojanin-1 is constitutively phosphorylated in nerve terminals, undergoing dephosphorylation by calcineurin upon depolarization [103]. Serine phosphorylation by Cdk5 reduces synaptojanin-1 5-phosphatase activity, similar to tyrosine phosphorylation by EphB2, down-stream of ephrin stimulation. Cdk5 phosphorylation affects endophilin and amphiphysin binding, whereas EphB2 phosphorylation affects the ability of synaptojanin-1 to bind to endophilin [119,120].

In the neuronal synapse, efficient endocytosis of internal membranes is required for the maintenance of a synaptic vesicle pool. Knockout or depletion studies of synaptojanin-1 in a number of experimental systems have confirmed a role for the 5-phosphatase in regulating clathrin-mediated endocytosis. Synaptojanin-1 knockout mice die shortly after birth, exhibiting neurological defects, correlating with increased levels of PtdIns(4,5)\(_2\) in neurons [121]. An accumulation of CCVs (clathrin-coated vesicles) in nerve terminals was also observed. It has been proposed that synaptojanin-1-mediated hydrolysis of PtdIns(4,5)\(_2\) promotes uncoating of CCVs. In cultured cortical neurons from synaptojanin-1 knockout mice the accumulation of CCVs was stimulus dependent, reducing the pool of neurotransmitter-containing vesicles ready for exocytosis [122]. Knockout of the unc-26 gene, encoding the only synaptojanin orthologue in Caenorhabditis elegans, causes an increase in clathrin-coated pits and vesicles and a decrease in synaptic vesicles, with cytoskeletal defects seemingly causing tethering of endocytic vesicles [123]. Verstreken et al. [124] identified both endophilin and synaptojanin single knockouts in Drosophila during a genetic screen of mutations that caused abnormalities in the synaptic vesicle cycle. Similarly to mouse and C. elegans mutants, arrays of coated vesicles in both synaptojanin- and endophilin-deficient nerve terminals were observed [124]. Also shown was the requirement for endophilin for the stabilization and localization of synaptojanin in the nerve terminal [124,125]. Examination of Drosophila synaptojanin and endophilin null mutants has demonstrated that classical clathrin-mediated endocytosis, although impaired, does persist in these mutants, and even under these conditions the vesicles undergo full fusion and re-formation [124]. This would therefore argue against the ‘kiss-and-run’ hypothesis of synaptic vesicle recycling, that proposes vesicles do not fully fuse with the membrane prior to endocytosis, but rather release transmitter via a transient fusion pore.

Synaptojanin-2 also regulates the early stages of endocytosis. Depletion of synaptojanin-2 in A-549 cells causes a decrease in the number and size of vesicles at all stages of endocytosis [126]. Synaptojanin-2B interacts with GTP-Rac1, and unlike synaptojanin-1, localizes to the PM upon co-transfection of constitutively active Rac1 (Rac1V12) or EGF (epidermal growth factor) stimulation [111,127]. EGF internalization is reduced in cells overexpressing a membrane targeted form of synaptojanin-2B, [127] indicating that synaptojanin-2 may contribute to the Rac1 inhibitory effect on endocytosis. Targeted depletion of synaptojanin-2 in glioblastoma cells also inhibits the formation of lamellipodia and invadopodia, and cell migration and invasion [128].

© 2007 The Biochemical Society
Synaptojanin-2A regulates the distribution of mitochondria within the cell, being recruited to the mitochondrial outer membrane via a PDZ-mediated interaction with OMP25 [110]. The endocytic protein binding properties of synaptojanin-2B1 and -2B2 and nerve terminal localization indicate that these isoforms are more similar in function to synaptojanin-1 [111].

OCRL

OCRL is the only 5-phosphatase which is specifically associated with an inherited human disease. Lowe’s oculocerebrorenal syndrome is a rare X-linked disorder characterized by renal failure, growth and mental retardation and cataracts [21]. Mapping of the breakpoint in two separate unrelated female Lowe’s patients identified the OCRL gene, encoded across 24 exons on the X chromosome [129]. The ubiquitously expressed OCRL protein carries a central 5-phosphatase domain, and a C-terminal region showing homology to the RhoGAP domain. The 5-phosphatase domain hydrolyses Ins(1,3,4,5)P$_4$, Ins(1,4,5)P$_3$, PtdIns(3,4,5)P$_3$ and PtdIns(4,5)P$_2$, showing preference for PtdIns(4,5)P$_2$ [14,130]. Some activity against PtdIns(3,5)P$_2$ has also been reported in vitro.

