REVIEW ARTICLE

The role of the inositol polyphosphate 5-phosphatases in cellular function and human disease

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Phosphoinositides are membrane-bound signalling molecules that regulate cell proliferation and survival, cytoskeletal reorganization and vesicular trafficking by recruiting effector proteins to cellular membranes. Growth factor or insulin stimulation induces a canonical cascade resulting in the transient phosphorylation of PtdIns(4,5)P_2 by PI3K (phosphoinositide 3-kinase) to form PtdIns(3,4,5)P_3, which is rapidly dephosphorylated either by PTEN (phosphatase and tensin homologue deleted on chromosome 10) back to PtdIns(4,5)P_2, or by the 5-ptases (inositol polyphosphate 5-phosphatases), generating PtdIns(3,4)P_2. The 5-ptases also hydrolyse PtdIns(4,5)P_2, forming PtdIns4*P*. Ten mammalian 5-ptases have been identified, which share a catalytic mechanism similar to that of the apurinic/apyrimidinic endonucleases. Gene-targeted deletion of 5-ptases in mice has revealed that these enzymes regulate haemopoietic cell proliferation, synaptic vesicle recycling, insulin signalling, endocytosis, vesicular trafficking and actin polymerization. Several studies have revealed that the molecular basis of Lowe's syndrome is due to mutations in the 5-ptase OCRL (oculocerebrorenal syndrome of Lowe). Futhermore, the 5-ptases SHIP [SH2 (Src homology 2)-domain-containing inositol phosphatase] 2, SKIP (skeletal muscle- and kidney-enriched inositol phosphatase) and 72-5ptase (72 kDa 5-ptase)/Type IV/Inpp5e (inositol polyphosphate 5-phosphatase E) are implicated in negatively regulating insulin signalling and glucose homoeostasis in specific tissues. SHIP2 polymorphisms are associated with a predisposition to insulin resistance. Gene profiling studies have identified changes in the expression of various 5-ptases in specific cancers. In addition, 5-ptases such as SHIP1, SHIP2 and 72-5ptase/Type IV/Inpp5e regulate macrophage phagocytosis, and SHIP1 also controls haemopoietic cell proliferation. Therefore the 5-ptases are a significant family of signal-modulating enzymes that govern a plethora of cellular functions by regulating the levels of specific phosphoinositides. Emerging studies have implicated their loss or gain of function in human disease.

Key words: cancer, diabetes, inflammation, Lowe's syndrome, 5-phosphatase, phosphoinositide.

INTRODUCTION

Phosphoinositides are ubiquitous phospholipid constituents of cell membranes that comprise a fatty acid backbone which anchors the lipid moiety in the membrane, attached to a six-sided inositol ring, which can be phosphorylated at the 3, 4, or 5 position to generate an array of signalling molecules with discrete functions [1]. PI3Ks (phosphoinositide 3-kinases) are a family of critical intracellular enzymes that catalyse the addition of a phosphate molecule specifically to the 3-position of the inositol ring to generate the D3 phosphoinositides, which include PtdIns(3,4,5)P_3, PtdIns(3,4)P_2, PtdIns3*P* and, via PIKfyve, PtdIns(3,5)P_2 [2,3]. PI3K initiates signalling pathways that regulate vesicular trafficking, cell proliferation, differentiation, protein translation, inhibition of apoptosis and actin cytoskeletal rearrangements and thereby cell migration [4–6] (Figure 1).

Activated cell-surface receptors recruit and activate class I PI3K, which phosphorylates PtdIns(4,5)P_2 to transiently generate PtdIns(3,4,5)P_3. PtdIns(3,4,5)P_3 recruits and localizes signalling proteins that contain lipid-binding domains such as PH (pleckstrin homology) domains to the inner wall of the plasma membrane, leading to their activation. These include the serine/threonine

Abbreviations used: AP-2, activator protein 2; APPL1, adaptor protein containing PH (pleckstrin homology) domain, PTB (phosphotyrosine-binding) domain and leucine zipper motif 1; ARF, ADP-ribosylation factor; ASH, ASPM (abnormal spindle-like microcephaly-associated protein)/SPD2 (spindle pole body 2)/hydin; BCR, B-cell receptor; Btk, Bruton's tyrosine kinase; Cdk5, cyclin-dependent kinase 5; CHO, Chinese-hamster ovary; CHO-IR, CHO cells expressing human insulin receptor; CIU, chronic idiopathic urticaria; CML, chronic myelogenous leukaemia; CNS, central nervous system; Dok, downstream of tyrosine kinase; DYRK1A, dual-specificity tyrosine-phosphorylated and -regulated kinase 1A; EH, Eps15 homology; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; F-actin, filamentous actin; GEF, guanine-nucleotide-exchange factor; GIPC, GAIP (G_α-interacting protein)-interacting protein C-terminus; GK, glucokinase; GLUT4, glucose transporter 4; G6Pase, glucose-6-phosphatase; Grb2, growth-factor-receptor-bound protein 2; GSK3β, glycogen synthase kinase 3β; IL, interleukin; Inpp5b, inositol polyphosphate 5-phosphatase B; Inpp5e, inositol polyphosphate 5-phosphatase E; IR, insulin receptor; IRS, IR substrate; ITAM, immunoreceptor tyrosine-based activator motif; KLF2, Krüppel-like factor 2; LPS, lipopolysaccharide; MDS, Miller–Dieker syndrome; MNB, minibrain; mTOR, mammalian target of rapamycin; NCA, Na^+/Ca^{2+} antiporter; NGF, nerve growth factor; OCRL, oculocerebrorenal syndrome of Lowe; $p70^{S6K}$, p70 S6 kinase; PDK1, phosphoinositide-dependent kinase 1; PEPCK, phosphoenolpyruvate carboxykinase; PH, pleckstrin homology; PI3K, phosphoinositide 3-kinase; PIPP, proline-rich inositol polyphosphate 5-phosphatase; PLC, phospholipase C; PPIPase, polyphosphoinositide phosphatase; PRD, proline-rich domain; 3-ptase, inositol polyphosphate 3-phosphatase; 4-ptase, inositol polyphosphate 4-phosphatase; 5-ptase, inositol polyphosphate 5-phosphatase; 72-5ptase, 72 kDa 5-ptase; PTEN, phosphatase and tensin homologue deleted on chromosome 10; RhoGAP, RhoGTPase-activating protein; RNAi, RNA interference; SAM, sterile α motif; SH2, Src homology 2; Shc, Src homology and collagen homology; SHIP, SH2-domain-containing inositol phosphatase; SHIP2-Tg, transgenic mice overexpressing SHIP2; SKIP, skeletal muscle- and kidney-enriched inositol phosphatase; SKICH, SKIP carboxyl homology; SNP, single nucleotide polymorphism; TAT-5′, tyrosine aminotransferase; TGN, *trans*-Golgi network; TLR, Toll-like receptor; TNFα, tumour necrosis factor α; TrkA, tropomyosin receptor kinase A; UTR, untranslated region.

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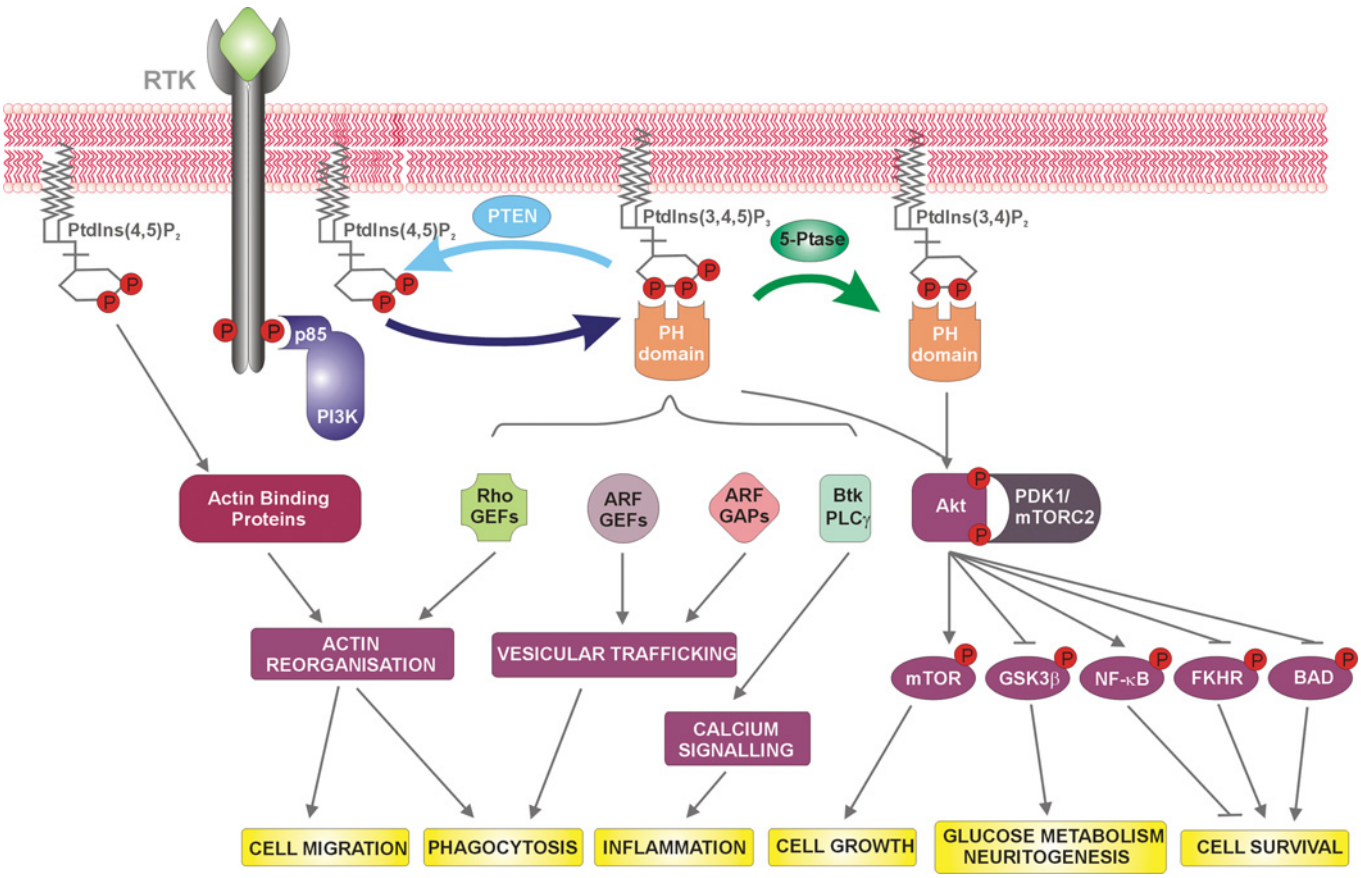

Figure 1 Diverse roles of phosphoinositides

Activation of receptor tyrosine kinases (RTK) leads to the recruitment of the PI3K to the receptor providing access to PtdIns(4,5)P_2, which it phosphorylates transiently, forming PtdIns(3,4,5)P_3 on the inner wall of the plasma membrane. PtdIns(3,4,5)P_3 binds PH-domain-containing proteins and thereby recruits to the plasma membrane a variety of effectors including Rho-GEFs, ARF-GEFs, non-receptor tyrosine kinases such as Btk, PLCγ and Akt, leading to their allosteric activation and initiation of downstream signalling cascades that promote a variety of cellular effects. PtdIns(3,4,5)P_3 signalling may be terminated by the lipid phosphatase PTEN, which hydrolyses this phosphoinositide to form PtdIns(4,5)P_2. Alternatively, in growth-factor-stimulated cells, PtdIns(3,4,5)P_3 is hydrolysed at the 5-position phosphate by 5-ptases to form PtdIns(3,4)P_2. Some PI3K effectors such as Btk only bind and are activated by PtdIns(3,4,5)P_3, some, such as Akt, bind both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2. BAD, Bcl-2/Bcl-X_L-antagonist, causing cell death; FKHR, forkhead in rhabdosarcoma; mTORC2, mTOR complex 2; NF-κB, nuclear factor κB. An animated version of this Figure can be seen at http://www.BiochemJ.org/bj/419/0029/bj4190029add.htm.

kinase, Akt (also known as protein kinase B), as well as its activating kinase PDK1 (phosphoinositide-dependent kinase 1), Btk (Bruton's tyrosine kinase), ARNO [ARF (ADP-ribosylation factor) nucleotide-binding-site operator], Grp1 (general receptor for phosphoinositides 1) and Rac-GEFs (guanine-nucleotide-exchange factors) such as P-Rex1 and P-Rex2 [7,8]. The PH domain of Akt binding to PtdIns(3,4,5)P_3/PtdIns(3,4)P_2 facilitates a conformational change allowing Akt phosphorylation on Thr^{308} and Ser^{473} [9–11]. The Akt 'catalytic T-loop' phosphorylation on Thr^{308} is mediated by PDK1 [10], whereas the C-terminal hydrophobic motif of Akt is phosphorylated on Ser^{473} by mTORC2 (mammalian target of rapamycin complex 2) [or mTOR (mammalian target of rapamycin)–rictor (rapamycin-insensitive companion of mTOR)] [11]. Phosphorylated Akt disassociates from the plasma membrane to the cytosol to elicit a plethora of cellular responses via phosphorylation of downstream effectors such as GSK3β (glycogen synthase kinase 3β), Mdm2 (murine double minute 2), mTOR, $p70^{S6K}$ (p70 S6 kinase), BAD (Bcl-2/Bcl-X_L-antagonist, causing cell death), CREB (cAMP-response-element-binding protein), Forkhead and NF-κB (nuclear factor κB), which in turn promote glucose uptake, cell proliferation and inhibition of apoptosis and many other cellular responses [12].

