Chapter 7
Phosphoinositide Phosphatases: Just as Important as the Kinases

Jennifer M. Dyson, Clare G. Fedele, Elizabeth M. Davies, Jelena Becanovic and Christina A. Mitchell

Abstract Phosphoinositide phosphatases comprise several large enzyme families with over 35 mammalian enzymes identified to date that degrade many phosphoinositide signals. Growth factor or insulin stimulation activates the phosphoinositide 3-kinase that phosphorylates phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] to form phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3], which is rapidly dephosphorylated either by PTEN (phosphatase and tensin homologue deleted on chromosome 10) to PtdIns(4,5)P_2, or by the 5-phosphatases (inositol polyphosphate 5-phosphatases), generating PtdIns(3,4)P_2. 5-phosphatases also hydrolyze PtdIns(4,5)P_2 forming PtdIns(4)P. Ten mammalian 5-phosphatases have been identified, which regulate hematopoietic cell proliferation, synaptic vesicle recycling, insulin signaling, and embryonic development. Two 5-phosphatase genes, *OCRL* and *INPP5E* are mutated in Lowe and Joubert syndrome respectively. SHIP [SH2 (Src homology 2)-domain inositol phosphatase] 2, and SKIP (skeletal muscle- and kidney-enriched inositol phosphatase) negatively regulate insulin signaling and glucose homeostasis. *SHIP2* polymorphisms are associated with a predisposition to insulin resistance. SHIP1 controls hematopoietic cell proliferation and is mutated in some leukemias. The inositol polyphosphate 4-phosphatases, INPP4A and INPP4B degrade PtdIns(3,4)P_2 to PtdIns(3)P and regulate neuroexcitatory cell death, or act as a tumor suppressor in breast cancer respectively. The Sac phosphatases degrade multiple phosphoinositides, such as PtdIns(3)P, PtdIns(4)P, PtdIns(5)P and PtdIns(3,5)P_2 to form PtdIns. Mutation in the Sac phosphatase gene, *FIG4*, leads to a degenerative neuropathy. Therefore the phosphatases, like the lipid kinases, play major roles in regulating cellular functions and their mutation or altered expression leads to many human diseases.

Keywords Inositol polyphosphate 5-phosphatases · Inositol polyphosphate 4-phosphatases · Sac phosphatases · Trafficking · Hematopoietic system

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7.1 Introduction

Phosphoinositide phosphatases are a complex series of enzyme families that play critical roles in the regulation of insulin signaling and glucose metabolism, the progression and invasion of cancer, neurodegenerative diseases and myopathies and are implicated in the pathogenesis of many other human diseases. In mammalian cells, over 35 phosphoinositide phosphatases have been identified and some, but not all, have been extensively characterized. The results of recent mouse gene knockout studies and emerging evidence of mutations or epigenetic change in specific phosphoinositide phosphatases have revealed non-redundant roles for these phosphoinositide-metabolizing enzymes *in vivo*.

Many phosphoinositide phosphatases were originally identified and classified following their purification from tissue homogenates using enzyme assays that recognized the ability of the lipid phosphatase to degrade specific phosphoinositides and/or or inositol phosphates *in vitro*. This approach classified these phosphoinositide phosphatases on the basis of the phosphate group that was removed by the phosphatase from the inositol ring of the phosphoinositide or inositol phosphate substrate, hence 3-phosphatases, 4-phosphatases and 5-phosphatases were characterized. More recently many phosphoinositide phosphatases have been identified based on homology within specific catalytic domains and several large families of enzymes including the inositol polyphosphate 5-phosphatases (10 mammalian family members) have been identified. Other smaller families including the SAC phosphatases, and the 4-phosphatases have also been characterized. The substrates of these various lipid phosphatases are shown in Fig. 7.1.

There is some commonality of catalytic mechanism of action amongst the different lipid phosphatases. 4-phosphatases and the SAC phosphatase contain a CX_5R catalytic motif. In contrast the inositol polyphosphate 5-phosphatases are members of the apurinic/apyrimidinic (AP) endonuclease family of enzymes. Below we have described the major characteristics of the 5-, 4-phosphatases and the SAC phosphatases, concentrating on recent studies that delineate the roles these phosphatases play in human diseases. Given the breadth of enzymes and their many functions we refer throughout the text to recent excellent reviews for areas that are not extensively covered.

7.2 Inositol Polyphosphate 5-Phosphatases

The inositol polyphosphate 5-phosphatases are a relatively large family of 10 mammalian and 4 yeast enzymes and all contain a conserved 300 amino acid catalytic domain. The yeast enzymes will not be described here but are reviewed elsewhere (Strahl and Thorner 2007). These enzymes remove the 5-position phosphate from phosphorylated phosphoinositides and inositol phosphates and exhibit overlapping substrate specificities and also many share common protein-protein interaction modules. Many of the 5-phosphatases are widely expressed, in many of the

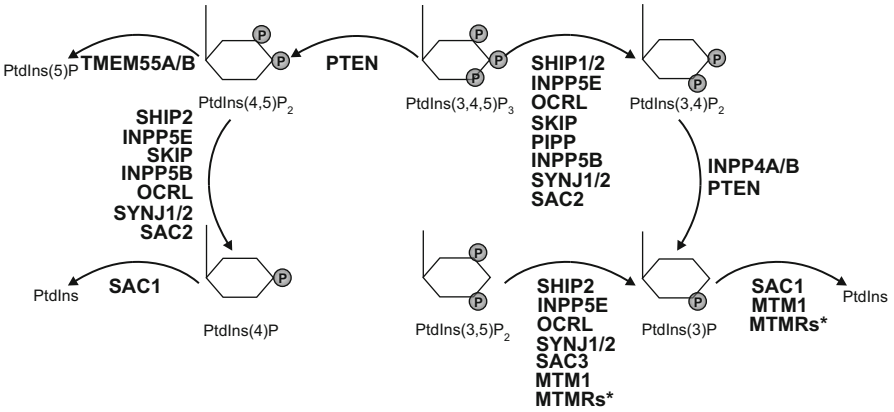

Fig. 7.1 *In vitro* and *in vivo* activities of mammalian phosphoinositide phosphatases. The reported activities of the mammalian phosphatases and their *in vitro* and known *in vivo* phosphoinositide substrates are shown. The repertoire of *bona fide in vivo* substrates of many of these enzymes remains to be delineated. *MTM and MTMR refers to myotubularin and related family members, not discussed here. Abbreviations: *PtdIns* Phosphatidylinositol, *SYNJ* synaptojanin

same cells and tissues and exhibit apparently similar enzymatic properties. Emerging evidence describing the phenotypes of 5-phosphatase knockout mice and the recent demonstration that some 5-phosphatases such as *OCRL* and *INPP5E* are mutated in human diseases indicates that although these enzymes share common features, there is little functional redundancy. It is likely specificity of function is established via distinct subcellular localization, and/or binding partners and association into specific signaling complexes. Here we describe the common features of the 5-phosphatase family and highlight some recent interesting studies that reveal the functional diversity of this complex enzyme family. The 5-phosphatases regulate glucose homeostasis, various aspects of hematopoietic function, embryonic development, neuronal and kidney development, protein trafficking, synaptic vesicle recycling, actin cytoskeleton dynamics and thereby cell migration, and cell viability to name a few.

7.2.1 5-Phosphatase Structure and Enzyme Activity

The inositol polyphosphate 5-phosphatases are Mg^{2+}-dependent phosphoesterases that hydrolyze the 5-position phosphate from the inositol ring of the water-soluble inositol phosphates Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 and the lipid-bound PtdIns-derived second messengers PtdIns(4,5)P_2, PtdIns(3,4,5)P_3, and PtdIns(3,5)P_2, reviewed by (Astle et al. 2007; Ooms et al. 2009). Within the 5-phosphatase family the majority of the enzymes share overlapping substrate specificities. A notable exception is the 43 kDa 5-phosphatase (5-phosphatase-1, INPP5A) which only hydrolyzes the

soluble inositol phosphates, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 forming Ins(1,4)P_2 and Ins(1,3,4)P_3 respectively. In contrast the 72 kDa 5-phosphatase (Inpp5e) only metabolizes phosphoinositides and is the most potent PtdIns(3,4,5)P_3 5-phosphatase *in vitro* (Kisseleva et al. 2000). Some of the other nine family members also hydrolyze soluble inositol phosphates, such as Ins(1,4,5)P_3 and/or Ins(1,3,4,5)P_4 and potentially other inositol phosphates, but this has not been extensively characterized (Ooms et al. 2009). It has been challenging to determine what the *bona fide in vivo* substrates of the 5-phosphatases are that result in specific phenotypes following their loss of function, since these enzymes *in vitro* degrade both PtdIns(3,4,5)P_3, PtdIns(4,5)P_2 and in some cases also PtdIns(3,5)P_2, in addition to inositol phosphates. Few studies have attempted to correlate total cellular phosphoinositide and inositol phosphate levels with functional defects in knockout mice, siRNA-depleted cells and/or human cell lines with 5-phosphatase mutations. This is made even more challenging for the 5-phosphatases, synaptojanin-1 and 2, which contain an additional catalytic Sac domain, which contains a Cx_5R motif that facilitates mono- and bis-phosphorylated phosphoinositide metabolism, hydrolyzing PtdIns(3)P, PtdIns(4)P and PtdIns(3,5)P_2 to PtdIns (Guo et al. 1999; Nemoto et al. 2001). The Sac domain is also present in two (Inp52/3p) of the four yeast 5-phosphatases (Strahl and Thorner 2007). How the two catalytic domains function together is not clearly defined but it is interesting to speculate that given the 5-phosphatase domain of synaptojanin hydrolyzes PtdIns(4,5)P_2 to PtdIns(4)P, this may then allow access of the Sac domain to PtdIns(4)P, which it degrades to PtdIns. To add to this complexity, unlike PTEN which directly opposes the activity of PI3K, 5-phosphatase degradation of PtdIns(3,4,5)P_3 generates a new signal, PtdIns(3,4)P_2, which functions to activate some of the same effectors as PtdIns(3,4,5)P_3, including Akt (Franke et al. 1997; Scheid et al. 2002; Ma et al. 2008). In addition, 5-phosphatase degradation of PtdIns(4,5)P_2, generates another signaling molecule, PtdIns(4)P. Both 5-phosphatase substrates and products of their catalysis are also targets for other lipid phosphatases which degrade phosphates from the inositol ring at the 4- and 3-position (to be discussed below) thereby providing a complex metabolic cascade of signaling molecules.

Common to all 5-phosphatases is a 300 amino acid catalytic domain that folds in a manner similar to the apurinic/apyrimidinic repair endonuclease family of DNA-modifying enzymes (Whisstock et al. 2000; Tsujishita et al. 2001). Many 5-phosphatases contain additional domains that facilitate their subcellular localization and/or interaction with other proteins. These include SH2, proline-rich domains, CAAX motifs at the C-terminus, WW, SAM, ASH domains, inactive RhoGAP (RhoGTPase-activating protein) and SKICH (SKIP (skeletal muscle- and kidney-enriched inositol phosphatase)) carboxyl homology domains (see Fig. 7.2). Below we describe the characteristics of each of the 10 mammalian 5-phosphatases. These enzymes have a complex nomenclature and the reader is referred to Table 7.1 which describes their various names and also provides a summary of the reported phenotypes for mouse knockouts of each 5-phosphatase.

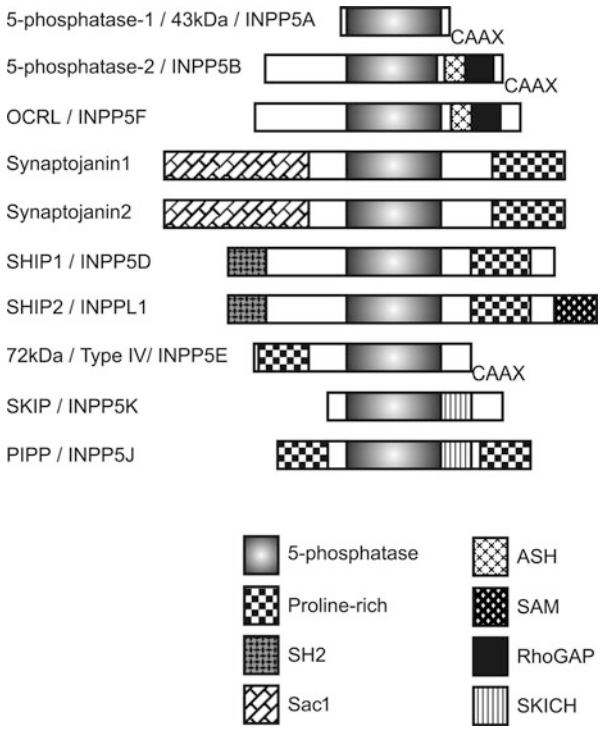

Fig. 7.2 Domain structure of the 10 mammalian inositol polyphosphate 5-phosphatases. Inositol polyphosphate 5-phosphatases contain a central 300 amino acid catalytic domain and distinct structural elements, which facilitate specific sub-cellular localization or define protein-protein or lipid-protein interactions, and may direct substrate specificity. The carboxyl-terminal CAAX motifs of INPP5A, INPP5B and INPP5E mediate plasma membrane targeting. Plasma membrane localization of SKIP and PIPP is regulated by the SKICH domain. The SH2 domains of SHIP1 and SHIP2 mediate protein interactions with tyrosine-phosphorylated receptors. The SAC domains of synaptojanin 1 and synaptojanin 2 mediate hydrolysis of PtdIns(3)P, PtdIns(4)P, or PtdIns(3,5)P_2. Proline-rich regions present in synaptojanin 1, synaptojanin 2, SHIP1, SHIP2, PIPP and INPP5E are proposed to mediate protein-protein interactions. ASH domains within INPP5B and OCRL are typically associated with ciliary proteins and may interact with microtubules

7.2.2 SHIP1

The SHIP (SH2-containing inositol phosphatase) family comprises SHIP1 (also known as INPP5D) and SHIP2 (also called INPP5L1). SHIP1 regulates many aspects of hematopoesis. Full length SHIP1 (SHIP1α) may be spliced to generate three shorter isoforms called SHIP1β, SHIP1δ and s-SHIP1 (Lucas and Rohrschneider 1999; Tu et al. 2001). SHIP1α and SHIP1δ exhibit a restricted distribution to hematopoietic and spermatogenic cells (Liu et al. 1998b). SHIP1, and the most closely related 5-phosphatase SHIP2, exhibit a similar domain structure, with an N-terminal SH2 domain, and central 5-phosphatase domain that hydrolyzes PtdIns(3,4,5)P_3 and Ins(1,3,4,5)P_4, but contain divergent C-terminal proline rich

Table 7.1 Inositol polyphosphate 5-phosphatases and phenotype of knockout mouse models

Protein name	Alias(es)	Gene name (s)	Knockout animal models	References
INPP5A	IP5-P-1 Type I IP5-P 43 kDa IP5-P	*INPP5A*	Not reported	
INPP5B	IP5-P-2 75 kDa IP5-P	*INPP5B*	Males—progressive testicular degeneration leading to sterility Double KO with *Ocrl* results in embryonic lethality	(Hellsten et al. 2001; Janne et al. 1998)
SHIP1	SHIP SHIP-1 IP5-P D	*INPP5D* *SHIP* *SHIP1*	Hematopoietic perturbations, lung pathology and shortened life span (Constitutive KO) Alterations in cytokine-mediated activation that influences Th2 response and cell cytotoxicity (T cell specific KO) Reduced B cells and an increase in basal serum IgGs. Splenic B cells exhibited increased proliferation and enhanced MAPK activation ($Ship1^{-/-}/Rag^{-/-}$ chimera)	(Helgason et al. 1998; Tarasenko et al. 2007; Liu et al. 1998a)
SHIP2	SHIP-2 51C protein	*INPPL1* *SHIP2*	Reduced body weight; normal insulin tolerance on standard chow diet but resistant to weight gain on high fat diet (mouse constitutive KO and rat transient SHIP2 knockdown)	(Sleeman et al. 2005; Buettner et al. 2007)
INPP5E	Pharbin Type IV IP5-P IP5-P E 72 kDa IP5-P	*INPP5E*	Embryonic or early post natal lethality. Features consistent with ciliopathy syndrome including, bilateral anophthalmos, postaxial hexadactyly, kidney cysts, anencephaly and exencephaly and ossification defects	(Jacoby et al. 2009)
OCRL	Lowe oculocerebrorenal syndrome protein IP5-P F	*OCRL* *INPP5F*	Testicular degeneration after sexual maturity	(Janne et al. 1998)

Table 7.1 (continued)

Protein name	Alias(es)	Gene name (s)	Knockout animal models	References
Synaptojanin-1	SJ1, SYNJ1	*SYNJ1* *INPP5G*	Born at mendelian frequencies however 85% of homozygotes died within 24h of birth, whilst remaining 15% failed to thrive and died within 15 days of birth. Homozygotes exhibited an accumulation of clathrin coated vesicles in nerve terminals. Brain cytosol from homozygotes had increased potency to generate clathrin coated liposomes (compared to WT)	(Cremona et al. 1999)
Synaptojanin-2	SYNJ2	*SYNJ2* *INPP5H*	Not reported	
PIPP	Phosphatidylinositol (4,5) bisphosphate 5-phosphatase A	*PIB5PA* *INPP5* *INPP*	Not reported	
SKIP	None	*SKIP* *INPP5K*	*Homozygotes:* Embryonic lethality (E10.5)—cause not reported *Heterozygotes:* Increased glucose tolerance and insulin sensitivity	(Ijuin et al. 2008)

**IP5*-P (*i*nositol *p*olyphosphate *5-p*hosphatase)

domains. The SHIP1 C-terminus contains two NPXY motifs, that following phosphorylation, bind proteins with PTB domains including Shc, Dok 1, Dok 2; and four PxxP motifs that bind SH3-containing proteins such as Grb2, Src, Lyn, Hck, Abl, PLCg1, and PIAS1 (Liu et al. 1994), reviewed in (Hamilton et al. 2011; Sasaki et al. 2009). s-SHIP, a stem-cell specific 104 kDa isoform, lacks the SH2 domain. s-SHIP associates with receptor complexes that are important for embryonic and hematopoietic stem cell growth and survival. Recently using a transgenic mouse model, s-SHIP promoter activity was characterized in actively functioning mammary stem cells, the proposed precursor cells to basal-like human breast cancers (Bai and Rohrschneider 2010).