Lowe’s patients demonstrate an accumulation of lysosomal enzymes in their plasma [131], and OCRL has been localized to lysosomal membranes in a human kidney proximal tubule cell line [132], however, in most cells OCRL is predominantly found at the TGN (trans-Golgi network) and also at endosomal structures [133–135]. OCRL localizes to clathrin-coated intermediaries and following growth factor-stimulation translocates to the plasma membrane, in a Rac1-dependent manner [135,136].

OCRL regulates both actin dynamics and endosomal trafficking, mediated by both PtdIns(4,5)P$_2$ hydrolysis and stimulation of the GTPase activity of Rac. Cells from Lowe’s patients exhibit decreased actin stress fibres and increased actin punctae [137]. The isolated RhoGAP-like domain inhibits Rac-dependent actin polymerization [138]. OCRL binds the heavy chain of clathrin, and the α-adaptin subunit of AP-2, however, no co-localization of OCRL and AP-2 has been observed [135]. OCRL regulates trafficking between the endosome and the TGN, altering the intracellular distribution of AP-1 and the mannose-6-phosphate receptor [139]. Interestingly, knockout of OCRL in mice does not result in a Lowe’s phenotype, implying some functional redundancy of the 5-phosphatases. Double knockout with 5-phosphatase II is embryonically lethal [140]. The 5-phosphatase I, 5-phosphatase II and SHIP2 are also expressed in proximal kidney tubules in humans and are unable to compensate for OCRL function in Lowe’s patients, suggesting that OCRL performs a distinct non-redundant function in these cells [132]

5-phosphatase II

The ubiquitously expressed 5-phosphatase II, enriched in the kidney, testis, liver and lung, was first purified from human platelet cytosol as a 75 kDa
protein, exhibiting catalytic activity against PtdIns(4,5)\(P_2\), PtdIns(3,4,5)\(P_3\), Ins(1,3,4,5)\(P_4\) and Ins(1,4,5)\(P_3\) [141–143]. A partial human cDNA was isolated from a screen searching for Ins(1,3,4,5)\(P_4\) binding proteins [144], with a complete cDNA including a C-terminal CAAX motif later isolated [145] and characterized. Encoded by the Inpp5b gene, 5-phosphatase II carries a central 5-phosphatase domain and the C-terminal CAAX motif and exhibits a high degree of homology to OCRL. The plasma and intracellular membrane localization of the protein has been shown to be mediated by both farnesylation of the CAAX motif and also N-terminal sequences [146]. Akin to other 5-phosphatase family members, differential RNA splicing is tissue specific and developmentally regulated, with two RNA transcripts producing proteins of 115 kDa and 105 kDa [146].

Inpp5b knockout mice were created in order to test the hypothesis that 5-phosphatase II could compensate for OCRL and explain the lack of a Lowe’s syndrome phenotype in OCRL knockout mice. Inpp5b knockout mice showed no obvious altered phenotype, apart from the observation that the males exhibit progressive testicular degeneration leading to sterility [140]. The Ocrl/Inpp5b double knockout is embryonically lethal, indicating possible overlapping function of these 5-phosphatases. Investigation of the cause of testicular degeneration in Inpp5b knockout mice supports a role for the 5-phosphatase in the regulation of intracellular trafficking. Ultrastructural examination of Inpp5b mutant testes has revealed vacuolarization of Sertoli cells, with proteins of adherens junctions and both apical and basolateral cell surfaces seemingly trapped in endosomal structures [147]. It is hypothesized that a reduction in functional adherens junctions causes premature shedding of germ cells from the seminiferous epithelium. The immature sperm produced in the Inpp5b knockout mice show reduced motility and have lower levels of processed β-fertilin (ADAM2) which results in decreased fertilization ability [148]. An inositol polyphosphate 4-phosphatase, as well as the 5-phosphatase II, exhibit GTP-dependent interaction with Rab5 thereby facilitating Rab5-directed production of PtdIns3P [149]. In complex with Rab5, 5-phosphatase II shows increased PtdIns(3,4,5)\(P_3\) 5-phosphatase activity, and 5-phosphatase II RNAi knockdown promotes Rab5-dependent receptor mediated endocytosis [149].