PtdIns(3,4,5)P_3 levels are governed by its synthesis by PI3K and its degradation by phosphoinositide phosphatases. Two major PtdIns(3,4,5)P_3-degradation pathways have been defined. The 3-ptase (inositol polyphosphate 3-phosphatase) PTEN (phosphatase and tensin homologue deleted on chromosome 10), which is a tumour suppressor that also regulates insulin signalling, hydrolyses the 3-position phosphate from PtdIns(3,4,5)P_3 forming PtdIns(4,5)P_2, thereby terminating PI3K signalling. *In vitro* studies have revealed that PTEN also hydrolyses the 3-position phosphate from PtdIns(3,4)P_2, but whether this occurs *in vivo* is unclear [13]. PtdIns(3,4,5)P_3 is also rapidly degraded at the 5-position phosphate by 5-ptases (inositol polyphosphate 5-phosphatases), forming PtdIns(3,4)P_2, which is in turn dephosphorylated by 4-ptases (inositol polyphosphate 4-phosphatases) to form PtdIns3*P*. This sequential dephosphorylation cascade results in the termination of PtdIns(3,4,5)P_3-dependent signalling, but generates another signalling molecule, PtdIns(3,4)P_2, which may also recruit and activate Akt signalling and other effectors. The relative levels of PtdIns(3,4,5)P_3 compared with PtdIns(3,4)P_2 are probably dependent on the cell type, receptor type activated, relative levels of PTEN, the tissue- and cell-specific expression of 5- and 4-ptases, and the signalling complexes with which they associate that regulate phosphatase activity

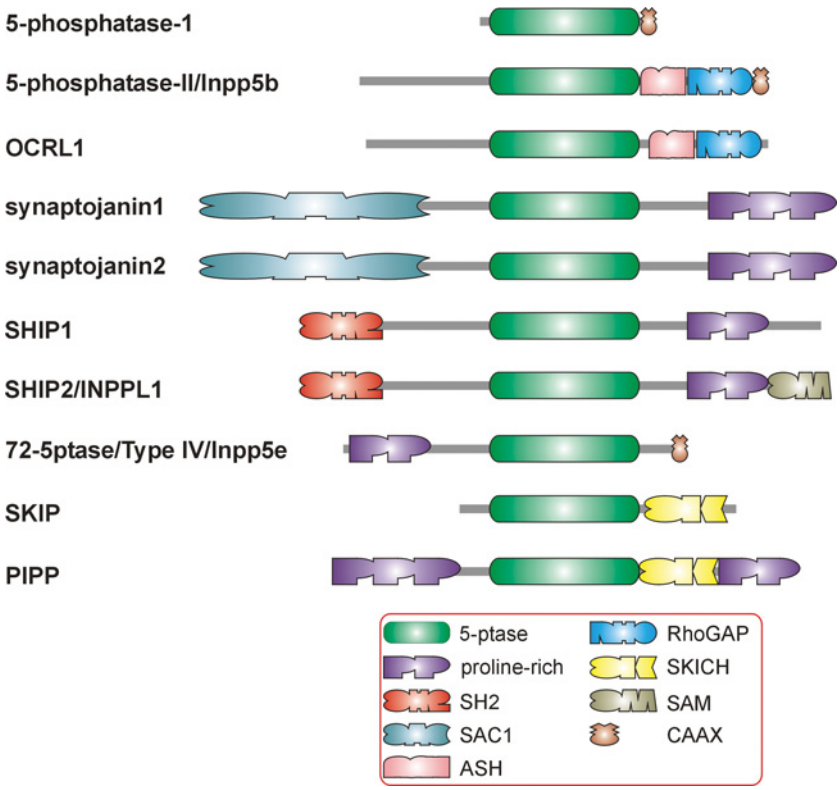

Figure 2 Domain structure of the ten mammalian 5-ptases

Each 5-ptase contains a conserved catalytic 5-ptase domain, with synaptojanin 1 and 2 also containing a catalytic Sac-1 domain. CAAX motifs and SKICH domains mediate protein localization, whereas other domains, including PRDs, SAM, SH2 and ASH RhoGAP are important for protein–protein interactions.

and/or subcellular localization. Some cell types exhibit sustained PtdIns(3,4)P_2 production, lasting for up to 45–60 min post-stimulation [14]. This is most notable following BCR (B-cell receptor)-mediated lymphocyte activation and thrombin-stimulated platelet aggregation [14,15]. PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 appear sequentially following agonist stimulation in many cell types, but show temporal overlap. It has therefore been challenging to delineate the precise role each phosphoinositide plays, independent of the other, in activating various effectors which exhibit both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 binding capacity. This analysis has been assisted in part by characterizing the signalling pathways and the functional consequences of altered PTEN, 4- or 5-ptase expression in cell lines or mice. The present review focuses on the 5-ptases and explores the available evidence that indicates that these enzymes may be important in the regulation of specific cellular functions and the pathogenesis of human diseases.

INOSITOL POLYPHOSPHATE 5-PHOSPHATASES

The 5-ptase family of phosphatases comprises ten mammalian (Figure 2) and four yeast enzymes. The 5-ptases, by degrading various 5-position phosphorylated phosphoinositides, regulate diverse cellular processes such as protein trafficking, phagocytosis and synaptic vesicle recycling [1,16,17]. Each 5-ptase contains a conserved 300-amino-acid catalytic domain which removes the 5′-position phosphate from the inositol ring of specific 5-position phosphorylated phosphoinositides including PtdIns(4,5)P_2, PtdIns(3,4,5)P_3 and/or PtdIns(3,5)P_2 [1]. In addition, the first family member purified and cloned, 5-phosphatase-I, hydrolyses only soluble inositol phosphates Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4, thereby regulating intracellular calcium signalling. 5-Phosphatase-I will not be discussed further here. The other nine family members also hydrolyse some soluble inositol phosphates, such as Ins(1,4,5)P_3 and/or Ins(1,3,4,5)P_4. The inositol phosphates and phosphoinositides have also been detected in the nucleus, where they regulate a number of cellular processes, including chromatin remodelling, gene expression, mRNA export, DNA repair, maintenance of telomere length and phosphorylation of nuclear proteins (reviewed in [18–21]). To date, SHIP [SH2 (Src homology 2)-domain-containing inositol phosphatase] 2 is the only 5-ptase reported to have a nuclear localization; however, its function in the nucleus has not been delineated [22]. No definitive role for the 5-ptases in the regulation of nuclear phosphoinositides or inositol phosphates has been established and this will not be discussed further here.

The central 5-ptase catalytic domain folds in a manner similar to that of the apurinic/apyrimidinic endonuclease family of DNA-modifying enzymes [23,24]. Numerous other domains and motifs including SH2, Sac-1, proline-rich, CAAX, RhoGAP (RhoGTPase-activating protein) and SKICH [SKIP (skeletal muscle- and kidney-enriched inositol phosphatase) carboxyl homology] domains have been identified in the 5-ptases which are important for mediating phosphatase subcellular localization and/or protein–protein interactions. These latter interactions enable the 5-ptases to function as molecular scaffolds which incorporate into large signalling complexes. The Sac-1 domain also represents an additional catalytic CX_5R motif in synaptojanin-1 and -2, which hydrolyses PtdIns3*P*, PtdIns4*P*

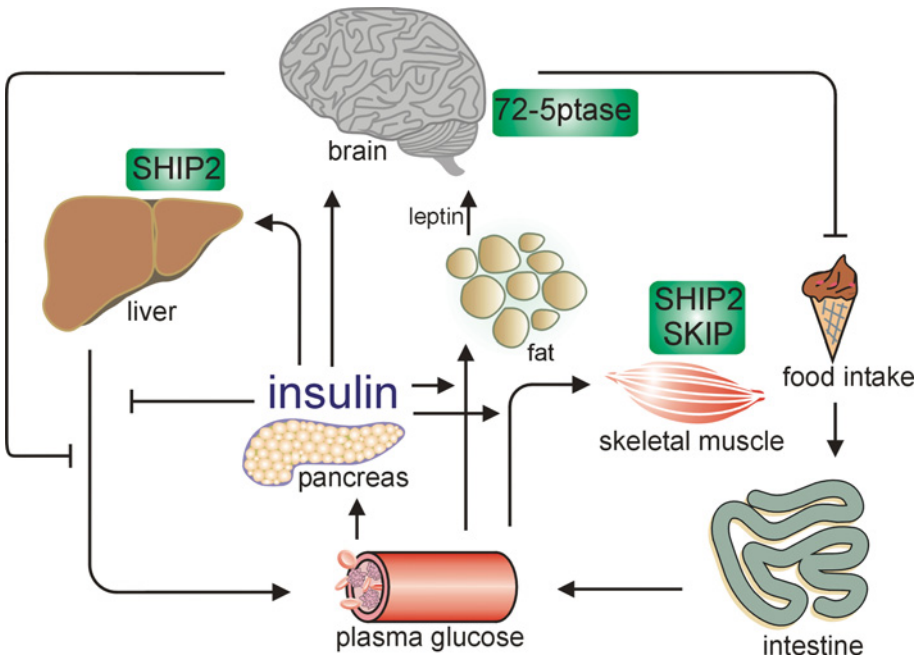

Figure 3 Regulation of insulin responses via PtdIns(3,4,5)P_3 5-ptases SKIP, SHIP2 and 72-5ptase/Type IV/Inpp5e

In response to increased plasma glucose levels, pancreatic β-cells secrete insulin which acts on target tissues to regulate glucose, lipid and protein metabolism. Plasma glucose disposal occurs in skeletal muscle and adipose tissue upon insulin stimulation, leading to subsequent storage. Leptin released by adipose tissue acts on the CNS to positively regulate metabolism, whereas insulin inhibits gluconeogenesis and glucose output in the liver. Insulin signalling in the hypothalamus also influences sympathetic nervous system activation of hepatic insulin sensitivity and appetite/food intake. There is therefore substantial cross-talk between the various insulin target tissues to collectively modulate nutrient metabolism, although each insulin-responsive tissue serves a unique role in this process. Tissue-specific knockout of mammalian 5-ptases involved in insulin signalling is lacking. However, global gene knockout, antisense oligonucleotide and adenoviral approaches have been utilized to manipulate the expression of SKIP, SHIP2 and 72-5ptase/Type IV/Inpp5e (72-5ptase) in mouse models, suggesting differential roles in specific insulin-responsive tissues.

and PtdIns(3,5)P_2 to PtdIns [25,26]. Whether the two catalytic domains function independently or co-ordinately is not well characterized, but, as the 5-ptase domain of synaptojanin hydrolyses PtdIns(4,5)P_2 to PtdIns4*P*, this would provide the substrate for its Sac-1 domain to potentially degrade PtdIns4*P* to PtdIns at specific subcellular sites. Many reviews have delineated the molecular characterization of 5-ptases [1,27–35], so this review focuses on evidence for the role that specific 5-ptases may play in diabetes, cancer, inflammation and neuronal function.

INSULIN SIGNALLING, DIABETES AND OBESITY

Type 2 diabetes is a complex disorder, caused by insulin resistance in skeletal muscle, liver and adipose tissues and impaired pancreatic islet insulin secretion. There is a global epidemic of Type 2 diabetes, as a consequence of both obesity and physical inactivity. Insulin binding to the IR (insulin receptor) causes phosphorylation of IRS (IR substrates), leading to the recruitment of the p85 subunit of PI3K, activating the p110 catalytic subunit and producing PtdIns(3,4,5)P_3 from PtdIns(4,5)P_2 [36–38]. PtdIns(3,4,5)P_3 mediates the binding and activation of Akt, leading to phosphorylation of AS160, GSK3β and other substrates. Akt activity is essential for the translocation and fusion of GLUT4 (glucose transporter 4) with the plasma membrane in skeletal muscle and fat tissue and thereby glucose uptake [39]. Insulin-mediated PI3K/Akt-dependent signalling is also critical for the regulation of mRNA expression of proteins required for gluconeogenesis, glycolysis and lipid synthesis in the liver [40]. Emerging evidence suggests that PI3K signals generated through insulin signalling contribute to the control of feeding and energy expenditure in the CNS (central nervous system) [41]. Three 5-ptases are implicated in the regulation of PI3K-dependent insulin signalling: SHIP2, SKIP and the 72-5ptase (72 kDa 5-ptase)/Type IV/Inpp5e (inositol polyphosphate-5-phosphatase E) (Figure 3).