SHIP1 has been extensively studied within the hematopoietic system and plays a regulatory role in B and T cells, dendritic cells, macrophages, mast cells, osteoclasts, platelets and neutrophils. *Ship1*$^{-/-}$ cells in general are more proliferative and exhibit

greater survival as a consequence of enhanced activation of both mitogen-activated protein kinase (MAPK) and PI3K/Akt pathways, reviewed in (Hamilton et al. 2011). *Ship1*-null mice develop a chronic myelogenous leukaemia (CML)-like myeloproliferative disease, with splenomegaly, failure to thrive, elevated macrophage and granulocyte counts, associated with massive myeloid infiltration of the lungs, leading to a shortened life span (Brauweiler et al. 2000b; Helgason et al. 1998, 2000; Liu et al. 1999). Stimulation of *Ship1*$^{-/-}$ hematopoietic progenitors by a number of agonists including monocyte colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), interleukin-3, or stem cell factor (SCF) results in hyperproliferative responses and increased resistance to apoptosis, associated with amplified Akt signaling (Helgason et al. 1998). SHIP1 is also the most significant phosphoinositide phosphatase that regulates neutrophil migration. S*hip1*$^{-/-}$ mice exhibit significant granulocytic infiltration of many organs including the lung, in contrast to the phenotype of mice with granulocyte-specific deletion of PTEN, which do not exhibit granulocytic tissue infiltration (Nishio et al. 2007). Genetic inactivation of *Ship1* significantly impairs neutrophil polarization and motility. Studies by Nishio et al revealed SHIP1 governs the formation of the actin-rich leading edge and thereby polarization of neutrophils by regulating the spatial distribution of PtdIns(3,4,5)P_3 which is required for chemotaxis (Nishio et al. 2007). The early death of *Ship1*$^{-/-}$ mice may be in part a consequence of altered neutrophil chemotaxis, which leads to the accumulation of neutrophils in the lungs.

7.2.2.1 B and T Lymphocyte Regulation by SHIP1

SHIP1 associates with ITIM's (immunoreceptor tyrosine-based inhibitory motif) in B cells and ITAM's (immunoreceptor tyrosine-based activation motif), FcγRIIa and 2B4, in macrophages and Natural Killer (NK) cells (Bruhns et al. 2000; Kimura et al. 1997; Osborne et al. 1996). SHIP1 plays a significant role in regulating B cell numbers and function. In B lymphocytes, SHIP1 associates with the Fcγ receptor II-B complex, where the 5-phosphatase inhibits signals stimulated by immune-complexed antigen (Isnardi et al. 2006; Poe et al. 2000; Eissmann et al. 2005; Nakamura et al. 2002). In B cells and mast cells, the non receptor tyrosine kinase Lyn and SHIP1 act together to negatively regulate M-CSF-dependent Akt activation (Baran et al. 2003). SHIP1 is also a target of Lyn-dependent phosphorylation and Lyn and SHIP1 cooperate in regulating FcγRIIb-inhibitory signaling in B cells and mast cells. SHIP1 also regulates autonomous B cell receptor (BCR) signaling (Brauweiler et al. 2000a). SHIP1 is linked to the suppression of B-cell activating factor (BAFF)-induced signaling and also functions independent of the BCR to suppress signaling mediated by chemokine receptors such as CXCR4 (Crowley et al. 2009; Brauweiler et al. 2007). *Ship1*$^{-/-}$ mice exhibit a reduction in the size of the peripheral B cell compartment and reduced BCR-induced proliferation (Brauweiler et al. 2000a; Helgason et al. 2000; Liu et al. 1998b). In addition as *Ship1*$^{-/-}$ mice age, the number of B lymphocytes reduces, as a consequence of elevated IL6 secretion by macrophages (Maeda et al. 2010). SHIP1 may also regulate B cell maturation. Irradiated mouse bone marrow

reconstituted with *Ship1*$^{-/-}$ hematopoietic cells shows a reduction in the immature and mature forms of B cells (Helgason et al. 2000; Liu et al. 1998a).

MicroRNA-155 (miR-155) is a critical regulator of immune cell development, function and disease (Baltimore et al. 2008). Recently *SHIP1* has been identified as a miR-155 target, which results in a reduction in SHIP1 protein expression in a group of diffuse large B cell lymphomas (Pedersen et al. 2009) and in a miR-155 transgenic B lymphoma mouse model (Costinean et al. 2009). MiR-155 represses SHIP1 through direct 3′UTR interactions. Retroviral delivery of a miR-155-formatted siRNA against SHIP1 results in a phenotype reminiscent of miR-155 transgenic mice or *Ship1* knockout mice, both result in a myeloproliferative-like syndrome (O'Connell et al. 2009). Moreover, in miR-155 transgenic mice, SHIP1 is gradually down-regulated in preleukemic and leukemic pre-B cells. Down regulation of SHIP1, as well as another IL-6 inhibitor, C/EBPβ (CCAAT enhancer-binding protein β), at the pre B stage (when miR-155 is maximally expressed) may block B-cell differentiation and induce a reactive proliferation of the myeloid cells (Costinean et al. 2009). Down-regulation of SHIP1 expression has also been described in macrophages responding to inflammatory stimuli (O'Connell et al. 2009).

Epigenetic down-regulation of SHIP1 expression in response to inflammation may play a role in promoting the transformation of B cells. Both PTEN and SHIP1 degrade PtdIns(3,4,5)P_3 and regulate Akt-dependent cell proliferation and survival. However, *Ship1* knockout mice do not exhibit a predisposition to lymphoma (Miletic et al. 2010). Interestingly, concomitant deletion of Pten and Ship1 (b*Pten/Ship1*$^{-/-}$) is associated with the development of spontaneous B cell neoplasms, consistent with marginal zone lymphoma, or with a lower frequency, follicular or centroblastic lymphoma. B cells from b*Pten/Ship1*$^{-/-}$ mice proliferate in response to BAFF, unlike single *Ship1* knockout cells. Therefore PTEN and SHIP1 may cooperatively suppress B cell lymphoma (Miletic et al. 2010). Ikaros are a family of transcription factors required for lymphoid development, and when functionally abnormal can contribute to lymphoid malignancy. Chip analysis reveals that the Ikaros bind to the promoter region of *INPP5D* (gene for SHIP1) and when Ikaros are not present SHIP1 expression is increased (Alinikula et al. 2010).

SHIP1 is expressed in both CD4 and CD8 lymphocytes. Peripheral T cells are constitutively active in *Ship1*$^{-/-}$ mice, consistent with previous *in vitro* studies suggesting SHIP1 functions downstream of T cell receptor (TCR) activation, reviewed in (Gloire et al. 2007; Parry et al. 2010). Ligation of CD3 or CD28 on T cells leads to SHIP1 phosphorylation, associated with activation of its 5-phosphatase activity (Edmunds et al. 1999). The expression of SHIP1 is reduced in a human Jurkat T-cell line. Re-expression of SHIP1 in Jurkat T-cells results in decreased levels of PtdIns(3,4,5)P_3, Akt activation and reduced cell proliferation (Horn et al. 2004; Fukuda et al. 2005). Expression of KLF2 (Krüppel-like factor 2), a negative regulator of T-cell proliferation, may be regulated by SHIP1 (Garcia-Palma et al. 2005). SHIP1 is also incorporated in multi-protein complexes which include LAT, Dok-2, Grb-2 and also interacts with and may contribute to the regulation of the Tec-kinase (Tomlinson et al. 2004; Dong et al. 2006). *Ship1*$^{-/-}$ mice exhibit some reduction in the total peripheral T cell numbers, and T cells show constitutive activation. Regulatory T cells

(Tregs) express CD4 and CD25 markers and limit the risk of autoimmune disease arising from TCR crossreactivity (Kashiwada et al. 2006; Stephens et al. 2005). *Ship1*$^{-/-}$ mice show increased numbers of Tregs that maintain their immunosuppressive capacity. However, in the constitutive *Ship1*$^{-/-}$ mice it was difficult to dissect the T cell specific phenotype, due to the complex effects of cytokines. In contrast T cell specific deletion of SHIP1 does not affect T cell or thymic development, T cell receptor signaling, or the number of Tregs. Rather T cell specific deletion of both SHIP1, and the smaller, s-SHIP isoform, reveals a role for SHIP1 in the generation of lymphocyte subsets and in the maintenance of inflammatory versus regulatory cells, Th1 and Th2 respectively (Tarasenko et al. 2007) favouring Th1 lymphocyte differentiation. SHIP1 thereby plays a significant role in controlling the levels of inflammatory T cells. T cell restricted *Ship1* knockout mice exhibit decreased expression of multiple cytokines including IL4, IL5 and IL13 (Th2 cytokines) (Tarasenko et al. 2007) with a diminished capacity to respond to *in vivo* challenge to this pathway.

Dendritic cells (DCs) are critical for the processing, and presentation of antigen to T cells. *Ship1*$^{-/-}$ mice exhibit an absence of allograft rejection (Ghansah et al. 2004; Wang et al. 2002) and SHIP1 regulates DC maturation and function, reviewed in (Hamilton et al. 2011). *Ship1*$^{-/-}$ splenic dendritic cells are increased in number and exhibit an altered morphology (Neill et al. 2007). SHIP1 also inhibits the generation of myeloid dendritic cells from bone marrow precursors, but promotes their maturation and function (Antignano et al. 2010).

7.2.2.2 SHIP1 Regulates Macrophage Activity and Function

Classically activated, or M1 macrophages are induced in response to the Type I cytokines, IFN-γ and TNF-α, which stimulate the production of pro-inflammatory cytokines, to phagocytose and kill intracellular microorganisms and tumor cells. Alternatively activated, or M2 macrophages are generated in response to stimulation by type II cytokines, such as IL-4, IL-10, and IL-13, anti-inflammatory cytokines that play important roles in killing extracellular microorganisms and parasites, and in promoting wound healing, reviewed in (Hamilton et al. 2011). In *Ship1*$^{-/-}$ mice, macrophages are M2 skewed in contrast to wildtype littermates which are M1 skewed (Rauh et al. 2005). Ship1-null peritoneal macrophages exhibit hyperactivation of components downstream of FcγR, resulting in an elevated IL-6 production, a cytokine that can exacerbate myelopoiesis (Maeda et al. 2010). SHIP1 expression can be increased in macrophages via activation of Toll-like receptors 4/9, which leads to the production of autocrine-acting TGFβ, that in turn stimulates a 10-fold increase in SHIP1 protein levels (Sly et al. 2009).

Several of the 5-phosphatases including SHIP1, SHIP2 and INPP5E inhibit macrophage phagocytosis, by degrading PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, signals that promote phagocytosis. SHIP1 and INPP5E are recruited to the macrophage phagocytic cup, the site of PtdIns(3,4,5)P_3 generation during phagocytosis. SHIP1 preferentially regulates phagocytosis mediated via the CR3 receptor, and to a lesser extent, also FcγR-mediated phagocytosis (Ai et al. 2006; Nakamura et al. 2002;

Cox et al. 2001; Horan et al. 2007). SHIP1 also regulates phagosome maturation and ROS production. The 5-phosphatase, INPP5E, hydrolyzes PtdIns(3,4,5)P_3 during phagocytosis, and like SHIP1, inhibits FcγR-mediated phagocytosis, but shows less activity for complement-mediated phagocytosis (Horan et al. 2007). The related SHIP2 also inhibits phagocytosis. SHIP2 may indirectly regulate PtdIns(4,5)P_2 levels, lipid signals that significantly regulate actin dynamics during phagocytosis via Rac-mediated activation of the PtdIns(4)P-5-kinase (Ai et al. 2006).

Some pathogens including *Francisella tularensis* (*F. tularensis*) escape phagolysosomal fusion allowing for their replication in the cytosol (Checroun et al. 2006; Chong et al. 2008). Upon host cell infection with *F. tularensis*, Fas expression increases, associated with activation of Fas-mediated apoptosis. In *Ship1*$^{-/-}$ macrophages, phagocytosis of *F. tularensis* is not affected, however, fusion events between the pathogen—containing phagocytes and lysosomes are significantly reduced, thereby promoting intra-macrophage growth of the pathogen, leading to activation of SP-1 and -3 transcription factors, induction of Fas and promotion of Fas-induced cell death (Rajaram et al. 2009). As the role of SHIP1 is only evident upon pathogenic bacterial infection, it is likely that SHIP1 may be acting indirectly. Interestingly, infection of macrophages with the less virulent form of *F. tularensis*, *F. novicida*, strongly induces miR-155 and reduces SHIP1 expression, however, infection with a virulent *F. tularensis* isolate, SCHU S4, does not alter miR-155. The differential induction of the miR-155 response in macrophages challenged with pathogenic *versus* non-pathogenic subspecies, and its subsequent effect on SHIP1 expression may contribute to the success of *F. tularensis* as an infectious agent (Cremer et al. 2009).

7.2.2.3 SHIP1 Regulation of Mast cells, Osteoclasts and Platelets

Mast cells regulate allergic reactions via interaction of surface FcεRI molecules with circulating IgE antibodies and when activated contribute to anaphylaxis and allergic asthma. Aggregation of IgE-bearing FcεRI molecules by polyvalent antigen leads to the release of proinflammatory mediators including histamine and TNFα, reviewed in (Galli and Tsai 2010). ITAMs within the β and γ subunits of the FcεRI multimer bind to the SH2 domain of SHIP1 (Kimura et al. 1997; Osborne et al. 1996). SHIP1 functions as a negative regulator of FcεRI-mediated cellular responses in mast cells by controlling the levels of PtdIns(3,4,5)P_3, signals which promote mast cell degranulation (Liu et al. 1999; Huber et al. 1998). SHIP1 is also recruited to the recently identified novel ITIM-like domain-containing receptor, Allergin 1, and facilitates Allergin-1's inhibition of IgE-stimulated mast cell degranulation and anaphylaxis in mice (Hitomi et al. 2010). Thus under normal conditions SHIP1 prevents degranulation signaling unless there is overwhelming challenge by antigens. However, SHIP1 is not required for mast cell differentiation (Rauh et al. 2003). *Ship1*$^{-/-}$ mast cells exhibit increased degranulation compared with *Ship1*$^{+/+}$ mast cells in response to IgE cross-linking (Huber et al. 2002). Naive *Ship1*$^{-/-}$ mice, under steady state conditions, show signs of allergic asthma such as airway inflammation

and remodeling, with mucus hyperproduction (Roongapinun et al. 2010). *Ship1*$^{-/-}$ mice also show systemic mast cell hyperplasia, increased levels of the cytokines IL-6, TNF, and IL-5, and heightened susceptibility to anaphylaxis (Haddon et al. 2009).