### 5-phosphatase I

5-phosphatase I, also called the 43 kDa 5-phosphatase, hydrolyses only the soluble inositol phosphates Ins(1,4,5)\(P_3\) and Ins(1,3,4,5)\(P_4\) and thereby regulates intracellular calcium signalling. This 5-phosphatase contains a central 5-phosphatase domain followed by a C-terminal CAAX motif that regulates its localization to the plasma membrane, the site of phospholipase C-mediated Ins(1,4,5)\(P_3\) production [150]. In activated platelets, 5-phosphatase I binds the phosphoprotein pleckstrin, which increases its catalytic activity. In addition, an interaction with 14-3-3\(\zeta\) also regulates enzyme activity, mediated by a phosphorylation-independent interaction.
The binding of Ins(1,4,5)P$_3$ to its receptor, IP$_3$R, results in the release of calcium from intracellular stores located in the endoplasmic reticulum. Increases in cytoplasmic calcium levels regulate many cellular functions including cellular proliferation, via activation of transcription factors both in the cytoplasm [NFAT (nuclear factor of activated T-cells) and in the nucleus [CREB (cAMP-response-element-binding protein), smooth muscle cell contraction, secretion of neurotransmitters at the neuronal synapse and fertilization.

Ins(1,4,5)P$_3$ may be phosphorylated at the 3´ position of the inositol ring by the Ins(1,4,5)P$_3$-kinase to form Ins(1,3,4,5)P$_4$, or dephosphorylated by 5-phosphatases to form Ins(1,4)P$_2$. The latter inositol phosphate does not promote intracellular calcium release. Ins(1,3,4,5)P$_4$ itself regulates calcium influx across the plasma membrane, and is also hydrolysed by 5-phosphatase I. Phosphorylation of the 5-phosphatase I by calcium/CaM (calmodulin) kinase II inhibits both Ins(1,4,5)P$_3$ and Ins(1,3,4,5)P$_4$ hydrolysis [151]. Low or high concentrations

![Figure 3 Intracellular localization of 5-phosphatases. (a) SHIP1, SHIP2, PIPP and SKIP regulate PM (plasma membrane) PtdIns(3,4,5)P$_3$ levels, following growth factor-stimulation or B cell receptor ligation. (b) Hydrolysis of PtdIns(4,5)P$_2$ by PLC (phospholipase C) creates Ins(1,4,5)P$_3$, a substrate of 5-phosphatase I (5-ptase I). 5-ptase I hydrolyses Ins(1,4,5)P$_3$ at the PM. (c) PtdIns(4,5)P$_2$ recruits endocytic proteins to the PM. Synaptojanin-1 and synaptojanin-2 regulate endocytosis from the PM, whereas OCRL regulates trafficking from endosomes to the TGN. 5-phosphatase II (5-ptase II), which localizes to the PM and intracellular membranes, may also regulate endosomal trafficking. The function of the 72 kDa 5-phosphatase (72 kDa 5-ptase) at the TGN is unknown. EE, LE and RE represent compartments of the early, late and recycling endosomal system respectively.

© 2007 The Biochemical Society
of Ins(1,4,5)P₃, differentially regulate the production of Ins(1,3,4,5)P₄ and the propagation of calcium signals [152]. The proposed molecular mechanisms mediating this effect appear to be a consequence of high concentrations of Ins(1,3,4,5)P₄ inhibiting the activity of 5-phosphatase I. ATP-induced calcium oscillations are abrogated in CHO cells overexpressing 5-phosphatase I [153], whereas fibroblasts underexpressing this 5-phosphatase show increased basal levels of Ins(1,4,5)P₃, spontaneous intracellular calcium oscillations in the absence of agonist and increased sensitivity to agonist upon stimulation [154]. These enhanced levels of Ins(1,4,5)P₃ correlate with a transformed cellular phenotype associated with increased proliferation, anchorage-independent growth and tumour formation in nude mice [155].

In C. elegans, targeted depletion of the 5-phosphatase I homologue ipp-5 affects the ovulation cycle due to aberrations in Ins(1,4,5)P₃ signalling [156]. The knockout of 5-phosphatase I results in a differing phenotype to the Ins(1,4,5)P₃-kinase knockout, reflecting that the complexity of Ins(1,4,5)P₃ regulation is conserved in this organism.

Summary

In conclusion, although many functional studies have been undertaken to characterize this enzyme family, there is much that remains to be explored. A summary of the intracellular sites of 5-phosphatase activity, and pathways which they regulate is found in Figure 3. Although the 5-phosphatases have not been strongly associated with cancer, genetic screens have implicated SKIP in MDS, PIPP in breast cancer, SHIP1 in CML, and the 72 kDa 5-phosphatase in cervical cancer, suggesting that these enzymes play yet to be discovered roles in human disease.

References

© 2007 The Biochemical Society
The inositol polyphosphate 5-phosphatases


© 2007 The Biochemical Society


© 2007 The Biochemical Society
The inositol polyphosphate 5-phosphatases 179


© 2007 The Biochemical Society

© 2007 The Biochemical Society
The inositol polyphosphate 5-phosphatases