SHIP2: THE PROMISE OF EATING WITHOUT WEIGHT GAIN

SHIP2 is a 142 kDa protein containing an N-terminal SH2 domain, a central catalytic 5-ptase domain and a C-terminal PRD (proline-rich domain) comprising a WW-domain-binding motif [PPLP (Pro-Pro-Leu-Pro)], an NPXY (Asn-Pro-Xaa-Tyr) motif and an SAM (sterile α motif) [42]. SHIP2 is highly homologous with the haemopoietic-specific 5-ptase, SHIP1, but exhibits sequence divergence in the PRD. SHIP2 substrate specificity was originally proposed to include 5-position phosphate hydrolysis of PtdIns(3,4,5)P_3 and Ins(1,3,4,5)P_4, generating PtdIns(3,4)P_2 and Ins(1,3,4)P_3 respectively [43]. However, a more recent analysis of SHIP2 enzyme kinetics *in vitro* using the recombinant SHIP2 catalytic domain proposed the preferred substrate specificity may be Ins(1,2,3,4,5)P_5 > Ins(1,3,4,5)P_4 > PtdIns(3,4,5)P_3 $\approx$ PtdIns(3,5)P_2 $\approx$ Ins(1,4,5,6)P_4 [44]. Additionally, SHIP2 has been shown to hydrolyse PtdIns(4,5)P_2 *in vitro* [45]. The biological *in vivo* significance of this proposed substrate preference is unknown. SHIP2 is expressed in both haemopoietic and non-haemopoietic tissues, such as brain, skeletal muscle and heart, and, to a lesser extent, liver and kidney [46,47].

The subcellular localization of SHIP2 in resting cells is mainly cytosolic; however, the enzyme translocates to the plasma membrane following growth factor/insulin stimulation, or cell-matrix contact. SHIP2 regulates the actin cytoskeleton by complexing with actin-regulatory proteins such as filamin,

p130^{Cas}, Shc (Src homology and collagen homology) and vinexin [48–50]. Although SHIP2 expression overlaps with that of SHIP1 in haemopoietic cells, it plays a non-redundant role in several haemopoietic cell lines such as platelets and macrophages [51,52]. The negative role SHIP2 plays in insulin signalling has been demonstrated in several *in vitro* cellular models such 3T3-L1 adipocytes, CHO (Chinese-hamster ovary) cells stably expressing the IR and L6 myotubes. SHIP2 overexpression decreases insulin-stimulated PI3K signalling and Akt activation, leading to reduced GLUT4 translocation to the plasma membrane and subsequent glucose uptake and glycogen synthesis [53–55]. However, RNAi (RNA interference)-mediated knockdown of SHIP2 in 3T3-L1 adipocytes does not alter insulin-stimulated PtdIns(3,4,5)P_3 levels or downstream Akt signalling [56], therefore the role SHIP2 plays in negatively regulating PI3K-dependent insulin signalling in adipocytes is uncertain.

Polymorphisms in the *SHIP2* gene (*INPPL1*) may contribute to the pathogenesis of Type 2 diabetes, hypertension and the metabolic syndrome. A 16 bp deletion located in the proximal region of the *SHIP2* gene 3′-UTR (untranslated region) is significantly associated with individuals with Type 2 diabetes, compared with healthy individuals, as shown by a cohort study of ethnically Caucasian residents from the U.K. and Belgium [57]. *In vitro* studies in HEK (human embryonic kidney)-293 cells have demonstrated that a SHIP2 3′-UTR lacking these 16 base pairs promoted increased expression of a reporter protein, suggesting that, *in vivo*, the mutation may lead to increased expression of SHIP2 and thus insulin insensitivity [57]. Analysis of diseased individuals from British Type 2 diabetic families observed a highly significant association between *SHIP2* gene SNPs (single nucleotide polymorphisms) and hypertension and diabetes [58]. However, analysis of the same polymorphisms in a French cohort did not detect any association with diabetic patients, but found a significant association with diabetic patients with hypertension compared with those without [58]. Further studies of these polymorphisms in British subjects with severe hypertension, but without diabetes or obesity, found no significant association with hypertension, suggesting that these particular *SHIP2* variants may only be associated with hypertension specifically in metabolic syndrome patients [59]. In a Japanese cohort of Type 2 diabetics, several polymorphisms (SNP1–SNP3) were found more commonly in control subjects than in diabetic subjects, suggesting that they may confer protection from insulin resistance [60]. Expression of SNP3-SHIP2, a polymorphism located in the 5-ptase domain, resulted in less efficient inhibition of insulin-stimulated PtdIns(3,4,5)P_3 levels and Akt phosphorylation in CHO-IR cells (CHO cells expressing human IR) compared with wild-type SHIP2, consistent with reduced 5-ptase activity [60]. A more recent study of a Japanese cohort revealed SNPs in the promoter and 5′-UTR of the *SHIP2* gene, which showed strong association with impaired fasting glycaemia and resulted in increased activity of a luciferase reporter [61]. Collectively, these studies suggest that some *SHIP2* polymorphisms identified in control subjects may protect individuals from Type 2 diabetes, whereas others identified in diabetic individuals exhibit a strong association with metabolic phenotypes such as hypertension and obesity.

SHIP2-KNOCKOUT MICE: HYPOGLYCAEMIA OR OBESITY RESISTANCE?

Two SHIP2-knockout mouse studies have been reported with different phenotypes. Clement et al. [62] generated *SHIP2*-knockout mice by targeted deletion of exons 19–29 of the *SHIP2* gene, which may produce a truncated SHIP2 protein, although this has not been demonstrated. *SHIP2*$^{-/-}$ pups appeared cyanotic, pale and lethargic within 24 h postpartum, failed to gain weight and died within 3 days of birth. Blood glucose and insulin concentrations were lower in *SHIP2*$^{-/-}$ mice, and this could be rescued by administering either D-glucose or insulin-neutralizing antibodies. Hepatic glucose output is regulated via PI3K-dependent insulin signalling, which suppresses gluconeogenic [G6Pase (glucose-6-phosphatase), PEPCK (phosphoenolpyruvate carboxykinase) and TAT-5′ (tyrosine aminotransferase)] and enhances glycogenic gene expression [GK (glucokinase) and glycogen synthase]. PEPCK, G6Pase and TAT-5′ mRNA levels were reduced in *SHIP2*$^{-/-}$ liver, consistent with increased hepatic insulin sensitivity. *SHIP2*$^{+/-}$ mice were viable and lived to adulthood, and glucose and insulin tolerance tests revealed rapid glucose uptake in these mice. Serum insulin levels were also decreased, indicating that the observed hypoglycaemia was a consequence of enhanced insulin sensitivity. These results suggest that SHIP2 functions as a negative regulator of insulin signalling [62].

Some controversy surrounds this knockout, however, as the third exon of an additional gene, *Phox2a*, was inadvertently deleted which would result in a non-functional Phox2a protein if expressed [62]. Phox2a is a homeodomain-containing transcription factor that exhibits limited expression in specific neurons and is involved in neuronal development and differentiation [63]. Loss of Phox2a function by gene deletion of its homeobox resulted in complete loss of the locus coeruleus, the prominent noradrenergic centre of the brain. *Phox2a*$^{-/-}$ pups did not demonstrate any gross anatomical abnormalities, but did not feed and died on the day of birth [64]. The effect of *Phox2a* loss-of-function on insulin signalling is still not fully understood; however, the severe hypoglycaemic phenotype observed in *Ship2*$^{-/-}$ mice may be the consequence of expression of a truncated inactive SHIP2 enzyme, truncated Phox2a or due to loss of function of both proteins, although this has yet to be clarified.

Subsequent studies in which only the *Ship2* gene was deleted have been unable to reproduce this original *Ship2*$^{-/-}$ phenotype. More recently, *Ship2*$^{-/-}$ mice were generated by deletion of the first 18 exons of the *Ship2* gene, which encodes the SH2 domain and 5-ptase catalytic domain [65]. *Ship2*$^{-/-}$ mice were viable and survived until adulthood, and showed a slightly truncated facial profile and reduced body weight, despite increased food intake. On a standard chow diet, *Ship2*$^{-/-}$ mice exhibited enhanced insulin-stimulated Akt and p70^{S6K} activation in the liver and skeletal muscle. Surprisingly, however, no differences in fasting serum glucose, insulin levels, glucose and/or insulin tolerance tests were demonstrated. *Ship2*$^{-/-}$ mice exhibited reduced serum lipids and 6-fold lower serum leptin levels on a standard chow diet, possibly as a consequence of reduced body weight. On a 12-week high-fat diet, *Ship2*$^{-/-}$ mice were almost completely resistant to weight gain (although the level of food intake relative to total body weight in comparison with wild-type mice was not reported), exhibited decreased serum lipids, and did not develop hyperglycaemia or hyperinsulinaemia. Under these conditions, *Ship2*$^{-/-}$ mice exhibited increased glucose and insulin tolerance relative to wild-type mice, correlating with enhanced insulin-stimulated PI3K/Akt signalling [65]. Many questions remain to be resolved about the *Ship2*$^{-/-}$ mice phenotype. For example, *Ship2*$^{-/-}$ mice on a standard chow diet showed increased Akt/p70^{S6K} signalling, but no changes in peripheral glucose uptake. This can perhaps be explained by an amplified negative-feedback loop, which may occur as a result of increased p70^{S6K}/mTOR activation, which phosphorylates IRS protein on serine residues, rendering it inefficient for downstream signal

transduction [66]. Secondly, the molecular mechanisms by which *Ship2*$^{-/-}$ mice are resistant to obesity and diabetes have not yet been delineated. Another PtdIns(3,4,5)P_3-metabolizing enzyme, PTEN, also regulates insulin-stimulated PI3K/Akt signalling. Global PTEN knockouts are embryonically lethal, but tissue-specific PTEN knockout in adipose tissue or muscle results in hypersensitivity to insulin [67,68]. Muscle-specific PTEN knockouts are resistant to obesity-associated insulin resistance, reminiscent of *Ship2*$^{-/-}$ mice [68]. We therefore propose that increased insulin-mediated PI3K/Akt signalling in insulin-responsive tissues confers protection from obesity and the metabolic syndrome.

Insulin signalling is also important in the brain, particularly in the hypothalamus to control sympathetic nervous system activation [69], regulation of hepatic insulin sensitivity and glucose output [70], calorie intake/appetite [71] and energy homoeostasis. Insulin signalling in hypothalamic neurons occurs via PI3K activity, for example, ICV (intracerebroventricular) infusion of insulin results in increased Akt phosphorylation on Ser^{473} [72]. Insulin and leptin are proposed to function side by side on similar neurons to effectively reduce energy intake and body fat stores [71]. Genetic predisposition or environmental factors can result in weight gain and, subsequently, peripheral insulin resistance (including the CNS), which further exacerbates obesity and the progression of the metabolic syndrome. NIRKO (neuron-specific IR knockout) mice exhibit increased food intake and obesity, mild insulin resistance, hyperinsulinemia, hyperlipidaemia and increased leptin levels [73]. In this regard, loss of SHIP2 enzyme activity in the hypothalamus may ameliorate insulin signalling during obesity-induced insulin resistance.

The effect of whole-body SHIP2 knockdown using antisense oligonucleotides has been reported [74], and the phenotype observed is consistent with the SHIP2 knockout reported by Sleeman et al. [65]. Rats administered SHIP2 antisense oligonucleotides showed normal insulin tolerance on a standard chow diet [74]. However, on a high-fat diet, rats treated with SHIP2 antisense oligonucleotides exhibited increased glucose disposal. Muscle from these treated animals displayed enhanced insulin-mediated Akt activation which correlated with significantly reduced SHIP2 protein expression in this tissue. As SHIP2 oligonucleotide treatment was conducted 1 day before insulin-tolerance tests, the phenotype observed would be primarily due to the effects of short-term SHIP2 protein reduction and so the long-term effects still need to be evaluated [74].