Osteoclasts are bone resorbing cells of monocyte-macrophage origin. M-CSF and RANKL (receptor activator of nuclear factor-κB ligand) stimulate the differentiation of osteoclast precursors, and this is enhanced with loss of SHIP1 (Takeshita et al. 2002). In osteoclasts SHIP1 localizes to podosomes, sites of adhesion and metallomatrix proteinase secretion. *Ship1*$^{-/-}$ osteoclasts exhibit an increased ability to resorb mineralized matrix, and Ship1-null mice exhibit signs of severe osteoporosis, due to the presence of increased numbers of enlarged and hypernucleated osteoclasts (Takeshita et al. 2002). Interestingly, SHIP1, via its SH2 domain, complexes with the inhibitory adaptor and plasma membrane protein, DAP12, thereby restricting the recruitment of PI3K to DAP12 and reduces macrophage and osteoclast activation (Peng et al. 2010).

Both SHIP1 and the related SHIP2 are expressed in human platelets, essential components of the initial events in blood clot formation. SHIP1 is implicated in controlling irreversible platelet aggregation (Severin et al. 2007; Trumel et al. 1999). SHIP2 forms a complex with the adhesion receptor, glycoprotein Ib/IX, in human platelets, however the function of this complex remains to be determined (Dyson et al. 2003). In addition, studies by Giuriato et al suggest that SHIP1, rather than SHIP2, controls PtdIns(3,4,5)P_3 levels in response to a number of platelet agonists (Giuriato et al. 1997). Following thrombin stimulation SHIP1 is tyrosine phosphorylated, correlating with its relocation to the actin cytoskeleton and PtdIns(3,4,5)P_3 production (Giuriato et al. 1997). Collagen-related peptide stimulation of SHIP1-deficient platelets results in increased PtdIns(3,4,5)P_3 signals and activation of Bruton's tyrosine kinase (Btk), which promotes calcium entry. *Ship1*$^{-/-}$ platelets exhibit defects in platelet aggregation and abnormal platelet contractility. Ship1-null mice show prolonged bleeding and abnormal thrombus organization, suggesting SHIP1 is required for normal hemostasis (Severin et al. 2007).

7.2.2.4 SHIP1 and its Association with Human Leukemia

Emerging evidence has linked changes in SHIP1 expression and/or SHIP1 mutations in some leukemias. Recent analysis of 81 primary T-cell acute lymphatic leukemias (ALLs) has revealed inactivation of SHIP1 and PTEN, with sparing of *PIK3CA*, suggesting a role for loss of SHIP1 in disease pathogenesis (Lo et al. 2009). Mutations in the SHIP1 gene (*INPP5D*) have also been identified within the region encoding the 5-phosphatase domain that result in loss of 5-phosphatase activity in acute myeloid leukemia (Luo et al. 2003, 2004). Although there is little evidence for mutations in *SHIP1* in solid tumors, recent reports have revealed tumors grow more rapidly in *Ship1*$^{-/-}$ mice potentially resulting from SHIP1's role in regulating immune responses to tumor cells (Rauh et al. 2005) reviewed in (Hamilton et al. 2011).

In Friend murine leukemia virus (F-MuLV)-induced erythroleukemia, a proto-oncogene transcription factor, Fli-1, is activated. Fli-1 activity is downstream of PI3K in a negative feedback loop, in which SHIP1 and Fli-1 regulate each other to direct erythropoietin (Epo) signaling leading to either erythrocyte proliferation or differentiation (Lakhanpal et al. 2010). Ship1-null mice exhibit accelerated progression of F-MuLV-induced erthroleukemia and Fli-1 represses expression of Ship1 during this process.

The chronic myeloid leukemia (CML) causative fusion protein, BCR/ABL, inhibits SHIP1 expression, with elevated SHIP1 expression noted in primitive CML cells, but in terminally differentiating CML cells, SHIP1 levels are reduced at the post-transcriptional level (Jiang et al. 2003; Sattler et al. 1999). Recently it was proposed that BCR/ABL-mediated SHIP1 expression is regulated by BCR/ABL-modulated phosphorylation of SHIP1, which acts as a trigger for its ubiquitination, possibly by c-Cbl leading to its proteosomal degradation (Ruschmann et al. 2010). These findings suggest that SHIP1 may act as a tumor suppressor in CML.

7.2.3 SHIP2

The 142 kDa 5-phosphatase, SHIP2 (gene name *INPPL1*) regulates insulin signaling and metabolism. This enzyme is composed of an N-terminal SH2 domain, a central catalytic domain and a C-terminal proline-rich domain (PRD) which contains additional motifs including WW, NPXY and a sterile alpha (SAM) motif. This 5-phosphatase shares a high degree of homology and similar domain structure to the hematopoietic specific 5-phosphatase SHIP1, but differs in its PRD. SHIP2 expression overlaps in part with that of SHIP1 in hematopoietic cells, and plays a non-redundant role in macrophages (Giuriato et al. 2003; Bruyns et al. 1999; Wisniewski et al. 1999). However, SHIP2 is also widely expressed in many other cells and tissues (Muraille et al. 1999; Sleeman et al. 2005). *In vitro* studies have revealed SHIP2 utilizes a broad number of substrates including several inositol phosphates, and phosphoinositides including PtdIns(3,4,5)P_3, PtdIns(3,5)P_2 and PtdIns(4,5)P_2 (Taylor et al. 2000; Pesesse et al. 1997; Schmid et al. 2004). SHIP2 also hydrolyzes other inositol phosphates and phosphoinositides *in vitro* (Chi et al. 2004). However the majority of reports have concentrated on SHIP2's role in regulating PtdIns(3,4,5)P_3 signaling, rather than its regulation of other phosphoinositides or inositol phosphates and what role this activity plays *in vivo* is unclear.

As assessed by traditional microscopy techniques, SHIP2 is predominantly cytosolic but upon growth factor/insulin stimulation or cell-matrix contact, the 5-phosphatase translocates to the plasma membrane where it regulates the actin cytoskeleton via association with a number of actin-regulatory proteins including filamin, p130Cas, Shc, vinexin and LL5β (Takabayashi et al. 2010; Gagnon et al. 2003; Paternotte et al. 2005; Prasad et al. 2001; Dyson et al. 2001). More recently, using TIRF (total internal reflection fluorescence) microscopy, recombinant SHIP2 has been detected, like synaptojanin and OCRL, at clathrin-coated vesicles, mediated by

an interaction between the SHIP2 PRD with intersectin. At this site SHIP2 acts as a negative regulator of clathrin-coated pit growth (Nakatsu et al. 2010).

In a number of insulin-sensitive cells including podocytes, SHIP2 overexpression regulates insulin-stimulated PI3K-generated PtdIns(3,4,5)P_3 degradation and thereby Akt activation resulting in reduced plasma membrane GLUT4 association and as a consequence reduced glucose uptake and glycogen synthesis (Hyvonen et al. 2010; Sasaoka et al. 2001; Wada et al. 2001). Surprisingly, however, SHIP2-depleted 3T3 L1 adipocytes do not exhibit altered insulin-mediated PI3K/Akt signaling (Tang et al. 2005). Linking the actin cytoskeletal and insulin signaling properties of SHIP2 is its interaction in the glomeruli with CD2-associated protein, which also binds with the key podocyte adhesion protein, nephrin and actin (Hyvonen et al. 2010). Overexpression of SHIP2 induces a 2-fold increase in podocyte apoptosis. Endogenous Ship2 protein levels are significantly elevated in the glomeruli of diabetic mice and rats (Hyvonen et al. 2010).

Clinical studies have identified a correlation between insulin resistance and the development of diabetic nephropathy (Parvanova et al. 2006; Orchard et al. 2002). Polymorphisms in the SHIP2 gene, *INPPL1*, have been identified in diabetic and also control subjects and are implicated in the pathogenesis of Type 2 diabetes, hypertension and the metabolic syndrome. Additionally, some reports have demonstrated altered expression of polymorphic SHIP2 *in vitro*. For example, a 16bp deletion in the 3′-untranslated region (UTR) of *INPPL1* has been detected in Type 2 diabetic patients, compared to healthy subjects, which when expressed in HEK cells, increased expression of the reporter protein. Although these studies are *in vitro*, it does suggest the 16bp deletion in the 3′UTR may enhance SHIP2 expression *in vivo* resulting in altered phosphoinositide metabolism and insulin sensitivity (Marion et al. 2002). Additionally, single nucleotide polymorphisms (SNPs) have been identified in *INPPL1*. In a British cohort study, *INPPL1* SNPs are significantly associated with diabetes and hypertension (Kaisaki et al. 2004). Surprisingly, analysis of the same SNPs in a French and British cohort did not identify association of *INPPL1* SNPs with diabetic patients *per se*, rather, an association was observed with hypertensive metabolic syndrome patients (Kaisaki et al. 2004; Marcano et al. 2007). In contrast in a Japanese cohort, *INPPL1* SNPs have been identified in control, rather than diabetic patients. Analysis of one of the several *INPPL1* SNPs identified in control subjects, that is located in the 5-phosphatase domain, results in less efficient inhibition of insulin-stimulated PtdIns(3,4,5)P_3 levels and Akt phosphorylation, consistent with reduced 5-phosphatase activity. It has been proposed that *INPPL1* SNPs in control subjects may protect subjects from Type 2 diabetes (Kagawa et al. 2005). *INPPL1* SNPs have also been identified in non-coding sequences. SNPs in the promoter and 5′UTR of the *INPPL1* gene are associated with impaired fasting glycaemia. In cell culture systems, one *INPPL1* haplotype was examined, which like the 16bp deletion in the 3′UTR, resulted in increased promoter activity, suggesting increased SHIP2 expression (Ishida et al. 2006). Collectively these studies indicate that polymorphisms in *INPPL1* are associated with hypertensive metabolic subjects, whilst at the molecular level, these polymorphisms, or at least those examined, may act to increase SHIP2 expression.

SHIP2 is also highly expressed in the brain and in NGF-stimulated PC12 cells. It localizes to lamellipodia and neurite buds, suggesting that SHIP2 may play a role in the early events of neurite budding (Aoki et al. 2007). Supporting this, siRNA-mediated knockdown of Ship2 in Pten-deficient PC12 cells, results in an elevation in the number of neurites per cell but also hyper-elongation (Aoki et al. 2007). *Ship2* knockout mice do not exhibit an overt neurological phenotype, however, detailed analysis has not been reported.

7.2.3.1 *Ship2*$^{-/-}$ Mice: Regulation of Glucose Homeostasis and Weight Gain

Two studies have reported the phenotype of *Ship2*$^{-/-}$ mice with conflicting results. In the first report Ship2-null mice were generated by targeted deletion of exons 19–29 of the *Inppl1* gene (Clement et al. 2001). These mice die within 3 days of birth, associated with low blood glucose and insulin concentrations. However in this study the *Phox2a* gene was also inadvertently deleted, and this may contribute to the mouse phenotype. In a more recent study, *Ship2*$^{-/-}$ mice were generated by deletion of the first 18 exons of the *Inppl1* gene, which encodes the SH2 domain and 5-phosphatase catalytic domain. *Ship2*$^{-/-}$ mice are viable but showed reduced body weight, associated with reduced serum lipids and 6-fold lower serum leptin levels on a standard chow diet (Sleeman et al. 2005). No changes in fasting serum glucose, insulin levels, or glucose/insulin tolerance were detected. On a standard chow diet, *Ship2*$^{-/-}$ mice exhibit enhanced insulin-mediated Akt and p70S6K activation in the liver and skeletal muscle. On a high fat diet, Ship2-null mice are resistant to weight gain, hyper-glycemia or insulinemia and show decreased serum lipids. This latter study has been validated by more recent studies using a variety of approaches described below.

Acute knockdown of Ship2 using antisense oligonucleotides in rats essentially recapitulates the phenotype observed in *Ship2*$^{-/-}$ mice (Buettner et al. 2007). On a standard diet, Ship2 knockdown rats show normal insulin tolerance, whilst in contrast, on a high fat diet the rats exhibit increased glucose disposal. Enhanced insulin-stimulated Akt activation is detected in the muscle of Ship2 knockdown rats, correlating with reduced Ship2 expression in this tissue. Data from two rodent models with either chronic ablation of Ship2, or acute reduction of Ship2, has revealed a role for this enzyme in regulating diet-induced insulin resistance (Buettner et al. 2007; Sleeman et al. 2005). Therapeutically, whether long-term application of SHIP2 anti-sense therapy is a viable option for the treatment of insulin resistance is unclear, however, anti-sense therapies have been successful in treating some human diseases (Jason et al. 2004).

Transgenic mice overexpressing Ship2 (Ship2-Tg) exhibit significant weight gain (5%) and elevated fasting insulin, but not glucose, leptin, adiponectin or serum lipids levels compared to wildtype controls (Kagawa et al. 2008). Additionally Ship2-Tg mice display impaired glucose tolerance and insulin sensitivity, correlating with reduced insulin-stimulated Akt activation in the liver, skeletal muscle and fat. Surprisingly, decreased phosphorylation of insulin receptor substrate 1 (IRS1) is observed

in the liver of Ship2-Tg mice as well as impaired glucose homeostasis. Moreover, increased liver G6P (glucose-6-phosphatase) and PEPCK (phosphoenolpyruvate carboxykinase) mRNA coupled with reduced glycogen content and GK (glucokinase) mRNA in Ship2-Tg indicates hepatic insulin resistance. Liver-specific overexpression of Ship2 in a diabetic rodent model (db/db) has also been reported (Fukui et al. 2005). Leptin receptor-deficient db/db mice are leptin resistant and diabetic, and exhibit insulin resistance associated with decreased PI3K/Akt signaling, hyperglycemia, hyperinsulinemia, increase gluconeogenesis and other metabolic related symptoms (Kobayashi et al. 2000). Overexpression of wildtype Ship2 in db/+ m (heterozygote) mice decreases liver Akt activation, whilst overexpression of catalytically inactive Ship2 (ΔIP-Ship2) which acts as a dominant-negative, enhances liver Akt activation, but does not affect insulin signaling in peripheral insulin-sensitive tissues (Fukui et al. 2005). In contrast, overexpression of wildtype Ship2 in the liver results in reduced glucose tolerance, whilst mice expressing the catalytically inactive Ship2 display improved tolerance, suggesting Ship2 modulates liver-restricted insulin sensitivity to influence peripheral glucose and insulin tolerance. Insulin signaling is also critical for brain function (Muntzel et al. 1995). Endogenous Ship2 protein levels in db/+ m mice are elevated relative to controls and are further increased in homozygous mice (db/db). Ship2-Tg mice show impairment of brain insulin/insulin-like growth factor I (IGF-I) signaling, attenuation of the neuroprotective effects of insulin/IGF-I, and a decline in learning and memory. Inhibition of Ship2, using the specific inhibitor, AS1949490, in the db/db mice, improves synaptic plasticity and memory formation (Suwa et al. 2009). Liver-specific inhibition of Ship2 in a second diabetic mouse model, KKA^{y}, is associated with reduced liver G6P and PEPCK mRNA in response to pyruvate, probably as a consequence of increased gluconeogensis due to insulin resistance (Grempler et al. 2007). Collectively these studies reveal SHIP2 plays complex roles in regulating insulin signaling and metabolism in a number of insulin-sensitive tissues.

7.2.3.2 SHIP2 and Regulation of Cancer

SHIP2 has been implicated in regulating the cell cycle, although, the mechanisms are yet to be fully delineated. SHIP2 overexpression induces glioblastoma cell cycle arrest, and also suppresses platelet derived growth factor (PDGF)- and IGF-stimulated cell cycle progression in vascular smooth muscle cells (Taylor et al. 2000; Sasaoka et al. 2003). SHIP2 is predicted to be either a suppressor or enhancer of cell proliferation, depending on the cell type. In HeLa cells, SHIP2 expression regulates receptor endocytosis and ligand-induced epidermal growth factor receptor (EGFR) degradation. In breast cancer cells, SHIP2 controls the levels of this receptor, promoting cell proliferation and tumor growth and in this context SHIP2 does not act as a tumor suppressor (Prasad 2009). Under conditions of SHIP2 siRNA, EGF-stimulated Akt activation is suppressed, in contrast to findings in insulin-stimulated cells where Ship2 loss of expression enhances PI3K/Akt signaling (Prasad 2009). SHIP2 therefore plays a role in promoting EGF-stimulated Akt activation, possibly by regulating

EGFR internalization. This is consistent with recent findings that SHIP2 may regulate clathrin-coated pit formation (Nakatsu et al. 2010).