Transgenic mice overexpressing SHIP2 (SHIP2-Tg) under the control of a modified chicken β-actin promoter with CMV-IE (cytomegalovirus immediate-early) enhancer exhibited SHIP2 overexpression in the liver, skeletal muscle, white adipose tissue, pancreas, brown adipose tissue and brain. SHIP2-Tg mice gained more weight (5 %) and exhibited elevated fasting insulin levels, but not glucose, leptin, adiponectin or serum lipids, relative to wild-type mice on a standard chow diet [75]. SHIP2-Tg mice also showed a slight, but significant, decrease in glucose and insulin tolerance, correlating with reduced insulin-induced Akt activation in the liver, fat and skeletal muscle. Liver glucose metabolism in SHIP2-Tg mice was impaired, with G6Pase and PEPCK mRNA increased, and with GK mRNA and glycogen content reduced, indicating hepatic insulin resistance. In addition, a marker of metabolic rate, Ucp1 (uncoupling protein 1) mRNA, was reduced in epididymal white adipose tissue of SHIP2-Tg mice. Surprisingly, SHIP2-Tg mice exhibited comparable weight gain and insulin and glucose tolerance relative to wild-type mice on a high-fat diet [75]. Therefore SHIP2 may not exacerbate insulin resistance induced by high-fat feeding, perhaps due to compensatory activation of other metabolic responses elicited by a high-fat diet.

While tissue-specific knockout/knockin of SHIP2 in mice have yet to be reported, adenoviral mediated liver-specific expression of SHIP2 in two diabetic rodent models has been described [76,77]. Leptin-receptor-deficient *db*/*db* mice are leptin-resistant and diabetic. These mice exhibit insulin resistance, associated with decreased PI3K/Akt signalling, hyperglycaemia, hyperinsulinaemia, increased gluconeogenesis and develop symptoms relating to the metabolic syndrome [78]. Overexpression of wild-type SHIP2 in *db*/+*m* (heterozygote) and ΔIP-SHIP2 (catalytically inactive SHIP2 deletion mutant) in *db*/*db* mice have been reported [76]. The ΔIP-SHIP2 mutant acts as a dominant-negative, possibly through competition with endogenous SHIP2 for binding to PtdIns(3,4,5)P_3, leading to enhanced downstream Akt signalling [54,55]. Wild-type SHIP2 overexpression in *db*/+*m* mice decreased Akt activation, whereas ΔIP-SHIP2 expression improved the reduced Akt signalling in the liver of *db*/*db* mice [76]. Hepatic expression of SHIP2 or ΔIP-SHIP2, however, did not affect insulin signalling in either skeletal muscle or fat tissues in either mouse strain. Wild-type SHIP2-expressing *db*/+*m* mice showed reduced glucose tolerance, whereas ΔIP-SHIP2 expression in *db*/*db* mice resulted in improved tolerance [76]. These results suggest that SHIP2 modulation of liver-specific insulin sensitivity can influence peripheral glucose and insulin tolerance. Similarly, inhibition of SHIP2 in the liver in another hyperglycaemic and hyperinsulinaemic diabetic mouse model, KKA^y, resulted in ameliorated insulin-induced Akt activation and reduced the abundant G6Pase and PEPCK mRNA (as a consequence of increased gluconeogenesis owing to insulin resistance) in response to pyruvate [77]. Glucose disposal was also increased in ΔIP-SHIP2-expressing KKA^y mice [77]. Finally, the role that SHIP2 plays in regulating denervation-induced insulin resistance was examined in rats. Antisense oligonucleotide-mediated knockdown of SHIP2 improved insulin-stimulated glucose uptake, which is suppressed following denervation [79].

Collectively, these studies support the contention that SHIP2 plays a key role in negatively regulating insulin signalling. Although the precise molecular mechanisms by which SHIP2 controls insulin-stimulated PI3K-dependent responses in a tissue-specific manner need to be elucidated further, inhibition of SHIP2 expression and/or function may represent a therapeutic tool for treating Type 2 diabetes and obesity-induced insulin resistance.

SKIP

SKIP is a relatively uncharacterized 51 kDa 5-ptase that is expressed in the heart, skeletal muscle and kidney [80]. SKIP contains a central catalytic 5-ptase domain and a C-terminal SKICH domain, the latter being responsible for its membrane localization. SKIP localizes to the perinuclear region, overlapping with the ER (endoplasmic reticulum) in quiescent cells and translocates to the plasma membrane upon EGF (epidermal growth factor) or insulin stimulation [81,82]. Whereas *in vivo* studies have indicated SKIP's preferred substrate to be PtdIns(3,4,5)P_3 [82], a more comprehensive *in vitro* kinetic analysis of SKIP substrate specificity indicates a preference for PtdIns(4,5)P_2 over PtdIns(3,4,5)P_3 [83]. However, this latter analysis was performed using only the catalytic domain, without the SKICH domain. SKIP inhibits insulin-induced PI3K/Akt-mediated signalling responses. Overexpression of SKIP in CHO-IR cells negatively regulates Akt and p70^{S6K} phosphorylation in response to insulin stimulation, but not p44 and p42 phosphorylation [82]. SKIP overexpression also attenuates

insulin-stimulated GLUT4 translocation to the plasma membrane and the formation of membrane ruffles [82]. Glucose uptake and glycogen synthesis were decreased in SKIP-overexpressing L6 myotubes. Conversely, SKIP knockdown using antisense oligonucleotides or siRNA (short interfering RNA) resulted in increased Akt phosphorylation at both Ser^{473} and Thr^{308} following insulin stimulation [82]. The increased Akt phosphorylation could be suppressed by re-expression of SKIP, but not by expression of SHIP2 or PTEN, suggesting that SKIP plays a role in insulin signalling distinct from these other lipid phosphatases [84].

Homozygous deletion of SKIP in mice is embryonically lethal, with no $Skip^{-/-}$ embryos detected after E10.5 [84]. $Skip^{+/-}$ mice demonstrated increased glucose tolerance and insulin sensitivity on a standard chow diet. $Skip^{+/-}$ mice also showed higher levels of Akt activation in skeletal muscle compared with wild-type mice, but not in adipose tissue, where full-length SKIP is not expressed [84]. When fed on a high-fat diet, $Skip^{+/-}$ mice demonstrated increased insulin sensitivity and glucose tolerance. Although $Skip^{+/-}$ mice displayed weight gain and hyperglycemia on a high-fat diet, this was substantially lower than that observed in wild-type mice, suggesting that decreased SKIP expression also provides some protection against diet-induced obesity [84].

SKIP is proposed to play a major role in regulating insulin signalling in skeletal muscle, and, given that ~75 % of post-feeding glucose is taken up by skeletal muscle, increased insulin hypersensitivity in this tissue could at least partly explain the increased glucose tolerance observed in $Skip^{+/-}$ mice. However, as SKIP is highly expressed in brain as well as skeletal muscle, increased whole-body insulin sensitivity could also be mediated by central insulin effects on the brain [84]. Further studies on tissue-specific $Skip^{-/-}$ mice are required to fully elucidate the molecular mechanisms by which SKIP regulates insulin signalling.

72-5PTASE/TYPE IV/INPP5E

72-5ptase contains an N-terminal PRD, a central catalytic 5-ptase domain and a C-terminal CAAX motif. The human enzyme, which is called the Type IV 5-ptase, and the rat enzyme, known as pharbin, share 74 % amino acid sequence identity [85–87]. 72-5ptase/Type IV/Inpp5e is expressed in various tissues, including the brain, testis, breast and haemopoietic cells [85,86]. Its intracellular localization is mainly cytosolic, with prominent perinuclear/Golgi localization. The 72-5ptase/Type IV/Inpp5e hydrolyses the 5′-position phosphate from PtdIns(3,5)P_2, PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3, forming PtdIns3P, PtdIns4P and PtdIns(3,4)P_2 respectively, and is reported to be the most potent PtdIns(3,4,5)P_3 5-ptase on the basis of kinetic analysis [85–87]. Overexpression of 72-5ptase/Type IV/Inpp5e in unstimulated 3T3-L1 adipocytes results in the generation of PtdIns3P at the plasma membrane of transfected cells, via hydrolysis of PtdIns(3,5)P_2, promoting the translocation and insertion of GLUT4 into the plasma membrane without affecting glucose uptake [88]. However, these results are based on ectopic 72-5ptase/Type IV/Inpp5e expression and do not provide evidence that the 5-ptase plays a functional role in GLUT4 translocation in adipocytes *in vivo* [88]. 72-5ptase/Type IV/Inpp5e expression has been detected in the hypothalamus and, following insulin stimulation, the enzyme is tyrosine phosphorylated and interacts with IRS-1/IRS-2 and PI3K [89]. Antisense-mediated reduction of 72-5ptase/Type IV/Inpp5e in the hypothalamus increased PtdIns(3,4,5)P_3 levels, correlating with reduced food intake and weight loss [89]. These effects were reversed with concomitant treatment with the PI3K inhibitor LY294002, indicating that the responses were PI3K-dependent. Hypothalamic reduction of 72-5ptase/Type IV/Inpp5e also affected the metabolic parameters of treated rats, as shown by reduced serum insulin, leptin and glucose [89]. On the basis of these data, the 72-5ptase/Type IV/Inpp5e may, along with SHIP2 and SKIP, regulate glucose homoeostasis and energy metabolism. No mouse knockout of the 72-5ptase/Type IV/Inpp5e has been reported. Gene profiling has also demonstrated differential expression of this enzyme (*INPP5E*) in several cancers, which will be discussed below.

LOWE'S SYNDROME

Lowe's oculocerebrorenal syndrome, first described in 1952, is a rare X-linked disorder affecting approx. 1 in 200 000 births. The disease is characterized by growth and mental retardation, bilateral congenital cataracts and renal failure, with impaired solute and protein reabsorption in the kidney proximal tubule and renal tubular acidosis [29]. Female carriers exhibit punctate opacities in the lens [90,91]. Breakpoint mapping of two affected individuals identified a gene encoding a ubiquitously expressed 105 kDa 5-ptase, *OCRL* (oculocerebrorenal syndrome of Lowe) [92]. The OCRL domain structure comprises a central 5-ptase catalytic domain and a catalytically inactive C-terminal RhoGAP domain, distal to a recently identified ASH [ASPM (abnormal spindle-like microcephaly-associated protein)/SPD2 (spindle pole body 2)/hydin] domain [93,94]. ASH domains have been predicted to bind microtubules. Mutations in several other ASH-domain-containing proteins are associated with abnormalities in brain development and hydrocephalus [93,95,96]. Recently, mutations in OCRL have been identified in a subset of patients with Dent 2 disease, an X-linked renal tubulopathy characterized by low-molecular-mass proteinuria and renal failure [97–99]. Dent 2 disease patients, however, do not exhibit the renal tubular acidosis or cataracts observed in Lowe's syndrome.

OCRL preferentially hydrolyses PtdIns(4,5)P_2 as well as Ins(1,4,5)P_3, Ins(1,3,4,5)P_4, PtdIns(3,5)P_2 and PtdIns(3,4,5)P_3 [83,100]. PtdIns(4,5)P_2 levels are increased in cells from Lowe's syndrome [101,102] and MRI (magnetic resonance imaging) brain scans of affected individuals exhibit cystic abnormalities in the white matter, suggestive of PtdIns(4,5)P_2 accumulation [103]. This 5-ptase localizes to the TGN (*trans*-Golgi network), lysosomes and endosomes and is enriched in clathrin-coated vesicles [101,104,105]. High levels of lysosomal enzymes are found in the plasma of affected individuals suggesting an endosomal trafficking/secretion defect [106]. Upon growth factor stimulation, OCRL translocates to membrane ruffles in a Rac1-dependent manner where it co-localizes with polymerized actin and Rac1 [102].

PtdIns(4,5)P_2 binds and regulates the activity of a number of actin-binding proteins, including gelsolin, profilin, cofilin, vinculin, talin and α-actinin, and promotes actin polymerization [107]. OCRL regulates actin cytoskeleton reorganization, consistent with its role in hydrolysing PtdIns(4,5)P_2. Fibroblasts from Lowe's syndrome exhibit reduced actin stress fibres, increased F-actin (filamentous actin) puncta and enhanced sensitivity to actin-depolymerizing agents [108]. In addition, the actin-binding proteins gelsolin and α-actinin, which are regulated by PtdIns(4,5)P_2, exhibit altered localization in the fibroblasts derived from Lowe's syndrome [108].

While the molecular defects underlying Lowe's syndrome are currently emerging, one possible explanation for the renal tubular acidosis may be abnormal trafficking of receptors required for solute reabsorption from proximal tubules. Several recent

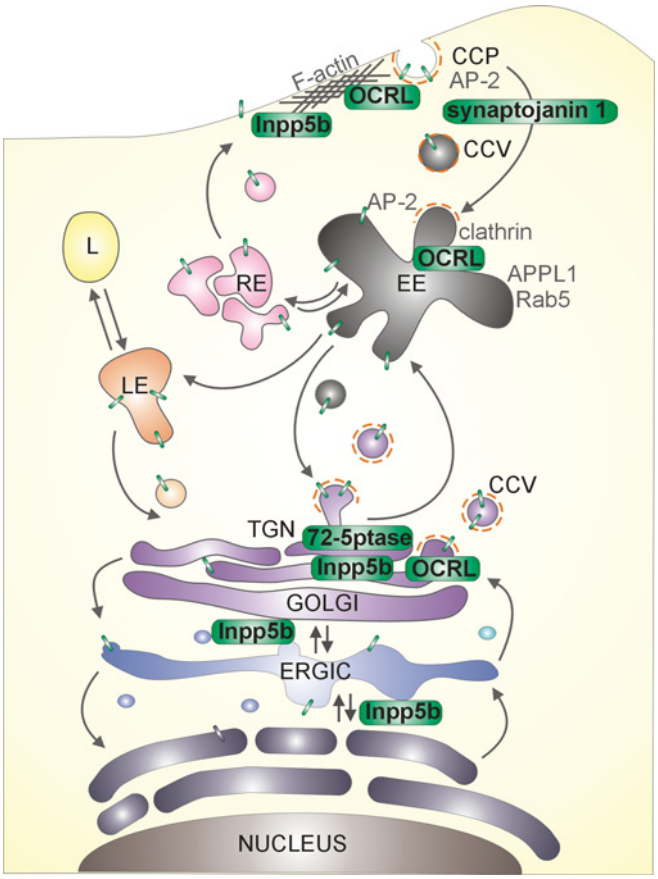

Figure 4 5-Ptase regulation of vesicular trafficking

Inpp5b may regulate the trafficking of biosynthetic cargo, such as receptors, through the ER and ERGIC, where they are sorted for delivery to the Golgi. Biosynthetic cargo is processed to the TGN, where OCRL, Inpp5b and the 72-5ptase/Type IV/Inpp5e (72-5ptase) are localized and may regulate trafficking of cargo to endocytic compartments, or endosome–TGN retrieval. In early endosomes (EE), OCRL may interact with Rab5, APPL1, clathrin and clathrin adaptor AP-2, to facilitate trafficking of receptors to the cell surface or other endocytic compartments, such as the late endosome (LE), recycling endosome (RE) or the lysosome (L). OCRL and Inpp5b can translocate to the plasma membrane in response to growth factor stimulation and co-localize with F-actin at membrane ruffles. Surface receptors can be internalized in clathrin-coated pits (CCP) which mature to clathrin-coated vesicles (CCV). Synaptojanin 1 hydrolyses PtdIns(4,5)P_2 to facilitate clathrin uncoating of vesicles.

studies have demonstrated that OCRL regulates protein trafficking (Figure 4). Overexpression of wild-type OCRL, or OCRL lacking the 5-ptase domain, results in Golgi fragmentation, the redistribution of the cation-independent mannose 6-phosphate receptor to enlarged endosomes and a block in clathrin-mediated transport from early endosomes to the TGN [104,109]. In addition, retrograde trafficking of Shiga toxin from endosomes to the TGN is blocked in cells overexpressing wild-type OCRL, or OCRL lacking the 5-ptase domain [104,109]. Knockdown of OCRL by RNAi also leads to impaired endosome/TGN trafficking as is evident from a redistribution of a cation-independent mannose 6-phosphate receptor reporter to early endosomes, consistent with OCRL playing a role in regulating endosome to Golgi trafficking [104].

A number of OCRL-binding partners have been identified, including clathrin, AP-2 (activator protein 2), Cdc42 (cell division cycle 42), Rac, APPL1 [adaptor protein containing PH domain, PTB (phosphotyrosine-binding) domain and leucine zipper motif 1], and the active, GTP-bound, forms of Rab1, Rab5 and Rab6 [105,109,110]. Both Rab5 and Rab6 directly stimulate the 5-ptase activity of OCRL in hydrolysing PtdIns(4,5)P_2 [109]. Missense, frameshift and truncating mutations have been identified in Lowe's syndrome (http://research.nhgri.nih.gov/lowe/). These mutations may result in the loss of binding to specific interacting proteins, and may, at least in part, explain the phenotype exhibited in Lowe's syndrome. Whereas many of the OCRL mutations identified in Lowe's syndrome occur within the 5-ptase domain, resulting in loss of enzyme activity, a number of mutations have been reported in the ASH and RhoGAP domains which may affect binding of a subset of proteins [94,105,110]. Interestingly, two point mutations in the inactive RhoGAP domain of OCRL lead to impaired 5-ptase activity, possibly due to altered protein conformation [94]. OCRL also localizes to early endosomes where it co-localizes with APPL1, a Rab5 effector protein which associates with a subset of peripheral early endosomes [105,110]. Mutations in OCRL which disrupt the interaction between the 5-ptase and APPL1, Rab1, Rab5 or Rab6 impair OCRL's endosomal localization and, in the case of the Rab proteins, also perturb Golgi targeting of the 5-ptase [109,110]. Six point mutations which disrupt OCRL/APPL1 binding have been identified in the ASH and RhoGAP domains of OCRL in Lowe's syndrome. Interestingly, each of these mutants retains the ability to bind clathrin and Rac, and some mutants are also still able to bind Rab5 [110]. Taken together, these results suggest that mutations in the 5-ptase domain of OCRL contribute to the Lowe's syndrome phenotype owing to loss of 5-ptase activity, whereas mutations in the ASH and RhoGAP domains result in mislocalization of the protein and thus deregulation of localized phosphoinositide signalling.

Failure of OCRL to associate with APPL1 may contribute to the neurological/cognitive defects observed in Lowe's syndrome. GST (glutathione transferase)–OCRL pull-down experiments using rat brain lysates, revealed a complex between OCRL, APPL1 and GIPC [GAIP (G_α-interacting protein)-interacting protein C-terminus], an endocytic adaptor protein [105]. Both APPL1 and GIPC bind the TrkA (tropomyosin receptor kinase A) NGF (nerve growth factor) receptor and together regulate TrkA endocytic trafficking [105,111,112]. Mutations in *OCRL* may lead to impaired TrkA signalling and thereby the neurological defects observed in the disease [105]; however, this has yet to be verified experimentally. Interestingly, GIPC and APPL1 were identified in pull-down experiments with megalin, a receptor expressed in the kidney proximal tubule which facilitates the uptake of low-molecular-mass proteins [105]. Both GIPC- and megalin-knockout mice exhibit low-molecular-mass proteinuria, similar to that found in Lowe's syndrome, suggesting that the proximal tubular kidney defects observed in these patients may result from impaired receptor trafficking [113,114]. Both Lowe's syndrome and Dent 2 disease-affected individuals exhibit reduced levels of megalin in the urine, suggesting that a defect in megalin recycling to the apical surface of renal proximal tubule cells leads to proteinuria [115]; however, further experimental evidence is required to confirm this hypothesis.

Surprisingly, gene-targeted deletion of *OCRL* in mice does not lead to Lowe's syndrome or Dent 2 disease [116]. This may be due to functional compensation by other 5-ptases such as Inpp5b (inositol polyphosphate 5-phosphatase B; also known as Type II 5-phosphatase) which shares 45 % sequence identity with OCRL and has a similar domain structure [29]. OCRL and Inpp5b also share similar phosphoinositide substrates, although Inpp5b does not hydrolyse PtdIns(3,5)P_2 [83,117,118]. OCRL is expressed in a wide range of human and mouse tissues; however, murine OCRL exhibits lower expression than the human orthologue in the testis [116]. In contrast, Inpp5b is more highly expressed

in mouse brain and kidney, compared with human, two tissues which are particularly affected in Lowe's syndrome, suggesting that Inpp5b may be able to functionally compensate for loss of OCRL in the mouse [116]. Double knockout of both OCRL and Inpp5b is embryonically lethal, supporting the hypothesis that these 5-ptases have overlapping roles [116]. Although much is now known about OCRL, further studies are still required to elucidate fully the molecular mechanisms by which mutations in this 5-ptase result in Lowe's syndrome and Dent 2 disease.

INPP5B

Inpp5b was first identified and purified from human platelets as a 75 kDa polypeptide, which was later shown to be a cleaved fragment of the larger intact protein [119,120]. Inpp5b exhibits 71 % sequence similarity to and 45 % amino acid identity with OCRL [92], as well as a similar domain organization with a central 5-ptase catalytic domain, which preferentially hydrolyses 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 followed by an ASH domain and a catalytically inactive RhoGAP domain ([105], and reviewed in [1]). Inpp5b also contains a CAAX motif at the C-terminus which facilitates its membrane attachment via isoprenylation; however, both the N- and C-terminal domains are required for its full membrane localization [120]. Deletion of the CAAX domain results in decreased 5-ptase activity [118], whereas recombinant Inpp5b lacking both N-and C-terminal domains retains 5-ptase activity [120].

Inpp5b localizes to the Golgi and endocytic pathway and, upon growth factor stimulation, translocates to lamellipodia where it co-localizes with Rab5 and actin [121,122] (Figure 4). Such translocation may facilitate access of the enzyme to its substrate PtdIns(3,4,5)P_3 and/or PtdIns(4,5)P_2. Inpp5b binds Rab5 via the ASH domain, and mutation of conserved key residues within this domain abolishes the interaction as well as perturbing the Golgi localization of the 5-ptase [121,122]. Rab5 binding also increases the 5-ptase catalytic activity of Inpp5b *in vitro*. Co-expression of an activated Rab5 mutant, together with Inpp5b, recruits the 5-ptase to a population of enlarged early endosomes [105,121]. Inpp5b interacts with Rab1, Rab2 (*cis*-Golgi), Rab6 (Golgi stack), Rab9 (late endosome, TGN) and APPL1, suggesting an involvement in the early secretory/trafficking steps between the ER and the Golgi, or retrograde trafficking between the Golgi and the ER [105,122]. Inpp5b co-localizes with ERGIC53, an ERGIC (ER–Golgi intermediate compartment)-resident protein. When HeLa cells were incubated at 15 °C, which blocks ERGIC to Golgi trafficking, overexpression of Inpp5b resulted in an accumulation of ERGIC53 in the ERGIC, with a concomitant decrease in the ER, independent of 5-ptase activity [122]. This suggests that Inpp5b may regulate retrograde ERGIC–ER transport; however, in a separate study, RNAi-mediated knockdown of Inpp5b expression failed to alter retrograde trafficking [121].

In contrast with OCRL, the RhoGAP domain of Inpp5b does not contain a clathrin-box-binding motif [105]. Not surprisingly, Inpp5b does not localize to clathrin-coated vesicles and does not interact with clathrin adaptors [122]; however, RNAi-mediated knockdown of Inpp5b inhibits endocytosis of transferrin [121]. Although catalytically inactive, the RhoGAP domain of Inpp5b has been shown to interact with the Rho family GTPases Rac and Cdc42, but not with Rho [105].

The *INPP5B* gene is located on human chromosome locus 1p34. Interestingly, a number of genes flanking this locus are implicated in genetic disorders involving lens development, kidney function and mental retardation, all characteristics of Lowe's syndrome [123–127]. In mice, the *Inpp5b* gene is located on chromosome 4, a region in which neighbouring genes are involved in lens development [128,129]. Studies of mouse *Inpp5b*$^{-/-}$ models have concentrated on the function of Inpp5b in the testis. Male sterility in some genetic backgrounds appears to be the only phenotype [116]. Histological examination of *Inpp5b*$^{-/-}$ testes has revealed the presence of vacuoles in seminiferous tubule epithelium. Ultrastructural studies showed an accumulation of membrane components such as adherens junctions in the vacuoles of Sertoli cells [130]. A severe disruption of spermatogenesis was detected at 1 year of age in these mice. In addition, *Inpp5b*$^{-/-}$ sperm showed decreased motility and fertilization, coinciding with abnormal processing of the sperm/egg adhesion molecule, β-fertilin [131]. *In vitro* fertilization studies have revealed decreased ability of *Inpp5b*$^{-/-}$ sperm to adhere and fuse with eggs. Sertoli cells act as a support for the maturation and release of sperm in the seminiferous tubules and aid in the processing of β-fertilin. Accumulation of cell adhesion molecules in vacuoles may induce premature shedding of germ cells from the seminiferous epithelium [130]. The recent findings of Williams et al. [122] in the context of ER–Golgi trafficking and secretion, support this contention. Alternatively, inhibition of receptor endocytosis from the plasma membrane, as shown by inhibition of transferrin recycling in Inpp5b RNAi knockdown cells, may indicate abnormal vesicular trafficking and recycling of adhesion proteins, resulting in non-functional cell junction formation at the plasma membrane.

OCRL is not highly expressed in the mouse testis and is therefore unlikely to compensate for the loss of Inpp5b activity. This may, in part, explain why the testis appears to be the only mouse tissue affected by *Inpp5b* gene knockout. There is as yet no known human disease linked to Inpp5b.