Recently SHIP2 has been implicated in the pathogenesis of squamous cell carcinoma (SSC) (Sekulic et al. 2010; Yu et al. 2008). The SHIP2 gene, *INPPL1*, is a target of small non coding microRNA (miRNA)-205, which suppresses its expression in stratified squamous epithelia. Aggressive SCCs show reduced expression of SHIP2 with increased miRNA-205 expression. Interfering with miRNA-205 by distinct mechanisms, increases SHIP2 expression and suppresses Akt activation, and increases keratinocyte cell death (Yu et al. 2008). miRNA-205 is upregulated in several carcinomas, so it will be of interest in future studies to examine its effects on SHIP2 expression and Akt regulation in these cancers (Iorio et al. 2005, 2009; Majid et al. 2010).

7.2.4 Synaptojanin 1 and 2

Synaptojanin 1 and 2 (gene names *SYNJ1* and *SYNJ2*, respectively) are two related 5-phosphatases that share amino acid sequence homology and a similar domain structure and both contain two distinct catalytic domains. Both enzymes contain an N-terminal Sac1 domain, a central 5-phosphatase domain and divergent C-terminal proline-rich domains. The 5-phosphatase domain hydrolyzes the 5-position phosphate from PtdIns(3,4,5)P_3, PtdIns(4,5)P_2, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, whilst the Sac1 domain contains a CX_5R motif that mediates the dephosphorylation of phosphates from the inositol ring of PtdIns(3)P, PtdIns(4)P and PtdIns(3,5)P_2 to form PtdIns (Guo et al. 1999; Nemoto et al. 2001; McPherson and Marshall 1996). Synaptojanin 1 and 2 can be alternatively spliced in the C-terminal proline rich domain giving rise to 145 and 170 kDa isoforms of synaptojanin 1 and up to six isoforms of synaptojanin 2 (Nemoto et al. 1997, 2001; Ramjaun and McPherson 1996; Seet et al. 1998). The 145 kDa isoform of synaptojanin 1 is highly expressed in presynaptic nerve terminals, however, the 170 kDa synaptojanin 1 isoform is not expressed in neuronal cells (McPherson et al. 1996; Ramjaun and McPherson 1996).

Synaptojanin 1 is highly expressed in the brain and localizes to presynaptic nerve terminals where it complexes with multiple interacting proteins including Grb2 (growth-factor-receptor-bound protein 2), dynamin, syndapin, endophilin, amphiphysin I and II, Eps15, clathrin, AP-2, DAP160 (dynamin-associated protein 160)/intersectin, myosin 1E, Snx9 (sortin nexin 9) and phospholipase C (Krendel et al. 2007; Yeow-Fong et al. 2005; Haffner et al. 2000; Ahn et al. 1998; McPherson et al. 1996) reviewed by (Astle et al. 2007). In general these interactions promote synaptojanin 1 subcellular localization and/or enhance its catalytic activity (Ringstad et al. 1997; Schuske et al. 2003; Lee et al. 2004). Many of these interacting partners function to regulate endocytosis. Intersectin 1 is an endocytic scaffolding protein that may coordinate clathrin coat assembly with synaptojanin 1-mediated PtdIns(4,5)P_2 hydrolysis (Adayev et al. 2006). AP2, the adaptor protein that binds clathrin, also

binds to intersectin 1, and it has been proposed this interaction may occlude the recruitment of PtdIns(4,5)P_2-bound synaptojanin 1 (Pechstein et al. 2010).

Synaptojanin 1 interacts with endophilin, which targets the 5-phosphatase to sites of endocytosis and promotes its 5-phosphatase catalytic activity in degrading PtdIns(4,5)P_2 (Ringstad et al. 1997; Schuske et al. 2003; Lee et al. 2004). The enzymatic activity of synaptojanin 1 is also regulated by its phosphorylation, including its constitutive phosphorylation in unstimulated nerve terminals by EphB2 and Cdk5 (Lee et al. 2004; Irie et al. 2005). Cdk5 (cyclin-dependent kinase 5) phosphorylation of synaptojanin 1 impairs its 5-phosphatase activity and its interaction with endophilin 1 and amphiphysin 1 (Lee et al. 2004). Following nerve depolarization, synaptojanin 1 is dephosphorylated by the serine threonine phosphatase calcineurin (McPherson et al. 1996; Bauerfeind et al. 1997). EphB2 tyrosine kinase phosphorylation of synaptojanin 1 also impairs its association with endophilin (Irie et al. 2005). In contrast, phosphorylation of synaptojanin 1 by the dual-specificity tyrosine-phosphorylated and regulated kinase 1A (MNB/DYRK1A) increases synapojanin 1 PtdIns(4,5)P_2 5-phosphatase activity and regulates its interaction with amphiphysin 1 and intersectin 1 (Adayev et al. 2006). The subcellular targeting of synaptojanin 1 is also influenced by the fatty acid groups on PtdIns(4,5)P_2. Synaptojanin 1 exhibits a preference for PtdIns(4,5)P_2-containing long chain polyunsaturated fatty acids over synthetic PtdIns(4,5)P_2 with two saturated fatty acids (Schmid et al. 2004). In *Caenorhabditis elegans* (*C.elegans*) mutants which lack the preferred fatty acids groups on PtdIns(4,5)P_2, synaptojanin 1 is mislocalized resulting in defective synaptic vesicle recycling (Marza et al. 2008).

Deletion of synaptojanin 1 homologs in mice, *D. melanogaster* and *C.elegans* has revealed synaptojanin 1 regulates clathrin-mediated endocytosis and neuronal function. Most *Synj1*$^{-/-}$ mice die shortly after birth, and the 15% of knockout mice that survive up to 15 days exhibit progressive weakness, ataxia and convulsions. Neurons from mice with loss of synaptojanin show increased PtdIns(4,5)P_2, correlating with an accumulation of clathrin-coated vesicles, associated with decreased synaptic vesicles (Cremona et al. 1999). A similar phenotype is observed in synaptojanin (unc-26) deficient *C.elegans* and *D. melanogaster* (Harris et al. 2000). PtdIns(4,5)P_2 regulates the releasable pool of synaptic vesicles and secretory granules at nerve synapses (Gong et al. 2005; Milosevic et al. 2005; Di Paolo et al. 2004), and the formation of new synaptic vesicles at the plasma membrane (Cremona et al. 1999; Verstreken et al. 2003; Mani et al. 2007; Van Epps et al. 2004; Harris et al. 2000). PtdIns(4,5)P_2 may affect several steps in the synaptic vesicle cycle, a process that relies on clathrin-dependent endocytosis, and functions to supply new synaptic vesicles during nerve stimulation (Kasprowicz et al. 2008; Haucke 2003). Synaptic vesicles are released following stimulation and fuse with the presynaptic plasma membrane, and are then in turn recycled via endocytosis via clathrin-mediated reinternalization. Clathrin-coated pit formation requires the assembly of endocytic proteins at PtdIns(4,5)P_2-enriched membrane sites. By degrading PtdIns(4,5)P_2, synaptojanin 1 directs the uncoating of clathrin, and thereby the recycling of the vesicles. Hence in the absence of synaptojanin 1, there is an accumulation of clathrin-coated vesicles. Interestingly, in *D. melanogaster*, disruption of the NCA (Na^+/Ca^{2+} antiporter) ion

channel, or *unc-80*, which encodes a novel protein required for ion channel subunit localization, partially suppresses the synaptojanin 1 knockout phenotype, whilst overexpression of endophilin can also partially rescue the synaptojanin-null phenotype, suggesting that aberrant NCA ion channel and endophilin function, may be required for proper synaptic vesicle recycling (Jospin et al. 2007).

Finally another function for synaptojanin 1 has been identified in *Danio rerio* (*D. rerio*). An ENU mutagenesis screen for *D. rerio* larvae with vestibular defects identified mutant *Synj1* induces abnormalities in hair cell basal blebbing and may function to regulate the number and release of synaptic vesicles at hair-cell ribbon synapses (Trapani et al. 2009).

7.2.4.1 Association of Synaptojanin 1 with Human Disease

Increased expression of synaptojanin 1 has been described in Down's syndrome by several groups using various experimental approaches. The synaptojanin gene (*SYNJ1*) is localized to chromosome 21q22.2, a region implicated in Down's syndrome, bi-polar disorder and schizophrenia (Cheon et al. 2003; Arai et al. 2002). In a Down's syndrome mouse model, Ts65Dn, and also in transgenic synaptojanin 1 mice, increased expression of synaptojanin 1 is associated with a decrease in PtdIns(4,5)P_2 levels, leading to cognitive defects (Voronov et al. 2008). The *D. melanogaster* orthologs of three genes implicated in Down's syndrome, *syn* (synaptojanin), *dap160* (intersectin) and *nla* (nebula), when overexpressed individually in *D. melanogaster* lead to abnormal synaptic morphology. However, overexpression of all three candidate genes concomitantly is required for defective endocytosis, impaired vesicle recycling and locomotor defects. Moreover, dissection of the activities of the functional complex formed between synaptojanin, intersectin and nebula, has revealed nebula regulates 5-phosphatase catalytic activity, whilst intersectin directs the subcellular localization of synaptojanin (Chang and Min 2009). Synaptojanin mutations also exacerbate polyglutamine toxicity in *C.elegans*, suggesting a potential protective role for synaptojanin, like endophilin, in Huntington's disease (Parker et al. 2007).

7.2.4.2 Synaptojanin 2

Synaptojanin 2 is much less characterized than synaptojanin 1 however, it plays a role in the regulation of clathrin-mediated endocytosis. Synaptojanin 2 knockout mice have not been described to date. Synaptojanin 2 may play a protective role as a regulator of hair cell survival and hearing. However recently, a mutant mouse called Mozart which exhibits recessively inherited non syndromic progressive hearing loss, has been shown to result from mutation in *SYNJ2*. This mutation occurs in the catalytic 5-phosphatase domain resulting in loss of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 degrading activity. *SYNJ2* but not *SYNJ1* is expressed in the inner and outer hair cells within the cochlea. Mozart mutant mice show progressive hearing loss with hair loss (Manji et al. 2011). RNAi-mediated depletion of synaptojanin 2 in a lung carcinoma

cell line impairs clathrin-mediated receptor internalization, resulting in a reduction of clathrin-coated pits and vesicles (Rusk et al. 2003). Synaptojanin 2 splice variants specifically interact with Rac1 in a GTP-dependent manner resulting in translocation of synaptojanin 2 from the cytosol to the plasma membrane, facilitating inhibition of endocytosis (Malecz et al. 2000; Nemoto et al. 2001). A role for synaptojanin 2 in Rac1-mediated cell invasion and migration has also been identified via the regulation of lamellipodia and invadipodia formation. RNAi-mediated depletion of either Rac1 or synaptojanin 2 in glioblastoma cells equally inhibits cell migration and invasion, in addition to reducing the formation of invadipodia and lamellipodia, suggesting synaptojanin 2 may act downstream of Rac1 within this signaling pathway (Chuang et al. 2004).

7.2.5 INPP5E: A Lipid Phosphatase Linked to Cilia

INPP5E (also called the Type IV 5-phosphatase, or pharbin, gene name *INPP5E*) is widely expressed in the brain, kidneys, testis and other tissues and plays a critical role in the regulation of embryonic development (Kisseleva et al. 2000; Kong et al. 2000; Asano et al. 1999). Human INPP5E and the rat enzyme, pharbin, share 74% amino acid sequence identity (Kisseleva et al. 2000). This lipid phosphatase contains the central 5-phosphatase domain, flanked by regions with proline rich motifs, with a C-terminal CAAX motif. The 5-phosphatase has been localized to the cytosol and a perinuclear/Golgi localization in proliferating cells (Kong et al. 2000). In macrophages, during phagocytosis INPP5E localizes to the phagocytic cup and regulates FcγR1-mediated phagocytosis (Horan et al. 2007). In cells which have exited the cell cycle INPP5E localizes to cilia (Jacoby et al. 2009), sensory projections that co-ordinate cell signaling pathways and play a role in regulating cell division and differentiation. Cilia are also important for many aspects of embryonic development (Tobin and Beales 2009).

INPP5E hydrolyzes the 5-position phosphate from PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, forming PtdIns(4)P and PtdIns(3,4)P_2 respectively, and is reported to be the most potent PtdIns(3,4,5)P_3 5-phosphatase *in vitro* (Kisseleva et al. 2000; Kong et al. 2000). Overexpression of mouse Inpp5e in unstimulated 3T3-L1 adipocytes also generates PtdIns(3)P at the plasma membrane of transfected cells, via hydrolysis of PtdIns(3,5)P_2, promoting the translocation and insertion of the glucose transporter, GLUT4, into the plasma membrane, without affecting glucose uptake (Kong et al. 2006). However, these results are based on ectopic Inpp5e overexpression and this may not occur *in vivo*. Inpp5e is expressed in the hypothalamus and, following insulin stimulation, is tyrosine phosphorylated and interacts with IRS-1/IRS-2 and PI3K (Bertelli et al. 2006). Antisense-mediated reduction of Inpp5e in the hypothalamus results in reduced food intake and weight loss, accompanied by altered metabolic parameters including reduced serum insulin, leptin and glucose levels (Bertelli et al. 2006).

7.2.5.1 INPP5E is Mutated in Human Ciliopathy Syndromes

Recent exciting studies have reported the phenotype of *Inpp5e* homozygous null mice, demonstrating a significant role for this 5-phosphatase in embryonic development. In addition *INPP5E* is mutated in two human ciliopathy syndromes, genetic diseases that affect the function of cellular cilia, or anchoring structures such as the basal body (Tobin and Beales 2009). Homozygous deletion of *Inpp5e* in mice, results in late embryonic lethality or death shortly after birth, with exencephaly, polydactyly, and kidney cysts, features of a ciliopathy (Jacoby et al. 2009). An inducible global knockout of *Inpp5e* leads to polycystic kidneys by 6 months and elevated body weight, indicating the 5-phosphatase also plays a significant functional role in the adult animal (Jacoby et al. 2009). In human studies a SNP for *INPP5E* has been identified in MORM (mental retardation, obesity, congenital retinal dystrophy and micropenis in male), a rare ciliopathy syndrome that results in a truncated INPP5E protein lacking the C-terminal 18 amino acids, which encompasses the CAAX motif. This motif is required for correct 5-phosphatase cilia axoneme localization (Jacoby et al. 2009). Whether the elevated body weight in Inpp5e-null adult mice is due to a regulatory role of the 5-phosphatase in insulin signaling is unknown, however, patients with ciliopathy syndromes frequently exhibit obesity (Tobin and Beales 2009). A second study has identified SNPs in *INPP5E* in human subjects with Joubert syndrome, which is characterized by underdevelopment of the cerebral vermis, polydactyly, retinal degradation, mental retardation and other phenotypes (Bielas et al. 2009). All six SNPs localize to regions encoding the catalytic domain of the INPP5E protein. These mutants exhibit reduced activity in hydrolyzing PtdIns(3,4,5)P_3 and to a lesser extent PtdIns(4,5)P_2. Overexpression of the Joubert SNP INPP5E mutants does not suppress Akt signaling, unlike expression of wildtype INPP5E. Interestingly, a homozygous mutation in *INPP5E* has been identified in a Joubert Syndrome (ciliopathy) patient that has only mild clinical features, including minimal truncal ataxia and oculomotor apraxia and normal cognitive function. Neuroimaging of the brain revealed the classical molar tooth sign which occurs in Joubert Syndrome (Poretti et al. 2009). The molecular mechanisms by which loss of function of INPP5E leads to similar complex phenotypes in both mice and humans is yet to be fully delineated, specifically how this relates to INPP5E regulation of PtdIns(3,4,5)P_3/Akt signaling is unknown.

7.2.5.2 INPP5E and Cancer

Gene expression profiling has revealed altered expression of *INPP5E* in a variety of cancers including cervical cancer, in which *INPP5E* is one of the top five (of 74 genes) with altered expression, showing an over 50-fold change (Yoon et al. 2003). Additionally *INPP5E* is one of the top six genes upregulated in non-Hodgkin's lymphoma following treatment (Chow et al. 2006). In uterine leiomyosarcoma compared to normal myometrium *INPP5E* RNA levels are increased greater than 5-fold (Quade et al. 2004). Decreased *INPP5E* gene expression has also been identified in stomach cancer and metastatic adenocarcinoma (Ramaswamy et al. 2003; Kim et al. 2003).

Interestingly, co-overexpression of INPP5E and another 5-phosphatase, SKIP, has been detected in gemcitabine-resistant pancreatic cell lines (Akada et al. 2005). It is tempting to speculate whether the different profiles of *INPP5E* gene expression in cancer are related to the newly identified function of INPP5E at cilia, as these organelles have a recently identified role in cancer, and depending on the initiating event, may either hinder or instigate tumor growth (Toftgard 2009). Moreover, cilia assembly and disassembly is linked to the cell cycle. It is therefore of interest that overexpression of INPP5E decreases cell growth due to increased apoptosis, whilst mouse embryonic fibroblasts (MEFs) isolated from the Inpp5e-null mice exhibit cell cycle arrest, albeit at moderate levels, possibly due to altered cilia disassembly in response to platelet-derived growth factor (PDGF) stimulation (Jacoby et al. 2009).

7.2.6 OCRL

OCRL (also called Lowe protein, gene name *INPP5F*) is a 5-phosphatase that is mutated in Lowe oculocerebrorenal (OCRL) syndrome and in some cases of Dent-2 disease (Attree et al. 1992; Hoopes et al. 2005; Sekine et al. 2007; Utsch et al. 2006). Lowe syndrome is an X-linked disorder affecting ~1 in 200,000 births that is characterized by growth and mental retardation, bilateral congenital cataracts and renal impairment, associated with impaired solute and protein reabsorption in the kidney proximal tubule and renal tubular acidosis (Lowe 2005). Female carriers show punctate opacities in the lens (Gardner and Brown 1976; Roschinger et al. 2000). Dent-2 disease is characterized by low molecular weight proteinuria and renal failure (Bokenkamp et al. 2009). OCRL shares 45% amino acid identity and a similar domain structure to the related 5-phosphatase, INPP5B. Both enzymes contain a recently identified NH2-terminal PH domain, a central catalytic 5-phosphatase domain, followed by an ASH domain (ASPM (abnormal spindle-like microcephaly-associated protein)/SPD2 (spindle pole body 2)/hydin) and a catalytically inactive C-terminal Rho-GAP domain (Mao et al. 2009). Mutations in some ASH-domain-containing proteins are associated with abnormalities in brain development, such as hydrocephalus (Kumar et al. 2004; Bond et al. 2003; Ponting 2006). The OCRL sequence also contains two clathrin box binding motifs, one in its N-terminal PH domain and the second within the inactive Rho-GAP domain, as well as a clathrin adaptor AP-2-binding motif that is flanked by the PH and 5-phosphatase catalytic domains (Mao et al. 2009). OCRL and INPP5B share overlapping substrate specificity, hydrolyzing the 5-position phosphate from the inositol rings of PtdIns(4,5)P_2, PtdIns(3,4,5)P_3, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, whilst OCRL also hydrolyzes PtdIns(3,5)P_2 (Schmid et al. 2004; Zhang et al. 1995). However, several studies suggest the major substrate that OCRL regulates in intact cells is PtdIns(4,5)P_2 (Zhang et al. 1998). For example the total cellular levels of PtdIns(4,5)P_2, but not PtdIns(3,4,5)P_3, are increased in cells from Lowe syndrome patients (Zhang et al. 1995).

OCRL is expressed in a wide range of human and mouse tissues (Janne et al. 1998). Surprisingly, although mutations in human OCRL cause significant human disease, deletion of Ocrl in mice does not lead to either Lowe or Dent-2 disease-like phenotypes (Janne et al. 1998). This lack of functional redundancy in mice may be

due to compensation by other 5-phosphatases, most probably Inpp5b (Janne et al. 1998). Mice which lack both Ocrl and Inpp5b are embryonic lethal (Bernard and Nussbaum 2010).

7.2.6.1 OCRL Forms Multiple Protein Complexes and Regulates Vesicular Trafficking

OCRL interacts with clathrin, AP-2, cdc42 (cell division cycle 42), Rac, APPL1 (adaptor protein containing PH domain) Ses1/2 (from the word "sesquipedalian"), GIPC (GAIP-interacting protein, C terminus) and active forms of Rab1 and Rab5/6, the latter also stimulates the PtdIns(4,5)P_2 activity of OCRL (Swan et al. 2010; Hyvola et al. 2006). Various types of mutations, including missense and truncating, have been identified in Lowe syndrome, many in the region encoding the 5-phosphatase catalytic domain. However, mutations in the ASH/RhoGAP domain have also been identified. Interestingly, APPL1 and Ses1/2 proteins bind to the same region of the ASH/RhoGAP domain of OCRL, and their association with OCRL is mutually exclusive. Furthermore, like APPL1, Ses1/2 localizes to the endocytic compartment (Swan et al. 2010). APPL1 resides on a subset of peripheral OCRL-positive endosomes that are derived from clathrin-coated pits that receive internalized receptors.

OCRL mutations occur in both Lowe and Dent-2 patients (Shrimpton et al. 2009) and although Lowe syndrome and Dent-2 disease share common kidney defects, for unknown reasons, Dent-2 disease patients do not exhibit all the abnormalities that are commonly present in Lowe syndrome (Dent and Friedman 1964; Cho et al. 2008; Hoopes et al. 2005; Utsch et al. 2006; Shrimpton et al. 2009). Disease causing mutations in OCRL have been identified throughout the coding sequence, some of which result in reduced protein expression, by generating a non-sense transcript whilst others are located in the ASH/RhoGAP domain, a region of OCRL that commonly mediates its association with other proteins (McCrea et al. 2008; Addis et al. 2004; Swan et al. 2010). Interestingly, regardless of the site of mutation in OCRL, Lowe patient fibroblasts all exhibit elevated PtdIns(4,5)P_2, suggesting OCRL localization and association with other proteins is, in addition to its 5-phosphatase activity, critical for OCRL function (Lichter-Konecki et al. 2006; Kawano et al. 1998; Lin et al. 1997). The association of OCRL with APPL is abolished by disease causing mutations in the ASH/RhoGAP domain of OCRL (Erdmann et al. 2007; McCrea et al. 2008). Significantly, 10 of the Lowe syndrome and one of the Dent-2 disease-causing mutations in OCRL inhibit its association with APPL1 or Ses1/2, suggesting that selective loss of APPL1 or Ses1/2 binding to OCRL cannot explain the clinical differences between Lowe and Dent-2 disease (Swan et al. 2010). The only disease-causing mutation in *OCRL*, A861T, that is able to associate with the endocytic proteins APPL1 and Ses1/2, is both missense and a splice-site mutation, and has been proposed to regulate OCRL protein expression by generating a non-sense transcript (Swan et al. 2010; Kawano et al. 1998). It remains to be determined whether loss of the association between OCRL and APPL1 and/or Ses1/2 is involved in Lowe and/or Dent-2 disease. It has been suggested that using its ASH/RhoGAP binding surface, OCRL may bind

a progression of endocytic proteins via the same motif, as is the case for APPL and Ses1/2, and that cumulative defects in this process may contribute to altered OCRL function causing Lowe and/or Dent-2 disease (Swan et al. 2010). APPL1 binding to OCRL is abolished by disease causing mutations in the OCRL-ASH-Rho GAP domain (Swan et al. 2010). Failure of OCRL to associate with APPL1 may contribute to the neurological/cognitive defects observed in Lowe syndrome. Both APPL1 and GIPC bind the TrkA (tropomyosin receptor kinase A) NGF (nerve growth factor) receptor and together regulate TrkA endocytic trafficking (Erdmann et al. 2007; Lin et al. 2006; Varsano et al. 2006). GIPC and APPL1 also associate with megalin, a receptor expressed in the kidney proximal tubule which facilitates the uptake of low-molecular-mass proteins. Both GIPC- and megalin-knockout mice exhibit low-molecular-mass proteinuria, similar to that found in Lowe syndrome (Norden et al. 2002).

Mutations in the 5-phosphatase domain of OCRL contribute to the Lowe syndrome phenotype due to loss of PtdIns(4,5)P_2 hydrolysis, whereas mutations in the ASH and RhoGAP domains result in mislocalization of the protein (http://research.nhgri.nih.gov/lowe/) (Bond et al. 2003; Erdmann et al. 2007; McCrea et al. 2008). Some point mutations in the inactive RhoGAP domain of OCRL lead to impaired 5-phosphatase activity, possibly due to altered protein conformation (Lichter-Konecki et al. 2006). Mutations in OCRL which disrupt the interaction between the 5-phosphatase and Rab1, Rab5 or Rab6 perturb the Golgi targeting of the enzyme (Hyvola et al. 2006).

There is growing evidence OCRL regulates vesicular trafficking, however, despite many interesting studies it still remains to be determined how OCRL degradation of PtdIns(4,5)P_2 regulates many of these events. PtdIns(4)P localizes to the Golgi, but there is limited evidence for PtdIns(4,5)P_2 at this site. In non stimulated cells OCRL localizes to the TGN (*trans*-Golgi network), lysosomes and endosomes and is enriched in clathrin-coated vesicles (Zhang et al. 1998; Choudhury et al. 2005; Erdmann et al. 2007). Overexpression of OCRL (and OCRL that lacks the 5-phosphatase domain) fragments the Golgi (Choudhury et al. 2005; Hyvola et al. 2006). In addition, OCRL and mutant OCRL expression, redistributes the cation-independent mannose-6-phosphate receptor (M6PR) to enlarged endosomes, and blocks clathrin-mediated transport from early endosomes to the Golgi, and retrograde trafficking (Choudhury et al. 2005). Moreover, siRNA-mediated knockdown of OCRL leads to impaired endosome to *trans*-Golgi trafficking, as shown by an accumulation of M6PR in endosomes (Choudhury et al. 2005). Increased levels of circulating lysosomal enzymes are found in the plasma of Lowe syndrome affected individuals suggesting an endosomal trafficking/secretion defect (Ungewickell and Majerus 1999). OCRL mutant expression also affects endocytosis of the transferrin receptor, independent of its Golgi function (Choudhury et al. 2005; Hyvola et al. 2006). In addition perhaps by regulating PtdIns(4,5)P_2 levels, OCRL regulates actin cytoskeletal dynamics. Fibroblasts derived from affected individuals with Lowe syndrome exhibit abnormal cell migration, cell spreading and fluid phase uptake and an altered actin cytoskeleton, a phenotype that can be rescued by expression of INPP5B (Mao et al. 2009; Coon et al. 2009). OCRL translocates to membrane ruffles in a Rac1-dependent manner, in

response to growth factor stimulation, where it co-localizes with polymerized actin and Rac1 (Faucherre et al. 2005).

The underlying molecular defects in Lowe Syndrome and Dent-2 disease are emerging, however, the kidney defects that are common to both syndromes and the significant role OCRL plays in vesicular trafficking, have lead to the prediction that the renal tubular acidosis observed in affected individuals may be the result of abnormal trafficking of receptors, that promote solute reabsorption from proximal tubules (Norden et al. 2002). Specifically, OCRL may regulate the recycling of cubulin and megalin, the latter is a proximal tubule renal receptor that facilitates the uptake of low molecular weight proteins. Significantly, megalin associates, like OCRL, with GIPC and APPL1 (Swan et al. 2010; Hyvola et al. 2006). Homozygous deletion of megalin, or GIPC in mice leads to low molecular weight proteinuria, similar to that observed in Lowe syndrome and Dent-2 disease and affected individuals exhibit reduced megalin levels in their urine. Collectively these studies suggest that OCRL may regulate the recycling of megalin to the apical surface of renal proximal tubule cells, and in its absence, this may lead to proteinuria.

7.2.7 INPP5A

INPP5A (also called the type I 5-phosphatase, 5-phosphatase-1 or 43 kDa 5-phosphatase, gene name *INPP5A*) hydrolyzes the second messenger molecules inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) and inositol 1,3,4,5-tetrakisphosphate ($Ins(1,3,4,5)P_4$) to form $Ins(1,4)P_2$ and $Ins(1,3,4)P_3$ (Laxminarayan et al. 1993, 1994; De Smedt et al. 1994). These inositol phosphate signaling molecules play significant roles in regulating calcium release from intracellular stores, and at the plasma membrane (reviewed by (Berridge and Irvine 1989)). There is little evidence to date that this enzyme hydrolyzes the membrane bound phosphoinositides, $PtdIns(3,4,5)P_3$ and/or $PtdIns(4,5)P_2$. INPP5A contains the 5-phosphatase domain and a C-terminal CAAX motif, that mediates its plasma membrane localization (De Smedt et al. 1996). Although INPP5A was one of the first 5-phosphatase enzymes to be identified and purified, its function *in vivo* remains relatively uncharacterized. No mouse knockout for this enzyme has been reported, although in some databases it is suggested to be embryonically lethal. INPP5A enzyme activity is regulated *in vitro* by binding the adaptor protein, 14.3.3 (Campbell et al. 1997), and also by Ca(2+)/calmodulin kinase II (De Smedt et al. 1997). Underexpression of INPP5A using an antisense strategy results in enhanced intracellular calcium oscillations and cellular transformation (Speed et al. 1996). However, there are few studies reporting a role for this enzyme in human disease. Recently expression of INPP5A was implicated in skin cancer pathogenesis (Sekulic et al. 2010). Deletion of a region on chromosome 10q, which contains the *INPP5A* gene, is detected in 24% of squamous cell carcinomas (SCC) of the skin. A decrease in INPP5A appears to be an early event in SCC development, as loss of its expression is also detected in (35%) of actinic keratoses, the earliest stage in SCC development (Sekulic et al. 2010).

7.2.8 *INPP5B*

INPP5B (also called 5-phosphatase-2 or 75 kDa 5-phosphatase, gene name *INPP5B*) shares 45% amino acid identity, as well the same domain structure as OCRL, but lacks the clathrin and AP-2 binding motifs (Attree et al. 1992; Erdmann et al. 2007). Although it was originally purified from human platelets as a 75 kDa 5-phosphatase, this is a cleaved fragment of a larger protein (Mitchell et al. 1989). Unlike OCRL, it contains a C-terminal CAAX motif, which facilitates its membrane attachment via isoprenylation (Matzaris et al. 1998). The N-terminal portion of INPP5B also contributes to its membrane localization. INPP5B hydrolyzes PtdIns(3,4,5)P_3, PtdIns(4,5)P_2, Ins(3,4,5)P_3 and Ins(1,3,4,5)P_4 (Matzaris et al. 1998; Jefferson and Majerus 1995; Schmid et al. 2004). Deletion of the CAAX motif reduces INPP5B catalytic activity (Erdmann et al. 2007), however deletion of both the N- and C-terminal domains in recombinant INPP5B has no effect on enzyme activity (Jefferson and Majerus 1995; Matzaris et al. 1998). INPP5B forms a direct complex with many of the endocytic Rab proteins including Rab1, Rab2 (*cis*-Golgi), Rab5 (early endosomes), Rab6 (Golgi stack) and Rab9 (late endosomes), suggesting the 5-phosphatase may regulate early secretory/trafficking events between the endoplasmic reticulum (ER) and Golgi, and/or retrograde trafficking from the Golgi to ER (Erdmann et al. 2007; Shin et al. 2005). In quiescent cells, INPP5B localizes to the Golgi, mediated by its C-terminal RhoGAP domain interaction with the Rab proteins, whilst upon growth factor stimulation it translocates to lamellipodia where it co-localizes with Rab5 and actin (Shin et al. 2005; Williams et al. 2007). INPP5B binds Rab5 via its ASH domain. Mutation of conserved key residues within the ASH domain that mediate the interaction of the 5-phosphatase with Rab5 results in mislocalization of INPP5B from the Golgi (Shin et al. 2005; Williams et al. 2007). Co-expression of an activated Rab5 mutant, together with INPP5B, recruits the 5-phosphatase to a population of enlarged early endosomes. Rab5 binding to INPP5B increases 5-phosphatase catalytic activity (Erdmann et al. 2007; Shin et al. 2005). Additionally the inactive RhoGAP domain of INPP5B interacts with the Rho family GTPases, Rac and cdc42, but not Rho. Functionally, overexpression of INPP5B regulates retrograde transport from the ER-Golgi intermediate compartment to the ER, probably dependent on its interaction with Rab proteins, but independent of its 5-phosphatase activity (Williams et al. 2007). However, in a separate study siRNA-mediated knockdown of INPP5B did not alter retrograde trafficking but rather inhibited transferrin endocytosis (Choudhury et al. 2005; Shin et al. 2005).