5-PTASES AND NEURONAL FUNCTION

The PI3K/Akt signalling pathway plays a significant role in regulating neuronal differentiation and polarity, processes that are essential for the establishment and maintenance of neuronal networks. Stimulation with NGF activates PI3K, generating PtdIns(3,4,5)P_3 which recruits downstream effectors such as Akt and ILK (integrin-linked kinase). Activated Akt phosphorylates GSK3β, inactivating the kinase, which in turn leads to dephosphorylation of APC (adenomatous polyposis coli) which promotes microtubule assembly and neurite elongation [132–135]. Regulated activation of the PI3K/Akt pathway in neurons is crucial for controlled neurite extension as shown by experiments using constitutively active Akt (myr-Akt), or RNAi-mediated knockdown of GSK3β, which both result in neurite hyperelongation [136,137]. In addition, the PI3K/Akt pathway is critical for establishing axon–dendrite polarity, a process which facilitates the unidirectional flow of signals in neurons [135].

Several 5-ptases regulate neurite elongation. The PIPP (proline-rich inositol polyphosphate 5-phosphatase) is a 108 kDa 5-ptase that is highly expressed in brain, as well as heart, kidney, stomach, small intestine and lung [138]. PIPP contains N- and C-terminal PRDs, that flank a central 5-ptase domain and a SKICH domain [81,138]. The SKICH domain, which is also present in the 5-ptase SKIP, mediates the constitutive localization of PIPP to the plasma membrane [81]. PIPP hydrolyses PtdIns(3,4,5)P_3 *in vivo*, but also shows activity against PtdIns(4,5)P_2, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 *in vitro* [138,139].

Studies in the PC12 rat pheochromocytoma cell line have revealed a role for PIPP in regulating neurite elongation [139]. PIPP localizes to the growth cone and shaft of extending neurites as well as the plasma membrane of the cell body in

NGF-differentiated PC12 cells. PIPP regulates levels of PtdIns(3,4,5)P_3, phospho-Akt and phospho-GSK3β at the growth cone, negatively regulating microtubule polymerization and neurite elongation [139]. SHIP2 is also highly expressed in the brain in the germinal layer during embryonic development and in neural stem cells in the adult brain [140]. Following NGF stimulation of PC12 cells, SHIP2 rapidly translocates to lamellipodia and neurite buds [141]. This is in contrast with PIPP which does not localize to neurite buds during the initiation of neuritogenesis [139]. RNAi-mediated knockdown of SHIP2 in PTEN-depleted PC12 cells results in a sustained elevation in PtdIns(3,4,5)P_3 following NGF treatment and increased numbers of neurites per cell, as well as hyperelongation of neurites [141]. These studies suggest that SHIP2 regulates both neurite budding and elongation, whereas PIPP controls neurite elongation, but not initiation [139,141]. The role of SHIP2 in neuronal disease is unknown, but *Ship2*$^{-/-}$ mice do not exhibit an obvious neuronal phenotype. Both PIPP and 72-5ptase/Type IV/Inpp5e are highly expressed in the brain, but as knockout mice have yet to be reported, it remains to be determined whether either of these 5-ptases play an important role in neuronal function *in vivo*.

As well as regulating neurite outgrowth, 5-ptases also play a significant role in regulating synaptic vesicle recycling and thus neuronal function. The 5-ptase synaptojanin 1 is highly expressed in the brain, localizing to presynaptic nerve terminals [142] and may represent the major PtdIns(3,4,5)P_3 5-ptase in the brain [143]. This 5-ptase also hydrolyses the 5-position phosphate from PtdIns(4,5)P_2, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 [144]. Synaptojanin 1 exhibits a high degree of sequence homology with synaptojanin 2, with both 5-ptases containing an N-terminal Sac-1 and central 5-ptase domains, but divergent C-terminal PRDs. The Sac-1 domains of synaptojanin 1 and 2 contain a CX_5R motif which mediates catalytic activity against PtdIns3*P*, PtdIns4*P* and PtdIns(3,5)P_2 and dephosphorylates these phosphoinositides to PtdIns [25,26]. Multiple isoforms of both synaptojanin 1 and 2 have been described resulting from alternative splicing of the C-terminal region, giving rise to 145 and 170 kDa isoforms of synaptojanin 1 and up to six isoforms of synaptojanin 2 [26,145–147]. The 145 kDa isoform of synaptojanin 1 is highly expressed in presynaptic nerve terminals, whereas the 170 kDa isoform is not expressed in neuronal cells [144,145].

Synaptojanin 1 was first identified as a binding partner for Grb2 (growth-factor-receptor-bound protein 2) [148]. Since this original report, numerous other synaptojanin 1-interacting proteins involved in endocytosis and signalling have been described, including dynamin, syndapin, endophilin, amphiphysin I and II, Eps15, clathrin, AP-2, DAP160 (dynamin-associated protein 160)/intersectin, myosin 1E, Snx9 (sortin nexin 9) and PLC (phospholipase C) γ ([149–152] and reviewed in [1]). These interactions in general direct synaptojanin 1 subcellular localization and enhance its 5-ptase activity. Synaptojanin 1's interaction with endophilin is important for its subcellular localization to sites of endocytosis and this interaction stimulates synaptojanin 1 5-ptase activity [153–155]. The subcellular localization of synaptojanin 1 is also regulated by long-chain polyunsaturated fatty acids, which are highly enriched in synaptic membranes and vesicles [156]. 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 [83]. *Caenorhabditis elegans* mutants which lack long-chain polyunsaturated fatty acids exhibit impaired localization of synaptojanin 1 at release sites and defective synaptic vesicle recycling [156]. Hydrolysis of PtdIns(4,5)P_2 by synaptojanin 1 may facilitate uncoating of clathrin-coated vesicles after fission, by decreasing the affinity of adaptor proteins [157,158].

Synaptojanin 1 5-ptase catalytic activity is regulated by phosphorylation. In resting nerve terminals, synaptojanin 1 is constitutively phosphorylated and, following nerve depolarization, is dephosphorylated by calcineurin [142,159]. Synaptojanin 1 is phosphorylated by the serine kinase Cdk5 (cyclin-dependent kinase 5), which inhibits synaptojanin 1 5-ptase activity and also impairs its binding to endophilin 1 and amphiphysin 1 [155]. EphB2 tyrosine kinase phosphorylates synaptojanin 1 following ephrin stimulation, resulting in impaired binding to endophilin and inhibition of 5-ptase activity [160]. Another kinase, MNB (minibrain)/DYRK1A (dual-specificity tyrosine-phosphorylated and -regulated kinase 1A), also phosphorylates synaptojanin 1, resulting in the regulation of its binding to amphiphysin 1 and intersectin 1 [161]. In contrast with Cdk5 and EphB2, phosphorylation of synaptojanin 1 by MNB/DYRK1A leads to a slight increase in 5-ptase activity, which correlates with the degree of synaptojanin 1 phosphorylation [161].

Synaptojanin knockouts have been generated in mice, *Drosophila* and *C. elegans*, and all demonstrate a role for the 5-ptase in regulating clathrin-mediated endocytosis and neuronal function. Synaptojanin 1-knockout mice exhibit neurological defects, increased levels of PtdIns(4,5)P_2 in neurons and an accumulation of clathrin-coated vesicles in nerve terminals [162]. Most synaptojanin 1-knockout mice die shortly after birth, but $\sim$15 % survive up to 15 days, progressively displaying severe weakness, ataxia and convulsions [162]. Knockout of the *unc-26* gene, the *C. elegans* orthologue of synaptojanin 1, results in an increase in the number of clathrin-coated pits, a decrease in synaptic vesicles, cytoskeletal defects and locomotory abnormalities owing to aberrant neuronal function [163]. Disruption of genes encoding the NCA (Na^+/Ca^{2+} antiporter) ion channel, or *unc-80*, which encodes a novel protein required for ion channel subunit localization, can partially suppress the *unc-26* mutant phenotype, suggesting that activation of the NCA ion channel in synaptojanin mutants impairs synaptic vesicle recycling [164]. *Drosophila* synaptojanin 1 mutants exhibit defects in synaptic vesicle uncoating and recycling similar to the abnormalities observed in mice and *C. elegans* [165]. Overexpression of endophilin is able to partially rescue the functional defects observed in the synaptojanin 1 mutant [165]. The synaptic boutons from synaptojanin 1 mutant neurons exhibit depletion of synaptic vesicles and have increased satellite bouton structures with more active zones, possibly to compensate for reduced vesicle recycling [165,166].

Although several 5-ptases play important roles in neuronal differentiation and function, they have not been definitively linked with neurological disease; however, several studies have provided evidence of increased synaptojanin 1 expression in Down's syndrome [167,168]. Ts65Dn mice, commonly used as a model of Down's syndrome, and synaptojanin 1 transgenic mice exhibit increased synaptojanin 1 expression in the brain with a concomitant decrease in PtdIns(4,5)P_2 levels [169]. Both mouse strains also display defects in learning, suggesting that impaired PtdIns(4,5)P_2 regulation may play a role in the cognitive abnormalities observed in Down's syndrome [169–171]. Synaptojanin 1 localizes to chromosome locus 21q22.2 [172], a region linked to bipolar disorder and schizophrenia. Gene mutation screening of patients with bipolar disorder identified 11 mutations in the synaptojanin 1 gene; however, no further characterization has been undertaken [173]. In addition, mutations in synaptojanin 1 exacerbate polyglutamine toxicity in *C. elegans* neurons in a similar manner to huntingtin-interacting protein and endophilin, suggesting a potential role

for the 5-ptase in protecting against Huntington's disease [174].

Another 5-ptase, SKIP, has been implicated in the contiguous-gene syndrome MDS (Miller–Dieker syndrome), a severe form of lissencephaly (smooth brain) [175]. MDS results from heterozygous deletions of eight genes (*PRP8*, *RILP*, *SREC*, *PITPNa*, *SKIP*, *MYO1C*, *CRK* and *14-3-3ζ*) from chromosome locus 17p13.3 and is characterized by lissencephaly owing to defects in neuronal cell migration, mental retardation and craniofacial and limb abnormalities [176]. Although it is not yet known how or whether SKIP contributes to MDS, the 5-ptase may play a role in regulating neuronal migration.

LESSONS LEARNED FROM YEAST

Four 5-ptases have been identified in the yeast *Saccharomyces cerevisiae*, and characterization of these enzymes has given further insight into the cellular functions of 5-ptases. Three of the yeast 5-ptases, Sjl1p/Inp51p, Sjl2p/Inp52p and Sjl3p/Inp53p, have a similar domain structure to that of the synaptojanin proteins, with an N-terminal Sac-1 domain, central 5-phosphatase domain and C-terminal PRD [177,178]. Like synaptojanin 1 and 2, the Sac-1 domains of Sjl52p and Sjl53p can dephosphorylate PtdIns3*P*, PtdIns(4)*P* and PtdIns(3,5)P_2 to PtdIns, whereas the Sac-1 domain of Sjl51p lacks PPIPase (polyphosphoinositide phosphatase) activity probably owing to substitution of critical residues within the CX_5R(T/S) catalytic motif [25,179]. The fourth yeast 5-ptase, Inp54p, comprises a 5-ptase domain and a C-terminal leucine-rich motif [180]. All of the yeast 5-ptases hydrolyse PtdIns(4,5)P_2, with the 5-phosphatase domain of Sjl52p also able to degrade PtdIns(3,5)P_2 [25,179–182].

Sjl52p and Sjl53p localize diffusely in the cytoplasm with Sjl52p also detected at cortical punctate structures that partially co-localize with actin [182–185]. Cell fractionation studies have revealed that Sjl52p may localize to plasma-membrane-derived endocytic vesicles, whereas Sjl53p is likely to be associated with Golgi, endosomal or vesicular membranes [184,186]. Inp54p localizes to the ER [180].

Loss of any single Sac-1-domain-containing 5-ptase has little phenotypic effect; however, loss of any two Sac-1-domain-containing 5-ptases results in marked phenotypic changes, with a thickened cell wall, plasma membrane invaginations, fragmented vacuoles and osmosensitivity [177,181,182,185,187]. Cells lacking both *sjl51* and *sjl52* display severe impairment of both receptor-mediated and fluid-phase endocytosis, whereas the *sjl52*/*sjl53*-null strain exhibits a less severe endocytic defect [178]. Actin polymerization may promote invagination of the plasma membrane and mediate the internalization of endocytic vesicles [183,188]. Both *sjl51*/*sjl52*- and *sjl52*/*sjl53*-null mutants exhibit abnormal actin patch distribution [177,178,187,189]. The endocytic and cytoskeletal defects in the *sjl51*/*sjl52*-null and *sjl52*/*sjl53*-null strains also correlate with defects in cell polarity [178,187].