Interestingly, the human *INPP5B* gene is located on chromosome 1p34, and genes flanking this locus are associated with genetic disorders with features of Lowe syndrome including abnormal kidney function, lens development and mental retardation (Bisgaard et al. 2007; Konrad et al. 2006; Cormand et al. 1999; Shearman et al. 1996; Nicole et al. 1995). In contrast the murine *Inpp5b* gene is on chromosome 4, where neighbouring genes are involved in lens development (Janne et al. 1994, 1995). However, Inpp5b-null mice do not exhibit features of Lowe or Dent-2 disease, but rather show abnormalities in the testis (Janne et al. 1998). *Inpp5b*$^{-/-}$ testis exhibits vacuoles in seminiferous tubule epithelium and an accumulation of adherence junctions in the

vacuoles of Sertoli cells. Sperm from these mice exhibit reduced motility and fertilization; abnormal processing of the sperm/egg adhesion molecule, and are unable to penetrate zona-pellucida free eggs, due to a decrease in binding and fusion with the egg plasma membrane (Marcello and Evans 2010). Reduced ADAMs processing in Inpp5b-null mice may explain the observed infertility, as ADAMs deficient mice exhibit multi-faceted infertility including abnormal sperm/egg binding (Cho et al. 2000). However, more recent studies have shown little correlation between ADAMs processing levels and infertility in Inpp5b-null mice (Marcello and Evans 2010). Conditional knockout of *Inpp5b* in spermatids does not recapitulate the fertility defects observed in the global *Inpp5b*$^{-/-}$ mice, suggesting loss of Inpp5b function in somatic cells that support sperm function may contribute to the observed infertility (Hellsten et al. 2001).

7.2.9 PIPP

The proline-rich inositol polyphosphate 5-phosphatase, PIPP (gene name *INPP5J*), is a little characterized 5-phosphatase and its function remains elusive. PIPP is expressed in brain, breast, heart, kidney, liver, stomach, and lung (Mochizuki and Takenawa 1999). This 108 kDa 5-phosphatase contains N- and C-terminal proline rich domains which may facilitate protein-protein interactions and a central 5-phosphatase catalytic domain that degrades PtdIns(3,4,5)P_3 (Mochizuki and Takenawa 1999; Ooms et al. 2006; Gurung et al. 2003). PIPP also contains a SKICH domain that mediates its constitutive association with the plasma membrane (Gurung et al. 2003). PIPP hydrolyzes PtdIns(3,4,5)P_3 decreasing Akt activation and its downstream signaling and recent reports indicate PIPP directly opposes oncogenic PI3K/Akt signaling, and its overexpression decreases colony formation in soft agar and reduces proliferation mediated by oncogenic *PIK3CA* (Ooms et al. 2006; Mochizuki and Takenawa 1999; Denley et al. 2009). In NGF-differentiated PC12 cells, PIPP localizes to the plasma membrane, the shaft of extending neurites and the growth cone where it regulates PtdIns(3,4,5)P_3 levels and the activation of Akt and GSK3β. PIPP, like SHIP2, regulates neurite outgrowth and elongation (Ooms et al. 2006). To date the phenotype of PIPP knockout mice remains unreported.

The human PIPP gene, *INPP5J*, is located on chromosome 22q12. Loss of heterozygosity (LOH) of chromosome 22q is frequently detected in breast carcinomas with the most commonly deleted region 22q13 (Castells et al. 2000; Ellsworth et al. 2003; Iida et al. 1998). Allelic loss of chromosome 22q12 has also been reported (Ellsworth et al. 2003; Iida et al. 1998; Osborne and Hamshere 2000) with one study demonstrating LOH of markers within this region in $\sim$ 30% of breast tumors (Iida et al. 1998). LOH of the markers D22S1150 and D22S280 that map to chromosome 22q on either side of *INPP5J* has been detected in 41% and 45% of breast carcinomas respectively (Allione et al. 1998). *INPP5J* mRNA is expressed at higher levels in estrogen receptor (ER)$^{+ve}$ tumors compared to ER^{-ve} tumors (Gruvberger et al. 2001; van't Veer et al. 2002). In a screen of $\sim$ 5000 genes, *INPP5J* was one of 231 genes significantly associated with disease outcome (van't Veer et al. 2002). Higher expression of *INPP5J* correlates with a better prognosis, defined as no distant metastases

developing within 5 years of diagnosis (van't Veer et al. 2002). *INPP5J* has been reported to be one of the 10 highest ranked genes for predicting breast cancer patient outcome (Takahashi et al. 2004). However, the role that PIPP plays in controlling breast cancer proliferation is yet to be reported.

7.2.10 SKIP

SKIP (*s*keletal muscle and *k*idney *i*nositol *p*hosphatase, gene name *INPP5K*) is a relatively uncharacterized 51 kDa 5-phosphatase, which is most highly expressed in the heart, skeletal muscle and kidney, that regulates embryonic development, insulin signaling and glucose homeostasis (Ijuin and Takenawa 2003; Ijuin et al. 2000, 2008). SKIP contains the common catalytic 5-phosphatase domain, with a C-terminal SKICH domain (which is also found in the 5-phosphatase, PIPP), that mediates its plasma membrane association following growth factor stimulation (Ijuin and Takenawa 2003; Gurung et al. 2003). In unstimulated cells SKIP is distributed in a perinuclear distribution which may indicate its association with the ER (Gurung et al. 2003). Purified SKIP recombinant protein exhibits a preference for degrading PtdIns(4,5)P_2 over PtdIns(3,4,5)P_3, but studies in cell lines in which SKIP expression has been reduced by siRNA has revealed PtdIns(3,4,5)P_3 levels are significantly increased in response to insulin stimulation, associated with enhanced Akt activation indicating SKIP regulates PI3K/Akt signaling in intact cells (Ijuin and Takenawa 2003; Schmid et al. 2004). SKIP overexpression in L6 myotubes attenuates insulin-stimulated Akt and p70S6K phosphorylation and inhibits translocation of the glucose transporter, GLUT4, to the plasma membrane leading to decreased glucose uptake and inhibition of glycogen synthesis (Ijuin and Takenawa 2003). *Skip*$^{-/-}$ mice are embryonically lethal at E10.5 for unknown reasons (Ijuin et al. 2008). Interestingly *Skip*$^{+/-}$ mice exhibit increased glucose tolerance and insulin sensitivity, regardless of diet, however, on a high fat diet, these increases are lower compared to wild-type mice, suggesting that loss of Skip expression may provide some protection against diet-induced obesity (Ijuin et al. 2008). SKIP may play a significant role in regulating insulin signaling in skeletal muscle, the major site for post-prandial glucose uptake. However, SKIP is also highly expressed in the brain as well as skeletal muscle, therefore the observed increase in whole-body insulin sensitivity could also be mediated by SKIP regulation of brain PI3K/Akt signaling, although this has not yet been reported. The tissue-specific role of SKIP in the regulation of insulin signaling will be aided by the development of tissue-specific *Skip*$^{-/-}$ mouse models, which have not been described to date.

7.2.10.1 SKIP Association with Human Disease

The heterozygous deletion of eight candidate genes, including *SKIP*, is associated with the contiguous-gene syndrome Miller-Dieker syndrome (MDS), a severe form of lissencephaly (smooth brain) due to defects in neuronal cell migration, which leads to mental retardation and craniofacial/limb abnormalities (Kato and Dobyns 2003;

Cardoso et al. 2003). Recent further refinement of the critical genomic region reduced the candidate gene number to six, including *SKIP* (Bruno et al. 2010). Whether SKIP contributes to MDS is currently unknown.

The human *SKIP* gene is located on chromosome 17p13.3, a region reported to be deleted or hyper-methylated in numerous human cancers including brain, breast, and hepatocellular cancers (Cornelis et al. 1994; Saxena et al. 1992; Zhao et al. 2003a, 2003b; Biegel et al. 1992; Rood et al. 2002). Microarray analysis of gene expression profiles has revealed altered *SKIP* expression, both up and down, in a diverse range of human cancers. *SKIP* transcription is down-regulated in lung adenocarcinoma (Beer et al. 2002; Stearman et al. 2005; Su et al. 2007), prostate carcinoma (Dhanasekaran et al. 2005), chronic lymphocytic leukaemia (Haslinger et al. 2000), Burkitt lymphoma (Corcione et al. 2006) and hepatocellular carcinoma (Wurmbach et al. 2007; Ye et al. 2003b). Conversely, *SKIP* is up-regulated in bladder cancer (Sanchez-Carbayo et al. 2006), cutaneous melanoma (Talantov et al. 2005), multiple myeloma (Zhan et al. 2007) and gemcitabine-resistant pancreatic cancer cell lines (Akada et al. 2005). As yet there have been no functional studies showing the effects of altered expression of SKIP on human cancer cell proliferation and/or invasion.

SKIP also interacts with the human hepatitis B virus (HBV) core protein, leading to nuclear localization of the protein complex, resulting in the suppression of HBV gene expression and virion replication (Hung et al. 2009). The mechanism of suppression remains unknown; however, the suppressive effect is not mediated by the 5-phosphatase activity of SKIP, but by a newly identified functional domain of SKIP located within amino acids 199–226 (Hung et al. 2009). HBV infection can lead to severe liver disease including hepatocellular carcinoma (Bertoletti and Gehring 2007). The interaction of SKIP and HBV core protein is noteworthy in light of the reported LOH at 17p13.3 in hepatocellular carcinoma, and micro-array studies showing decreased *SKIP* transcription in hepatocellular carcinoma (Wurmbach et al. 2007; Ye et al. 2003a; Zhao et al. 2003a).

7.2.11 Future Studies on 5-Phosphatases

Many recent gene-linkage, *in vitro* and *in vivo* studies have demonstrated the significance of the 5-phosphatases in multiple aspects of embryonic and human development and disease. Over the last decade the 5-phosphatase enzymes have been re-classified from PI3K-terminating enzymes to PI3K-modifying enzymes, as many of the products of their catalysis exhibit signaling properties in their own right. Surprisingly, even though the 5-phosphatase family encompasses 10 mammalian enzymes there appears to be little functional redundancy.

The generation of 5-phosphatase knockout animals has provided valuable and sometimes unexpected information about these enzymes. The embryonic lethality or early death associated with some of the 5-phosphatase knockout mice indicates a significant role in development, however, the generation and characterization of inducible constitutive enzyme-null mouse models would be beneficial in determining their role in non-developmental processes. Moreover, tissue- or cell-specific

knockout models and/or crossing null mice with various cancer models will also assist in determining whether the 5-phosphatases function as *bona fide* tumor suppressors, for which there is currently little evidence with the exception of SHIP1. Furthermore, the use of 5-phosphatase expression as a diagnostic and/or prognostic indicator or as a druggable target should be evaluated in the coming years.

7.3 Inositol Polyphosphate 4-Phosphatases

Inositol polyphosphate 4-phosphatases preferentially hydrolyze the D4 position phosphate of target inositol head-groups. The 4-phosphatase enzyme family comprises two inactive members, and five active members (see Table7.2) which can be sub-classified based on substrate specificity into the PtdIns(4,5)P_2 4-phosphatases or PtdIns(3,4)P_2 4-phosphatases. The catalytic product of the PtdIns(4,5)P_2 4-phosphatases is PtdIns(5)P, while the PtdIns(3,4)P_2 4-phosphatases generate PtdIns(3)P.

7.3.1 PtdIns(4,5)P_2 4-Phosphatases

The PtdIns(4,5)P_2 4-phosphatases hydrolyze PtdIns(4,5)P_2 exclusively and there are two mammalian (TMEM55A and TMEM55B) and one bacterial (invasion plasmid gene D [IpgD]) isoenzymes identified to date. The mammalian TMEM55A/B enzymes share 52% amino acid identity, including a catalytic CX_5R motif, and are ubiquitously expressed in human tissues, however TMEM55A exhibits higher expression in the liver, spleen and thymus, and TMEM55B is more prevalent in regions of the brain (Ungewickell et al. 2005). In human cells, both enzymes localize to late endosomes/lysosomes. Overexpression of TMEM55A increases cellular PtdIns(5)P and is associated with increased EGFR degradation (Ungewickell et al. 2005; Zou et al. 2007). Following cell stress, TMEM55A translocates to the nucleus where it acts to promote p53 acetylation and stability, resulting in increased apoptosis through PtdIns(5)P-dependent ING2 (inhibitor of growth protein 2) activity (Zou et al. 2007). To date, no mouse knockout models or functional studies for these enzymes have been reported.

Interestingly, while the bacterial 4-phosphatase, IpgD, exhibits sequence homology with the mammalian PtdIns(3,4)P_2 4-phosphatases (Norris et al 1998), this enzyme actually functions to regulate PtdIns(4,5)P_2 levels in mammalian cells. The pathogen, *Shigella flexneri* (*S. flexneri*), responsible for causing bacillary dysentery in humans, directly injects IpgD into mammalian host cells through its type III secretion machinery during infection, resulting in PtdIns(4,5)P_2 dephosphorylation at the host cell plasma membrane, thereby facilitating actin reorganization and the formation of entry sites that promote bacterial uptake (Niebuhr et al. 2000, 2002; Allaoui et al. 1993). Interestingly, dephosphorylation of PtdIns(4,5)P_2 by IpgD also results in the accumulation of its phosphoinositide product, PtdIns(5)P, which activates the

Table 7.2 Inositol polyphosphate 4-phosphatase and murine models with gene targeted deletion of family members

Protein Name	Alias(es)	Gene	Animal models	References
Inositol polyphosphate 4-phosphatase type I	INPP4A	*INPP4A*	*Inpp4a*^{wbl} mutant mouse—early onset cerebellar ataxia, Purkinje cell loss, postnatal lethality at 2–3 weeks of age	(Nystuen et al. 2001; Sachs et al. 2009)
			Inpp4a$^{-/-}$ constitutive KO mouse—excitotoxic neuronal death in striatum, involuntary movements, postnatal lethality at 2–3 weeks of age	(Sasaki et al. 2010)
Inositol polyphosphate 4-phosphatase type II	INPP4B	*INPP4B*	Not reported	
PtdIns(4,5)P_2 4-phosphatase type I	TMEM55A	*TMEM55A*	Not reported	
PtdIns(4,5)P_2 4-phosphatase type II	TMEM55B	TMEM55B	Not reported	
P-Rex1		*P-Rex1*	*P-Rex1*$^{-/-}$ constitutive KO mouse—viable and healthy, decreased Rac activation, ROS and superoxide formation and chemotaxis of neutrophils and macrophages	(Welch et al. 2005; Wang et al. 2008)
			P-Rex1 GEF-dead transgenic mouse—develop normally, diminished Rac2 activation, chemotaxis, superoxide formation and actin reorganisation in neutrophils	(Dong et al. 2005)
P-Rex2	P-Rex2a, P-Rex2b	*P-Rex2*	*P-Rex2*$^{-/-}$ constitutive KO mouse—viable, fertile and healthy, abnormal cerebellar Purkinje cells and impaired motor coordination, more severe in females and age-related	(Donald et al. 2008)
			P-Rex1$^{-/-}$/*P-Rex2*$^{-/-}$ double KO mouse—more severe but similar phenotype as *P-Rex2*$^{-/-}$ global KO, reduced long-term potentiation in cerebellum	(Donald et al. 2008; Jackson et al. 2010)

PI3K/Akt pathway to signal for host cell survival (Pendaries et al. 2006; Guittard et al. 2010). In addition, increased production of IpgD-dependent PtdIns(5)P may also promote tyrosine phosphorylation of Src family kinases and their substrates to regulate immunogenic T-cell responses (Guittard et al. 2009, 2010).

An IpgD-related enzyme, SopB/SigD, has also been identified in *Salmonella* species. This enzyme is integral for *Salmonella*-mediated pathogenesis in host systems (Galyov et al. 1997), through a variety of mechanisms including chloride channel and tight junction regulation, leading to cell permeability (Boyle et al. 2006; Feng et al. 2001), regulation of vesicular trafficking, and the formation of the *Salmonella*-containing vacuole (SCV) (Hernandez et al. 2004; Mallo et al. 2008; Bakowski et al. 2010) and the promotion of Akt phosphorylation and host cell survival (Knodler et al. 2005, 2009). SopB exhibits 3-, 4- and 5-phosphatase activity towards multiple soluble and insoluble inositol phosphates, and initial studies reported its ability to hydrolyze the D4 phosphate from PtdIns(3,4)P_2 and Ins(1,3,4)P_3, but not PtdIns(4,5)P_2 (Norris et al. 1998). More recent studies, however, suggest that SopB does, in fact, utilize PtdIns(4,5)P_2 as a substrate and that negative regulation of this phosphoinositide by SopB may promote actin reorganization, opening of tight junctions and inhibition of SCV-lysosome fusion in host cells (Mason et al. 2007; Bakowski et al. 2010). Given the contrasting results concerning SopB substrates, this enzyme remains to be classified as a *bona fide* PtdIns(4,5)P_2 4-phosphatase.