Additional studies on the *sjl1*/*sjl2*-null mutant have delineated further a role for 5-ptases in endocytosis. Sjl52p is recruited to endocytic sites only after actin and endocytic coat assembly has reached maximal levels and internalization of coat proteins has been initiated [185]. In the *sjl51*/*sjl52*-null mutant, internalization of coat proteins is initiated; however, coat protein disassembly is significantly delayed. In addition, Sjl52p is required for dissociation of the PtdIns(4,5)P_2-binding coat proteins Sla2p, Ent1p and Ent2p from the endocytic vesicle, suggesting that the temporal hydrolysis of PtdIns(4,5)P_2 around the time of vesicle scission may regulate coat protein dissociation [185,190]. Furthermore, vesicle scission does not appear to occur in the *sjl51*/*sjl52*-null strain, consistent with the severe defect in fluid-phase and receptor-mediated endocytosis observed in this strain [185]. Defects in vesicle scission may also explain the large plasma membrane invaginations in the *sjl51*/*sjl52*-null mutant resulting from initiation of multiple unsuccessful rounds of endocytosis [185]. The phenotype of the yeast Sac-1 domain containing 5-ptase double knockouts is consistent with knockout of mammalian synaptojanin 1 in mice, *C. elegans* and *Drosophila*, which also results in cytoskeletal abnormalities and defects in endocytosis.

Null mutation of all three Sac-1-domain-containing 5-phosphatases is lethal; however, triple-mutant cells are able to grow on media supplemented with 1 M sorbitol [177,181,187]. Triple-mutant cells are misshapen with a thickened cell wall, fragmented internal membranes, severely abnormal plasma membrane and grossly abnormal bud site selection [181,187]. The triple *sjl51*/*sjl52*/*sjl53*-null mutant can be rescued by expression of Inpp5b which hydrolyses PtdIns(4,5)P_2 [187] or by *sjl2C446S* which lacks Sac-1 domain PPIPase activity, but not *sjl2D850S* in which the 5-ptase domain is catalytically inactive, indicating that regulation of PtdIns(4,5)P_2 is essential for cell viability [189]. Null mutation of *SJL52*/*SJL53* and the PtdIns3*P* 3-phosphatase *YMR1* is lethal, but can be rescued by expression of Sjl52p lacking 5-ptase activity or PtdIns3*P*-targeted Sac1p, indicating that regulation of PtdIns3*P* is also essential for cell viability.

Cells lacking *inp54* demonstrate normal morphology, but increased secretion of a mammalian reporter protein [180]. Deletion of *inp54* and the PPIPase *sac1* results in accumulation of PtdIns(4,5)P_2 derived from the plasma membrane on a subset of fragmented vacuoles, leading to defects in vacuole fusion and cargo-selective secretion as well as delays in ER–Golgi trafficking and endocytic trafficking to the vacuole [191].

Studies of the genetic and physical interactions between the 5-ptases and other proteins have provided further insight into the cellular roles of these enzymes. A genetic interaction between *SJL53* and the plasma membrane ATPase *PMA1* indicates a role for Sjl53p in regulating endosome–Golgi trafficking [192], whereas genetic and physical interactions between *SJL53* and the clathrin heavy chain gene predict a role for Sjl53p in clathrin-mediated TGN–endosome protein trafficking [179,184,193]. Additional studies have revealed direct interactions between Sjl2p and the actin patch proteins Bsp1p and Abp1p. Bsp1p localization appears to be phosphoinositide-dependent, and the protein is proposed to act as an adaptor linking Sjl52p to the actin cytoskeleton [194]. Mutation of *abp1* in *sjl51* cells results in a similar phenotype to that of *sjl51*/*sjl52*-null cells with plasma membrane invaginations and endocytic defects, suggesting that Abp1p may act to localize Sjl52p during endocytic vesicle formation [183].

Deletion of *sjl51* is lethal in combination with a mutation of *pan1*, an Eps15 homologue which contains two EH (Eps15 homology) domains and is necessary for endocytosis and actin cytoskeleton organization [195]. Sjl51p also physically interacts with two EH-domain-containing proteins, TAX4 and IRS4 [196]. TAX4 and IRS4 activate Sjl51p to down-regulate PtdIns(4,5)P_2 levels and negatively regulate the cell integrity pathway [196]. The complex between a 5-phosphatase and EH-domain-containing proteins is reminiscent of the interaction between the mammalian 5-ptase synaptojanin 1 and Eps15 [197]. The similarities in substrate-specificity, knockout phenotype and binding partners between the yeast Sac-1-domain-containing 5-ptases and mammalian synaptojanin 1 suggest that additional studies in yeast will provide further insights into the cellular functions of these enzymes.

5-PTASES AND CANCER

Activation of the PI3K signalling pathway occurs in many human cancers and may be a consequence of activation of receptor tyrosine kinases, oncogenic mutations in PI3K or loss of function of PTEN [198]. Genomic alterations in *PIK3CA*, including mutations and amplifications, have been identified in breast, cervical, lung, colon, ovarian, gastric and brain cancers [199,200]. Akt is also a target of genomic alterations. *AKT1* is amplified in gastric cancers, the *AKT2* gene is amplified in pancreatic, breast and ovarian tumours, and *AKT3* is overexpressed in hormone-insensitive breast and prostate cancers [199]. Furthermore, mutations in *AKT1* and *PDK1* have been identified in colorectal cancer and, more recently, *AKT1* has also been shown to be mutated in breast and ovarian cancers [199,201]. Loss of function of the tumour-suppressor PTEN is associated with inherited cancer predisposition syndromes such as Cowden's disease, Bannayan–Zonana syndrome and Lhermitte–Duclos disease as well as in sporadic cancers including gliomas, melanomas, prostate, endometrial, lung, renal, ovarian and breast cancers [199,202]. However, to date, there is only limited evidence that the 5-ptases may function as tumour suppressors, and, in some cancers, gene array data suggest that specific 5-ptases exhibit increased expression. Many of the 5-ptases hydrolyse PtdIns(3,4,5)P_3 to form PtdIns(3,4)P_2, a signalling molecule that also binds and may facilitate Akt activation [203–205]. Therefore the 5-ptases may function as both signal terminators and generators.

Gene-expression profiling of human cervical cancers has revealed 72-5ptase/Type IV/Inpp5e as one of the top five of 74 genes with altered expression, being up-regulated over 50-fold in cervical cancer [206]. 72-5ptase/Type IV/Inpp5e is also one of the top six genes up-regulated in non-Hodgkin's lymphoma patients following treatment [207]. In addition, 72-5ptase/Type IV/Inpp5e RNA levels are increased more than 5-fold in uterine leiomyosarcoma samples compared with normal myometrium [208]. Both the 72-5ptase/Type IV/Inpp5e and SKIP are overexpressed in gemcitabine-resistant pancreatic cell lines [209]. In contrast, 72-5ptase/Type IV/Inpp5e is one of 57 genes down-regulated in stomach cancer [210] and also demonstrates reduced expression in metastatic adenocarcinomas compared with primary tumours [211]. There has been no functional characterization of the role *INPP5E* plays in cancer; however, studies by Majerus and colleagues have shown that cells overexpressing 72-5ptase/Type IV/Inpp5e exhibit cell cycle arrest and decreased cell growth owing to increased apoptosis [212].

Several large-scale gene-expression-profiling studies have revealed a correlation between PIPP (encoded by *PIB5PA*) gene expression and tumour oestrogen receptor status [213,214]. PIPP mRNA is expressed at higher levels in ER-positive tumours relative to oestrogen receptor-negative tumours; the latter are in general more invasive and less responsive to treatment, and are associated with a worse prognosis [213,214]. Additionally, in a screen of $\sim$5000 genes, PIPP was one of 231 genes significantly correlating with breast cancer disease outcome [213]. Higher expression of PIPP is associated with a better prognosis, defined as no distant metastases developing within 5 years of diagnosis [213]. Although these studies suggest a role for PIPP in breast cancer, no functional studies have been reported to support this contention.

SHIP2 expression was reported to be increased in a panel of breast cancer cell lines compared with non-transformed breast cell lines [215]. Interestingly, experiments undertaken to decrease SHIP2 expression in the MDA-231 breast cancer cell line resulted in decreased cell proliferation, as well as tumour formation and metastasis in nude mice, suggesting that the 5-ptase may play a positive role in promoting breast cancer [215]. Given that obesity is a risk factor for some cancers, such as breast, endometrial, kidney, gastrointestinal tract, pancreas and prostate [216], and that *Ship2*$^{-/-}$ mice are resistant to obesity [65], this 5-ptase may be a potential drug target for both diabetes and cancer [215]. However, whereas expression of SHIP2 affects the proliferation and tumorigenic potential of a breast cancer cell line, it does not affect cell growth or apoptosis of a myeloma cell line [217], suggesting that its effects may be cell-type- or tissue-specific. In contrast, decreased expression of SHIP2 has been demonstrated in hepatocellular carcinomas compared with normal tissue [218]. Finally, in one study, the 5-ptase SKIP was shown to be one of 17 known genes located on chromosome locus 17p13, which map to a region that frequently displays loss of heterozygosity in hepatocellular carcinomas [219].

The related SHIP2 homologue, SHIP1, is predominantly expressed in haemopoietic cells [220]. SHIP1 is a 145 kDa protein, and several splice variants have also been identified, as well as a 104 kDa stem-cell-specific isoform which is only expressed during embryonic development [221,222]. SHIP1 contains an N-terminal SH2 domain, a central 5-ptase domain which hydrolyses PtdIns(3,4,5)P_3 and Ins(1,3,4,5)P_4 and a C-terminal PRD containing two NPXY motifs [223]. The N-terminal SH2 domain binds tyrosine-phosphorylated proteins such as Shc [223], the Gabs (Grb2-associated binders) [224,225], the Doks (downstream of tyrosine kinases) [226–228], SHP-2 (SH2-domain-containing protein tyrosine phosphatase 2) [229,230], c-Cbl [231] and LAT (linker for activation of T-cells) [232]. SHIP1 is recruited to the cytoplasmic tail of ITIM (immunoreceptor tyrosine-based inhibitory motif)-containing receptors FcγRBIIb and Cd22 following their co-ligation with the BCR via its interaction with Shc (reviewed in [1]). In addition, SHIP1 can interact with the ITAM (immunoreceptor tyrosine-based activator motif)-containing receptors FcγRIIa, FcεRI and 2B4 in macrophages, mast cells and natural killer cells respectively (reviewed in [1]). The PRD of SHIP1 also interacts with Grb2 [233], whereas phosphorylation of the NPXY sequences allows binding of SHIP1 to the phosphotyrosine-binding domains of Shc, Dok1 and Dok2 [234,235]. SHIP1 acts to negatively regulate cell proliferation [236], but overexpression of the 5-ptase appears to promote apoptosis [237]. SHIP1 plays critical roles in regulating the immune system (discussed below), bone maintenance, haemopoietic stem cell function and proliferation.

Ship1$^{-/-}$ mice develop a myeloproliferative disease which bears some resemblance to CML (chronic myelogenous leukaemia) [238]. These mice exhibit splenomegaly, massive macrophage infiltration of the lungs and failure to thrive [238]. Interestingly, the BCR/ABL oncogene, which causes CML, inhibits SHIP expression, suggesting that the 5-ptase may act as a tumour suppressor in CML [239]. Expression of SHIP1 is reduced in a human Jurkat T-cell line, derived from an individual with acute lymphoblastic leukaemia, as well as in adult T-cell leukaemia/lymphoma [240,241]. 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 [240]. In addition, SHIP1 can up-regulate expression of KLF2 (Krüppel-like factor 2), a negative regulator of T-cell proliferation, suggesting that SHIP may mediate inhibition of cell growth via KLF2 [242]. Mutations in the SHIP1 gene (*INPP5D*) have been identified in acute myeloid leukaemia as well as in acute lymphoblastic leukaemia, although the latter study only involved one patient [243–245]. These mutations occur within the 5-ptase domain, and result in loss of 5-ptase enzyme activity, as well as the PRD [243,245]. Exogenous expression of a SHIP1 V684E point mutant in K562 cells led to enhanced cell proliferation, even under low serum conditions and conferred resistance to etoposide, suggesting a potential role for

the SHIP1 5-ptase in the development of leukaemia and resistance to chemotherapy [243].