7.3.2 PtdIns(3,4)P_2 4-Phosphatases

There are two mammalian PtdIns(3,4)P_2 4-phosphatases; type I and type II (gene names; *INPP4A* and *INPP4B*, respectively), which share 37% amino acid identity, including a conserved amino-terminal C2 domain, responsible for their interactions with target phosphoinositides (Shearn and Norris 2007; Ivetac et al. 2005). Apart from a conserved CX_5R motif they share little resemblance to the PtdIns(4,5)P_2 4-phosphatases. INPP4A was the first mammalian 4-phosphatase identified, initially found to hydrolyze the soluble Ins(3,4)P_2 and Ins(1,3,4)P_3 (Bansal et al. 1987). It was later identified as a magnesium-, calcium- and lithium-independent phosphatase (Bansal et al. 1990) with additional activity towards insoluble PtdIns(3,4)P_2, 120-fold greater than Ins(3,4)P_2 (Norris and Majerus 1994), indicating PtdIns(3,4)P_2 is its preferred *in vivo* substrate. The catalytic activity of both enzymes is mediated by a conserved carboxy-terminal catalytic CX_5R (CKSAKDR) motif (Norris et al. 1997). INPP4A also binds the p85 subunit of PI3K, which may facilitate the recruitment of the phosphatase to substrate-enriched membranes (Munday et al. 1999). Alternatively spliced variants have been identified for both INPP4A and B, featuring a hydrophobic C-terminal transmembrane domain (Norris et al. 1997). The function of these spliced isoforms in mammalian cells remains unknown.

7.3.2.1 INNP4A Regulates Excitatory Neuronal Cell Death

INPP4A and B exhibit ubiquitous tissue distributions, with the highest expression in brain and heart (Norris et al. 1995, 1997). Evidence from recent knockout mouse studies and analysis of human tumors suggests non-redundant roles for these enzymes. Knockout of the *Inpp4a* gene in mice promotes postnatal neuronal degeneration and lethality by 2–3 weeks of age (Nystuen et al. 2001; Sachs et al. 2009; Sasaki et al. 2010). The naturally-occurring *Inpp4a* knockout mouse, *weeble* (*Inpp4a*^{wbl}), resulting from a spontaneous single nucleotide deletion in exon 10 of the *Inpp4a* gene (Δ744G), exhibits early onset cerebella ataxia, associated with Purkinje cell loss (Nystuen et al. 2001; Sachs et al. 2009). In a mouse model of targeted *Inpp4a* disruption (*Inpp4a*$^{-/-}$), neuronal degeneration is restricted to the striatum, associated with increased apoptosis of medium-sized spiny projection neurons (MSNs), resulting from glutamate receptor excitotoxicity (Sasaki et al. 2010). Both mouse models of Inpp4a loss reveal a neuroprotective role for this enzyme in postnatal animals, however, Inpp4a is dispensable during embryogenesis (Nystuen et al. 2001; Sachs et al. 2009; Sasaki et al. 2010). Although the precise molecular mechanisms by which Inpp4a fulfils its neuroprotective function during postnatal development are emerging, regulation of PtdIns(3,4)P_2 appears critical as *Inpp4a*^{wbl} Purkinje cells exhibit PtdIns(3,4)P_2 accumulation (Shin et al. 2005). Furthermore, treatment of wildtype MSNs with PtdIns(3,4)P_2 promotes glutamate-induced cell death (Sasaki et al. 2010).

Recent reports also indicate that the PtdIns(3,4)P_2 4-phosphatases can function to regulate a wide range of additional cellular processes. For example, INPP4A acts to control normal endosome function and is recruited to endosomal membranes by binding Rab5, thereby increasing its PtdIns(3,4)P_2 catalytic activity and contributing to an endosomal pool of PtdIns(3)P (Ivetac et al. 2005; Shin et al. 2005). Absence or depletion of INPP4A promotes the formation of dilated endosomes and impairs transferrin internalization and its overexpression rescues PI3K inhibition-induced endosomal dilation, dependent on its catalytic activity (Ivetac et al. 2005; Shin et al. 2005). Interestingly, the endosomal function of INPP4A is not recapitulated by the INPP4B isoform, which does not localize to endosomal membranes or contribute to the production of endosomal PtdIns(3)P. Rather, INPP4B regulates plasma membrane localized PtdIns(3,4)P_2 downstream of PI3K activation, a role also shared by INPP4A, which translocates to the plasma membrane upon growth factor stimulation (Ivetac et al. 2005; Shin et al. 2005). The role of the 4-phosphatases at the plasma membrane is presumably to regulate signaling events downstream of PtdIns(3,4)P_2, including activation of the proto-oncogene, Akt. Indeed, both INPP4A and INPP4B negatively regulate Akt activation and its downstream cellular processes including cell proliferation, tumor growth, anchorage-independent colony formation and cell migration (Ivetac et al. 2009; Vyas et al. 2000; Gewinner et al. 2009; Fedele et al. 2010; Hodgson et al. 2011).

7.3.2.2 Association of INPP4A and INPP4B with Human Disease

There is some evidence of altered *INPP4A* expression in human diseases, including asthma (Sharma et al. 2010), prostate cancer (LaTulippe et al. 2002) and leukemia (Erkeland et al. 2004), but no functional studies have been reported. In contrast, a significant role for INPP4B as a putative tumor suppressor in multiple human cancers has recently been identified. For example, INPP4B expression is lost during malignant proerythroblast progression and re-introduction of INPP4B into late-stage blasts decreases Akt activation (Barnache et al. 2006). In human prostate cancer cells, *INPP4B* is an androgen receptor (AR)-responsive gene (Hodgson et al. 2011), and its transcription is diminished in late-stage, androgen-independent prostate cancer xenografts in mice (Gu et al. 2005). Significantly, INPP4B protein is frequently lost in human prostate cancers, associated with reduced recurrence-free survival (Hodgson et al. 2011) and diminished *INPP4B* gene transcription is frequently observed in metastatic prostate carcinomas (Taylor et al. 2010). Interestingly, in the human mammary gland INPP4B is expressed in a sub-population of estrogen receptor (ER)-positive cells in the terminal ductal lobuloalveolar units (TDLU), where it may act to suppress cell proliferation (Fedele et al. 2010). Indeed, in human breast cancer, *INPP4B* is frequently deleted (Naylor et al. 2005) and its transcription and protein expression positively correlates with ER and progesterone receptor (PR) expression (Yang et al. 2005; West et al. 2001; Fedele et al. 2010). LOH maps to the *INPP4B* gene in a range of human cancers, including breast and ovarian carcinomas and melanomas, associated with decreased patient survival (Gewinner et al. 2009). In human breast cancer, *INPP4B* LOH and protein loss occur most frequently in the aggressive basal-like sub-type (Gewinner et al. 2009; Fedele et al. 2010). Significantly, loss of INPP4B protein expression is frequently observed in PTEN-null breast cancers, associated with increased Akt phosphorylation, indicating co-operative promotion of tumorigenesis through the loss of multiple phosphoinositide phosphatases (Fedele et al. 2010). Overall these studies highlight the importance of PtdIns(3,4)P_2-dependent signaling and its regulation by the 4-phosphatases in maintaining normal cell proliferation and implicates this pathway in the regulation of human cancer. Collectively these studies identify INPP4B as a significant new tumor suppressor in many human cancers.

7.3.3 Inactive 4-Phosphatases: P-Rex Family Guanine Nucleotide Exchange Factors

The PtdIns(3,4,5)P_3-dependent Rac exchanger (P-REX) enzymes are related to the PtdIns(3,4)P_2 4-phosphatases, as they contain a 4-phosphatase homology domain, which shows primary amino acid sequence identity with INPP4A, with a conserved carboxy-terminal CX_5R 4-phosphatase catalytic motif (Welch et al. 2002). In contrast to the PtdIns(3,4)P_2 4-phosphatases, P-REX enzymes do not exhibit phosphoinositide phosphatase activity for unknown reasons (Welch et al. 2002). Rather, these

proteins are critical for the regulation of signaling events downstream of PI3K activation in a phosphoinositide phosphatase-independent manner and are implicated in a range of human functions and diseases, including cancer and diabetes. Therefore the 4-phosphatase enzyme family is reminiscent of the myotubularins, with both active and inactive family members.

To date, three P-REX isoenzymes have been identified; P-REX1, P-REX2a and P-REX2b, all of which are multi-domain proteins, sharing 59% amino acid identity with similar protein structures, including tandem amino-terminal dbl-homology (DH) and pleckstrin-homology (PH) domains, two DEP domains and two PDZ domains (Welch et al. 2002; Donald et al. 2004; Rosenfeldt et al. 2004). The P-REX2a and P-REX2b isoforms are transcribed from the same gene, however P-REX2b lacks the carboxy-terminal 4-phosphatase domain (Rosenfeldt et al. 2004). The founding member of the P-REX family of proteins, P-REX1, was initially identified in neutrophils as the major guanine nucleotide exchange factor (GEF) required for activation of the Rho-family GTPase, Rac (Welch et al. 2002), and P-REX2a and 2b also exhibit Rac-GEF activity (Donald et al. 2004; Rosenfeldt et al. 2004; Li et al. 2005). Activation of these enzymes occurs synergistically via binding to PtdIns(3,4,5)P_3 and the G-protein, G$\beta\gamma$, at the plasma membrane in response to extracellular stimulation and receptor tyrosine kinase (RTK) and G protein-coupled receptor (GPCR) activation, dependent on their PH and DH domains, respectively (Welch et al. 2002; Hill et al. 2005; Donald et al. 2004; Barber et al. 2007). The multi-domain nature of the P-REX enzymes is critical for their activation and function. For example, the Rac-GEF activity is mediated by their DH/PH domains (Hill et al. 2005) and the PH domain may facilitate the specific recognition of certain Rac isoforms as substrates (Joseph and Norris 2005). In addition, the P-REX1 tandem DEP domains bind the mammalian target of rapamycin (mTOR) and this interaction is necessary for mTOR complex 2 (mTORC2)-dependent Rac activation (Hernandez-Negrete et al. 2007). Furthermore, the PDZ domains of P-REX1 can bind GPCR, suppressing receptor internalization and promoting downstream signaling and cell migration (Ledezma-Sanchez et al. 2010). Interestingly, the function of P-REX1 may also be self-regulated by inter-domain interactions between the DEP and PDZ domains, and also by the PH domain (Hill et al. 2005; Urano et al. 2008). Moreover, recruitment of P-REX proteins to activated RTKs at the plasma membrane may also serve to facilitate their phosphorylation and/or dephosphorylation and activation, possibly mediated by protein kinase A (PKA) (Montero et al. 2011; Mayeenuddin and Garrison 2006; Urano et al. 2008; Zhao et al. 2007). Interestingly, the 4-phosphatase domain of these enzymes appears to be dispensable for P-REX-mediated Rac activation, however it may play a role in promoting full P-REX function in an undefined manner (Hill et al. 2005; Waters et al. 2008). Once activated at the plasma membrane, P-REX enzymes catalyze the formation of functional Rac and are, therefore, critical for the regulation of a wide range of Rac-dependent cellular processes, including actin reorganization (Welch et al. 2002; Montero et al. 2011; Qin et al. 2009; Waters et al. 2008), cell migration, invasion and chemotaxis (Yoshizawa et al. 2005; Qin et al. 2009; Hernandez-Negrete et al. 2007; Ledezma-Sanchez et al. 2010; Li et al. 2005),

gene transcription (Li et al. 2005) and ROS and superoxide formation (Wang et al. 2008; Welch et al. 2005; Dong et al. 2005; Nie et al. 2010).

P-REX1 is expressed most abundantly in peripheral blood leukocytes (Welch et al. 2002), however the P-REX2 isoforms are notably absent from circulating leukocytes (Donald et al. 2004; Rosenfeldt et al. 2004). Mouse models of altered P-Rex1 expression, including *P-Rex1*$^{-/-}$ mice and a transgenic mouse expressing a Rac-GEF-dead P-Rex1 mutant, while viable and healthy, exhibit impaired neutrophil and macrophage function, including decreased Rac1 and Rac2 activation, diminished ROS and superoxide formation and decreased chemotaxis and recruitment to inflammation (Welch et al. 2005; Dong et al. 2005; Wang et al. 2008). Interestingly, both P-Rex1 and P-Rex2a are highly expressed in the brain (Welch et al. 2005; Yoshizawa et al. 2005; Donald et al. 2008) and P-Rex1 negatively regulates neurite differentiation, elongation and migration of rat pheochromocytoma PC12 and primary hippocampal and cerebral cortical neurons through the regulation of actin reorganization (Waters et al. 2008; Yoshizawa et al. 2005).

P-Rex2$^{-/-}$ mice, like *P-Rex1*$^{-/-}$ mice, are viable, fertile and healthy, however they exhibit abnormal Purkinje cell development with decreased dendritic diameter and length, associated with impaired motor coordination, which is more severe in females and worsens with age (Donald et al. 2008). Interestingly, *P-Rex1*$^{-/-}$ mice do not exhibit the same differences in Purkinje cell integrity or motor skills as *P-Rex2*$^{-/-}$ mice, however, depletion of both P-Rex1 and P-Rex2 (*P-Rex1*$^{-/-}$*P-Rex2*$^{-/-}$) exacerbates the phenotype observed in single *P-Rex2*$^{-/-}$ animals (Donald et al. 2008), attributed to reduced sustained post-synaptic long term potentiation (LTP) in the cerebellum (Jackson et al. 2010).

7.3.3.1 P-REX1 Regulation of the PI3K Pathway and Disease Associations

The P-REX enzymes are also critical for the regulation of phosphoinositide signaling, even in the absence of phosphoinositide phosphatase activity, and are implicated in human diseases associated with PI3K pathway alterations, including cancer and diabetes. These enzymes achieve their phosphoinositide-regulatory function indirectly through domains distinct from the 4-phosphatase domain. For example, the DH/PH domain of P-REX2a binds the 3-phosphatase and tumor suppressor, PTEN, negatively regulating the 3-phosphatase activity of this enzyme toward its substrate, PtdIns(3,4,5)P_3 (Fine et al. 2009). Significantly, overexpression of P-REX2a in breast cancer cells decreases PTEN phosphoinositide phosphatase activity, associated with increased Akt phosphorylation and cell proliferation and rescues PTEN-induced suppression of Akt phosphorylation. Conversely, P-REX2a deficiency decreases Akt activation and cell proliferation in cells expressing PTEN. Interestingly, the inhibitory function of P-REX2a on PTEN is dependent on its binding ability, but independent of its Rac-GEF activity (Fine et al. 2009). As a negative regulator of PTEN activity, it is not surprising that P-REX2 is implicated in human cancers. The *P-REX2* gene is located at 8q13, a region frequently amplified in breast, prostate and ovarian cancers (Fejzo et al. 1998; Dimova et al. 2009; Sun et al. 2007). In breast cancer, amplified

P-REX2a expression inversely correlates with PTEN loss and is frequently observed in breast cancers expressing mutant PI3K, and overexpression of both P-REX2a and mutant PI3K drives mammary cell transformation (Fine et al. 2009).

Utilizing an alternative mechanism, the P-REX1 isoform can also function to potentiate PI3K/Akt signaling through activation of Rac1, which, in turn, promotes Akt phosphorylation resulting in reciprocal activation of Rac1, in a positive feedback loop (Nie et al. 2010). This relationship is dependent on the Rac-GEF activity of P-REX1 and the kinase activity of Akt. Significantly, amplified P-REX1 expression is associated with decreased disease-free survival in human breast cancer (Montero et al. 2011) and metastatic prostate cancer (Qin et al. 2009). Interestingly, P-REX1 may also play a role in the promotion of angiogenesis, potentially facilitating tumor growth and metastasis (Carretero-Ortega et al. 2010). Conversely, P-REX1 protein depletion in breast and prostate cancer cells decreases Rac activation, lamellipodia formation, cell motility, invasion, proliferation and xenograft tumor growth and metastasis (Montero et al. 2011; Qin et al. 2009). Recently P-REX1 was identified as an essential mediator of ErbB2 signaling in breast cancer (Sosa et al. 2010). P-REX1 is highly overexpressed in breast cancers. ErbB2 and GPCR signaling converges on P-REX1 to facilitate Rac activation. Interestingly, P-REX1 is also implicated in diabetes. The gene encoding P-REX1 is located on chromosome 20q12-13, which is linked to Type 2 diabetes, and *P-REX1* may be a type 2 diabetes-susceptibility gene (Bento et al. 2008; Lewis et al. 2010). The precise mechanisms by which the P-REX enzymes function in health and disease are, however, largely unknown and are the focus of current research.

7.4 Sac Domain Phosphoinositide Phosphatases

Sac domain phosphoinositide phosphatases are characterized by the presence of a conserved Sac phosphatase domain that was first identified in the founding member, yeast suppressor of actin (ySac1) (Novick et al. 1989; Guo et al. 1999). The Sac domain exhibits broad specificity for phosphoinositide substrates, and its intrinsic catalytic $CX_5R(S/T)$ motif can hydrolyze both the mono-phosphorylated phosphoinositides, PtdIns(3)P, PtdIns(4)P and PtdIns(5)P, in addition to dual-phosphorylated PtdIns(3,5)P_2 (Guo et al. 1999). The Sac phosphatase domain comprises $\sim$ 400 amino acids arranged into 7 highly conserved motifs (Guo et al. 1999) and is unique in its structure and mechanism of substrate dephosphorylation, possibly involving the presence of a self-regulatory SacN domain (Manford et al. 2010). The functions of the Sac phosphatases are varied, and alterations in their expression are implicated in a range of diseases including cardiac hypertrophy and neurodegenerative disorders.

Generally, Sac domain phosphatases can be divided into two classes. The first class comprises the stand-alone Sac phosphatases that contain a conserved amino-terminal Sac domain with no other identifiable motifs, and examples include yeast ySac1 and yFig4, and human SAC1, SAC2/INPP5F and SAC3/FIG4 (Table7.3). The second class is the Sac domain-containing inositol phosphatases (SCIPs), which comprise

Table 7.3 Mammalian Sac domain phosphatases

Protein name	Alias(es)	Gene	Animal models	References
SAC1		*SAC1*	Not reported	
SAC2	INPP5F	*INPP5F*	*Inpp5f*$^{-/-}$ constitutive KO mouse—normal development, increased stress-induced cardiac hypertrophy	(Zhu et al. 2009)
			Sac2 transgenic mouse—normal development, resistant to stress-induced hypertrophy	(Zhu et al. 2009)
SAC3	FIG4	*FIG4*	*Fig4*$^{-/-}$ constitutive KO mouse—"pale tremor", severe tremor, abnormal gait, neurodegeneration, juvenile lethality	(Chow et al. 2007; Zhang et al. 2008; Ferguson et al. 2009)

both a Sac phosphatase domain in addition to a central inositol polyphosphate 5-phosphatase catalytic domain, and examples include human synaptojanin 1 and 2, and the yeast phosphatases, Inp51, Inp52 and Inp53, reviewed in (Hughes et al. 2000). While in most cases the Sac domain alone is incapable of hydrolyzing inositol headgroups with adjacent phosphates, such as PtdIns(3,4)P_2 and PtdIns(4,5)P_2, the 5-phosphatase domain of SCIPs allows this class of enzyme to utilize PtdIns(4,5)P_2 as a substrate (Guo et al. 1999). Here, we have outlined the major functions of the yeast and mammalian stand-alone Sac phosphatases, with particular emphasis on the role these enzymes play in mammalian cells and in human disease.

7.4.1 SAC1

The stand alone Sac phosphatase, ySac1, was the first enzyme of this family to be identified in the yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) as a regulator of actin organization and membrane trafficking events through the Golgi (Cleves et al. 1989; Novick et al. 1989; Whitters et al. 1993). In yeast, loss of ySac1 results in a range of cellular defects, including actin cytoskeletal disorganization and cold sensitivity for growth (Novick et al. 1989; Cleves et al. 1989), abnormal vacuole formation and trafficking (Foti et al. 2001; Tahirovic et al. 2005) and cell wall abnormalities (Schorr et al. 2001). Interestingly, however, ablation of ySac1 in yeast is not lethal. The function of Sac1 is evolutionarily conserved, with Sac1 orthologs identified as critical enzymes in a range of biological systems, including *D. melanogaster* (Wei et al. 2003; Yavari et al. 2010) and *Arabidopsis thaliana* (Despres et al. 2003; Zhong and Ye 2003; Zhong et al. 2005). SAC1 expression is essential for viability in mammalian systems and *Sac1*$^{-/-}$ mice exhibit pre-implantation lethality (Liu et al. 2008). Furthermore, SAC1 depletion in human cells results in decreased cell viability, inhibition of G_2-M cell cycle progression and suppressed cell growth rates (Liu et al. 2008; Cheong et al. 2010). Sac1 is ubiquitously expressed in adult and embryonic rat and mouse tissues (Nemoto et al. 2000; Liu et al. 2009), however, its expression is specifically up-regulated in the heart and regions of the brain during embryogenesis

(Liu et al. 2009), suggesting a critical tissue-specific role for this enzyme during development. Given the importance of SAC1 for cell viability and development, it is surprising that this enzyme has not yet been associated with any human disease.

7.4.1.1 Structure and Function

SAC1 localizes to the ER and Golgi compartments in both yeast and mammalian cells, anchored to the membranes by dual trans-membrane domains located in its carboxyl-terminus (Whitters et al. 1993). In quiescent cells, SAC1 resides predominantly in the Golgi, translocating to the ER upon growth factor stimulation (Blagoveshchenskaya et al. 2008; Cheong et al. 2010). While both yeast and human SAC1 exhibit Golgi-ER shuttling, the mechanism differs between species. In *S. cerevisiae*, retention of ySac1 in the ER is dependent on interactions between its carboxyl-terminal tail and the ER resident protein, Dpm1 (Dolichol phosphate mannosyltransferase) (Faulhammer et al. 2005), however, the mechanism for shuttling between intracellular compartments is not understood. In contrast, mammalian SAC1 forms oligomers in the ER via leucine zipper motifs located in its Sac domain, which interact with the coatomer protein complex II (COP-II) to promote its transport to the Golgi (Blagoveshchenskaya et al. 2008). Upon growth factor stimulation, activation of the mitogen activated protein kinase (MAPK) pathway promotes dissociation of SAC1 complexes to reveal a coatomer protein complex I (COP-I)-binding carboxyl-terminal "KXKXX" motif and induce retrograde trafficking back to the ER (Blagoveshchenskaya et al. 2008; Rohde et al. 2003; Liu et al. 2008). To date the precise function of SAC1 in distinct sub-cellular compartments remains poorly understood. SAC1 utilizes PtdIns(4)P as its predominant substrate, and depletion of this phosphatase in both yeast and mammalian cells results in increased cellular PtdIns(4)P levels (Guo et al. 1999; Nemoto et al. 2000; Rivas et al. 1999; Liu et al. 2008; Cheong et al. 2010). Sequestration of SAC1 in the ER following cell stimulation may allow accumulation of Golgi PtdIns(4)P, thereby promoting protein export to the plasma membrane (Blagoveshchenskaya et al. 2008). Conversely, Golgi-localized SAC1 may assist in the maintenance of normal Golgi organization through spatio-temporal regulation of PtdIns(4)P levels and its downstream effector proteins (Cheong et al. 2010). Indeed, RNAi-mediated SAC1 depletion results in disruption of Golgi integrity and distorted morphology, however, this does not affect overall rates of secretion (Liu et al. 2008; Cheong et al. 2010). Interestingly, however, expression of a Golgi-directed SAC1 mutant, resulting in PtdIns(4)P depletion, suppresses trafficking from this site (Szentpetery et al. 2010). Intriguingly, the ySac1 crystal structure suggests this phosphatase may function beyond its sub-cellular distribution, revealing the presence of a linker sequence located between the catalytic domain and the carboxyl-terminal trans-membrane domain, which may allow the Sac domain access to adjacent membranes even when embedded in the membranes of intracellular organelles (Manford et al. 2010). For example, ER-localized SAC1 could regulate phosphoinositides embedded in adjacent regions of the plasma membrane, however this function remains to be confirmed.

7.4.2 SAC2

The human SAC2 (gene name *INPP5F*) is an exceptional Sac phosphatase as it can hydrolyze the D5 position phosphate of PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, generating PtdIns(4)P and PtdIns(3,4)P_2 respectively (Minagawa et al. 2001). Although SAC2 contains a conserved catalytic phosphatase motif, it differs slightly from ySac1 in its surrounding modules, which may be responsible for the differential substrate specificities (Minagawa et al. 2001). Northern blot analysis indicates that this enzyme is ubiquitously expressed in human tissues, with highest levels detected in brain, heart, skeletal muscle, kidney and placenta (Minagawa et al. 2001). The heart-specific function of Sac2 has been characterized in mouse models with deficiency of this enzyme. Both *Inpp5f* knockout mice, and a histone deacetylase 2 (Hdac2) transgenic mice in which *Inpp5f* expression is transcriptionally repressed, exhibit stress-induced cardiac hypertrophy associated with elevated PtdIns(3,4,5)P_3 and hyper-phosphorylation of Akt and GSK3β (Trivedi et al. 2007; Zhu et al. 2009). Reciprocally, Sac2 transgenic mice are resistant to cardiac hypertrophy (Zhu et al. 2009), verifying the importance of this protein for normal cardiac myocyte function.

7.4.3 SAC3/Fig4

The related SAC3 (gene name *FIG4*) also serves a critical function in mammalian systems. Mutations in this enzyme are associated with human neuropathies. SAC3 is the human counterpart of the yFig4 protein (Rudge et al. 2004; Sbrissa et al. 2007), initially identified in *S. cerevisiae* as a pheromone-regulated or induced gene (Erdman et al. 1998). Both yFig4 and SAC3 utilize PtdIns(3,5)P_2 as their preferred *in vivo* substrate (Rudge et al. 2004; Sbrissa et al. 2007). Intriguingly, however, cellular depletion of these phosphatases results in a paradoxical suppression of PtdIns(3,5)P_2 levels, due to the loss of an activating interaction between the Sac phosphatases and the PtdIns(3,5)P_2-generating PtdIns(3)P 5-kinases, Fab1 in yeast and PIKfyve in mammalian cells (Duex et al. 2006a, 2006b; Gary et al. 2002; Rudge et al. 2004; Sbrissa et al. 2007; Chow et al. 2007; Botelho et al. 2008). This association is permitted by direct interactions between the phosphatases and the intermediate yeast Vac14 or mammalian ArPIKfyve proteins, allowing the formation of a stable ternary complex that promotes both activation of Fab1/PIKfyve, to generate PtdIns(3,5)P_2, in addition to stabilization of its agonist yFig4/SAC3 (Sbrissa et al. 2007; Ikonomov et al. 2010). This complex localizes to intracellular vesicular membranes to regulate PtdIns(3,5)P_2 production at these sites (Sbrissa et al. 2007; Rudge et al. 2004; Botelho et al. 2008).

7.4.3.1 SAC3 Function and Disease Associations

In the mouse, Sac3 protein and RNA is ubiquitously detected in all tissues, with highest expression in the brain, white adipose tissue and lung (Sbrissa et al. 2007; Chow et al. 2007). Sac3 may play a role in insulin-mediated GLUT4 translocation

and glucose entry in mouse adipocytes, potentially implicating this enzyme in insulin resistance (Ikonomov et al. 2009). The rat Sac3 homolog is also important for neurite elongation in neuronal PC12 cells (Yuan et al. 2007). A naturally occurring Sac3 knockout mouse (*Fig4*$^{-/-}$), termed "pale tremor", exhibits severe tremor and abnormal gait, associated with neurodegeneration and juvenile lethality (Chow et al. 2007; Zhang et al. 2008; Ferguson et al. 2009). *Fig4*$^{-/-}$ derived fibroblasts and neurons exhibit enlarged endosomal and lysosomal compartments and impaired organelle trafficking (Chow et al. 2007; Ferguson et al. 2009) and brains from *Fig4*$^{-/-}$ mice exhibit inclusion bodies with markers of defective autophagy (Ferguson et al. 2009). The clinical and pathological features of the pale tremor mouse are reminiscent of human neuropathies, in particular Charcot-Marie-Tooth (CMT) disorder. Indeed, an autosomal recessive form of CMT, type 4J (CMT4J), is caused by a pathogenic mutation at amino acid 41 (Ile-to-Thr) in SAC3 (Chow et al. 2007). While the resulting mutant protein exhibits no change in its PtdIns(3,5)P_2 phosphatase activity, it is no longer stabilized by its interaction with ArPIKfyve and is thereby readily degraded (Ikonomov et al. 2010). In addition, deleterious mutations in the human *FIG4* gene are associated with the severe human neuropathy, amyotrophic lateral sclerosis (ALS) (Chow et al. 2009), further highlighting the importance of this enzyme in the regulation of neuronal function.

7.5 Concluding Remarks

The characterization of the phosphoinositide phosphatases has lagged behind the kinases but the results of the many recent studies described here reveal they play just as important a role in cellular function and human disease as the phosphoinositide kinases. There is likely to be many more exciting results that emerge in the next few years on the role the lipid phosphatases play in human diseases. Many phosphoinositide phosphatases exhibit altered expression in many different diseases as shown by gene array expression analysis but the functional consequences remain to be determined but are likely to be significant. Characterization of mouse knockout models of some of the lipid phosphatases is still emerging, which may give significant insights into phosphatase function. Furthermore most reports to date have characterized the function of individual lipid phosphatases in isolation and it will be a challenge for the future to determine how these enzymes work together to regulate the complex interactive phosphoinositide signaling pathway.

Locus	Common name (s)	SwissProt/Protein [UniProt]	Accession nr. Hs	Reference MIM	Gene map Hs/Mm
5-phosphatases					
INPP5A	IP5-P-1 Type I IP5-P 43 kDa IP5-P	*INPP5A Human*	*NM_005539.3*	MIM 600106	*10q26.3* *7 F4*
INPP5B	IP5-P-2 75 kDa IP5-P	*INPP5B Human*	*NM_005540.2*	MIM 147264	*1p34* *4 D2*
INPP5D	SHIP SHIP-1 IP5-P D	*SHIP1 Human*	*NM_001017915.1*	MIM 601582	*2q37.1* *1 C5*
INPPL1	SHIP-2 SHIP2 51C protein	*SHIP2 Human*	*NM_001567.3*	MIM 600829	*11q13* *7 F1*
INPP5E	Pharbin Type IV IP5-P IP5-P E 72 kDa IP5-P	*INPP5E Human*	*NM_019892.4*	MIM 613037	*9q34.3* *2 A3*
OCRL	Lowe oculocerebrorenal syndrome protein IP5-P F OCRL INPP5F	*OCRL-1 Human*	*NM_000276.3*	MIM 300535	*Xq25* *X A4*
INPP5G SYNJ1	Synaptojanin-1 SJ1, SYNJ1	*Synaptojanin 1 Human*	*NM 003895.3*	MIM 604297	*21q22.2* *16 C3-C4*
INPP5H SYNJ2	Synaptojanin-2 SYNJ2	*Synaptojanin 2 Human*	*NM 003898.3*	MIM 609410	*6q25.3* *17 A2-A3*
INPP5J PIPP	INPP5J PIB5PA Phosphatidylinositol (4,5) bisphosphate 5-phosphatase A	*PIPP Human*	*NM 001 002837.1*	MIM 606481	*22q12.2* *11 A1*

(continued)

Locus	Common name (s)	SwissProt/Protein [UniProt]	Accession nr. Hs	Reference MIM	Gene map Hs/Mm
INPP5K SKIP	SKIP	*SKIP Human*	*NM 016532.3*	MIM 607875	*17p13.3* *11 B5*
4-phosphatases					
INPP4A	Inositol polyphosphate 4-phosphatase type I	*Type I 4-phosphatase Human*	*NM 004027.2*	MIM 600916	*2q11.2* *1 B*
INPP4B	Inositol polyphosphate 4-phosphatase type II	*Type II 4-phosphatase Human*	*NM 003866.2*	MIM 607494	*4q31.21* *8 C2*
TMEM55A	PtdIns(4,5)P_2 4-phosphatase type I	*Type I PtdIns(4,5)P2 4-phosphatase*	*NM 018710.2*	MIM 609864	*8q21.3* *4 A1-A2*
TMEM55B	PtdIns(4,5)P_2 4-phosphatase type II	*Type II PtdIns(4,5)P2 4-phosphatase*	*NM 001100814.1*	MIM 609865	*14q11.2* *14 C1*
P-Rex1	P-Rex1	*P-Rex1 Human*	*NM 020820.3*	MIM 606905	*20q13.13* *2 H3*
P-Rex2	P-Rex2a P-Rex2b	*P-Rex2 Human*	*NM 024870.2*	MIM 612139	*8q13.2* *1 A3*
Sac phosphatases					
SACM1L	SAC1	*SAC1 Human*	*NM 014016*	MIM 606569	*3p21.3* *9 F*
INPP5F	SAC2	*SAC2 Human*	*NM 014937.2*	MIM 609389	*10q26.11* *7 F3*
FIG4	SAC3	*SAC3 Human*	*NM 014845.5*	MIM 609390	*6q21* *10 B1*

**IP5-P* (*i*nositol *p*olyphosphate *5-p*hosphatase)

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