5-PTASE REGULATION OF HOST–PATHOGEN INTERACTIONS

Regulation of phagocytosis and phagosome maturation

Cells of the myeloid lineage, including macrophages, neutrophils and mast and dendritic cells, provide an important link between the innate and adaptive immune responses to invading pathogens. One of the first events upon recruitment of these cells to the site of infection is phagocytosis. Phagocytosis is the mechanism whereby immune cells engulf large particles ($< 1.0\ \mu$m), including pathogens and virally infected host cells, as well as tumour and apoptotic cells. Phagocytosis can be stimulated via many different receptors, including the IgG receptor, FcγR, and the complement component 3 receptor, CR3 [246]. In addition, other receptors such as the β-glucan receptor, dectin-1, and the phosphatidylserine receptors Tim4 and BAI1 are also important in the phagocytosis of invading pathogens and apoptotic cells [247–249]. PtdIns(4,5)P_2 generation occurs at FcγR-containing phagocytic cups in macrophages, where it regulates actin re-organization [250]. Both PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 accumulate at the phagocytic cup in response to FcγR and CR3 activation [16,251], although a functional role for PtdIns(3,4)P_2 in phagocytosis directed via the FcγR has not been demonstrated [16]. The importance of PtdIns(3,4,5)P_3 in phagocytosis is highlighted by studies using PI3K inhibitors and analysis of p85$^{-/-}$ mice, revealing that PtdIns(3,4,5)P_3 is required for completion of phagocytosis [251–254]. However, the molecular mechanisms by which PtdIns(3,4,5)P_3 promotes phagocytosis are complex [16,255,256]. Studies carried out in our laboratory [16] and in others [255] have shown that depletion of PtdIns(3,4,5)P_3 from the phagocytic cup does not inhibit focal exocytosis to this site. However, other studies have demonstrated a partial inhibition of focal exocytosis following inhibition of PI3K [256]. PtdIns(3,4,5)P_3 recruits myosin X to the phagocytic cup via its PH domain and this may facilitate scission of the phagosome from the plasma membrane [257]. In addition, PtdIns(3,4,5)P_3 increases the affinity of CR3 for *Mycobacterium bovis* BCG (Bacille Calmette–Guérin), enhancing phagocytosis of this bacterium [258]. Five lipid phosphatases have been implicated in the regulation of PtdIns(3,4,5)P_3 signalling during phagocytosis, the 5-ptases SHIP1, SHIP2, 72-5ptase/Type IV/Inpp5e, the *Dictyostelium* OCRL homologue Dd5P4 [16,17,259,260] and the 3-ptase PTEN, although the exact role of this latter phosphatase is contentious [261,262].

SHIP1, SHIP2 and 72-5ptase/Type IV/Inpp5e are recruited to FcγR-containing phagocytic cups [16,17,259], although only 72-5ptase/Type IV/Inpp5e has been shown to degrade PtdIns(3,4,5)P_3 directly during FcγR-mediated phagocytosis [16]. SHIP1 primarily regulates PtdIns(3,4,5)P_3 dynamics during phagocytosis directed via the CR3, although FcγR-mediated phagocytosis is also affected, but to a lesser extent [16]. The 72-5ptase/Type IV/Inpp5e contains a putative ITAM [85,86], the tyrosine residues of which are potential phosphorylation sites for Itk, a member of the Tec family of tyrosine kinases (http://scansite.mit.edu). Thus it is possible that the recruitment of the 72-5ptase/Type IV/Inpp5e to FcγR-containing phagocytic cups is regulated by phosphorylation, although this has not been shown. In addition, SHIP1 exhibits a restricted distribution only to the tips of the pseudopods of the phagocytic cup [263], which may limit its access to PtdIns(3,4,5)P_3. SHIP1 also regulates phagosome maturation and reactive oxygen species formation by generation of PtdIns3*P*, presumably via its participation in the sequential degradation of PtdIns(3,4,5)P_3 to PtdIns3*P* [264]. The 72-5ptase/Type IV/Inpp5e and SHIP2 both regulate Akt phosphorylation via dephosphorylation of PtdIns(3,4,5)P_3 during phagocytosis [16,265], perhaps regulating the activity of ROCK-1 and/or p70^{S6K} [266,267]. The contribution of SHIP2 to PtdIns(3,4,5)P_3 degradation during phagocytosis has not been examined, although an intact 5-ptase domain is required for SHIP2 inhibition of FcγR-mediated phagocytosis [17]. SHIP2 negatively regulates Rac activity during phagocytosis, presumably via regulation of PtdIns(3,4,5)P_3 [17]. PtdIns(4,5)P_2, but not PtdIns(3,4,5)P_3 is a significant regulator of actin dynamics during phagocytosis [250,252,253]. However, SHIP2 may indirectly affect PtdIns(4,5)P_2 and therefore actin dynamics during phagocytosis via Rac-mediated activation of the PIPK (PtdIns4*P* 5-kinase) family [268,269].

The studies discussed above in mammalian macrophages indicate a negative regulatory role for 5-ptases in phagocytosis. However, a recent study in *Dictyostelium* has proposed that the *Dictyostelium* homologue of OCRL, Dd5P4, positively regulates phagocytosis via the hydrolysis of PtdIns(3,4,5)P_3 at the phagocytic cup [260]. Although purified component enzyme assays indicate that OCRL may hydrolyse PtdIns(3,4,5)P_3 *in vitro* [83], there is limited evidence that mammalian OCRL hydrolyses PtdIns(3,4,5)P_3 *in vivo*. PtdIns(4,5)P_2 is important in regulating phagocytosis via regulation of actin dynamics at the phagocytic cup [250], therefore Rac1-dependent recruitment of OCRL may play a role in phagocytosis, although further investigation is required.

Inflammation and allergy

Hypersensitivity reactions, or allergies, are the development of an inappropriate immune response that leads to tissue damage and cause diseases, such as asthma. Many of these reactions are linked directly or indirectly to mast cell activation. Much of the body's IgE is bound to the high-affinity FcεR on mast cells. Activation of this receptor occurs upon cross-linking IgE with multivalent antigen, leading to degranulation, the rapid secretion of cytoplasmic granules which contain many inflammatory mediators, including histamine, which causes an increase in vascular permeability. Mast cell activation also leads to the production of inflammatory cytokines such as IL (interleukin)-13 and IL-4, which recruit other leucocytes and skew the immune response in a Th2/humoral direction (reviewed in [270]). Both PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 are implicated in the regulation of mast cell and basophil function, including the release of granules. PtdIns(4,5)P_2 hydrolysis by PLC generates diacylglycerol and Ins(1,4,5)P_3, leading to Ca^{2+} mobilization, which contributes to degranulation. PtdIns(3,4,5)P_3 also facilitates Ca^{2+} mobilization via activation of PLC and regulation of the Tec family of tyrosine kinases [271–273]. Recent studies have implicated both SHIP1 and SHIP2 in the regulation of mast cell activation and the development of allergic reactions.

Degranulation of mast cells follows a bell-shaped curve, with minimal response in the absence of antigen (suboptimal), followed by an increase to maximal response in the presence of antigen (optimal). Inhibition of degranulation is observed at excessive levels of antigen (supraoptimal), thus preventing inappropriate degranulation and thereby allergic responses. FcγRIIB is an inhibitory receptor that associates with and attenuates FcεR signalling [274]. SHIP1 forms complexes with both FcγRIIB and FcεR to negatively regulate mast cell activation [275,276]. Maximal degranulation in *SHIP1*$^{-/-}$ mast cells occurs in response to IgE alone, with no further increase in degranulation in response to FcεR cross-linking [275]. In addition, SHIP1 expression

is necessary for the inhibition of degranulation observed at supraoptimal antigen levels [277]. Enhanced degranulation observed in *SHIP1*$^{-/-}$ mast cells has been attributed to increased calcium influx [275]. SHIP1 hydrolysis of PtdIns(3,4,5)P_3 could mediate calcium influx via regulating Tec family kinases and PLC activity and hence DAG (diacylglycerol)/Ins(1,4,5)P_3 [273]. However, the exact mechanism by which SHIP1 regulates degranulation in mast cells is unclear. Recent studies have questioned the role of SHIP1 in regulating the activity of the Tec family kinase, Btk, whereas Akt activation is strongly inhibited by SHIP1 under the same conditions [277].

The role SHIP1 plays in regulating allergic reactions has been confirmed by studies in mice and humans. *Ship1*$^{-/-}$ mice develop massive infiltration of the lungs by myeloid cells, including macrophages and eosinophils [238,278]. The myeloid infiltration of the lungs of *Ship1*$^{-/-}$ mice is also characterized by increased mast cell number, histamine levels and the levels of Th2 cytokines IL-4 and IL-13, relative to that observed in wild-type mice [278], consistent with allergic inflammation. Low SHIP1 expression and phosphorylation and enhanced Akt activity correlates with increased histamine release in human subjects [279–281]. In addition, basophils from patients with CIU (chronic idiopathic urticaria), which are non-respondent to IgE cross-linking, exhibit increased SHIP1 and SHIP2 protein levels, whereas basophils from IgE-respondent CIU sufferers demonstrate decreased levels of these 5-ptases [282]. Interestingly, although basophils from IgE-respondent individuals (low SHIP1/2) exhibit Akt activation in the resting state, both IgE-respondent and non-respondent basophils have decreased Akt phosphorylation, compared with basophils from unaffected individuals [282].

The role of SHIP2 in degranulation and allergy has not been studied extensively, although recent work has revealed that SHIP2 regulates mast cell degranulation, via a mechanism distinct from SHIP1 [283]. Unlike SHIP1, SHIP2 does not affect the mobilization of calcium in response to IgE cross-linking, but regulates microtubule reorganization via Rac activation [283]. In addition, SHIP2 regulates GATA-1 phosphorylation via Akt activation in response to IgE cross-linking [283]. The control of IL-4 and IL-13 transcription by GATA-1 [284,285] provides a possible link between 5-ptases and the further development of allergic reactions.

TLRs (Toll-like receptors) recognize common features of invading pathogens, e.g. TLR4, a plasma-membrane-associated receptor, recognizes endotoxins such as LPS (lipopolysaccharide). LPS stimulation results in up-regulation of pro-inflammatory cytokines and other inflammatory mediators. However, chronic stimulation with LPS may lead to endotoxin tolerance, which prevents tissue damage (reviewed in [286]). The role SHIP1 plays in inflammatory signalling pathways is complex, with studies indicating that SHIP1 negatively regulates LPS induced TNFα (tumour necrosis factor α) secretion and FcγR-mediated IL-1β secretion via phosphatase-independent mechanisms [287,288]. In addition, SHIP1 negatively regulates pro-inflammatory IL-12 secretion in response to *Francisella novicida*, via regulating Akt activation [289,290]. These reports are supported by results which have demonstrated that *in vitro* differentiated *SHIP1*$^{-/-}$ macrophages and mast cells are hyperresponsive to LPS, as shown by increased pro-inflammatory cytokine and nitric oxide (NO) release. However, these cells also do not exhibit endotoxin tolerance [291]. In contrast, *SHIP1*$^{-/-}$ macrophages differentiated *in situ* produce significantly less NO in response to LPS, compared with wild-type macrophages [292]. In addition, *in situ* differentiated *SHIP1*$^{-/-}$ macrophages secrete lower levels of IL-6, IL-12 and TNFα, but higher levels of TGFβ (transforming growth factor β) and IL-10, compared with wild-type macrophages [292]. The low level of NO and the cytokine profile of the *in situ* differentiated *SHIP1*$^{-/-}$ macrophages is characteristic of alternatively activated macrophages, which contribute to healing and resolution of inflammation (reviewed in [293]). The reasons for these discrepancies are unclear, but may be due to the levels of TGFβ available during differentiation [292]. However, *Ship1*$^{-/-}$ mice have increased susceptibility to *Salmonella enterica* serovar serotype Typhimurium infection, perhaps as a consequence of this alternatively activated phenotype [294].

Alternatively activated macrophages are closely related to TAMs (tumour-activated macrophages), which promote angiogenesis, secrete low levels of NO, the inflammatory cytokines IL-12 and TNFα, and matrix metalloproteinases (reviewed in [293]). Thus SHIP1 may also play a role in the regulation of tumour immunosurveillance, via skewing the macrophage population to the alternatively activated macrophage lineage [292].

CONCLUDING REMARKS

Many studies have demonstrated the importance of the 5-ptases in a range of human diseases, including diabetes, cancer, Lowe's syndrome and allergy; however, there are still many questions to be answered concerning the molecular mechanisms by which the 5-ptases contribute to human health and disease. Elucidating these mechanisms has been difficult, particularly with respect to the PI3K signalling pathway, as the 5-ptases appear to act as both signal terminators and generators, in contrast with PTEN, which specifically terminates PI3K signalling. In addition, many of the 5-ptases are capable of hydrolysing multiple substrates *in vitro*, making it a challenge to delineate which of these are important *in vivo*. Emerging evidence suggests that the interactions between 5-ptases and their binding partners may also play a key role in regulating 5-ptase enzyme activity and/or localization or recruitment to specific subcellular microdomains. There is also evidence that 5-ptases may, in some cases, serve as adaptors for the assembly of signalling complexes to localize other proteins to sites of PI3K signalling.

Studies on 5-phosphatase-knockout mice have demonstrated that these enzymes play critical roles in regulating cellular function. In particular, homozygous deletion of SKIP is embryonically lethal, while heterozygous SKIP mice exhibit severe insulin hypersensitivity. Both synaptojanin 1 and *Ship1*$^{-/-}$ mice have a significantly shortened lifespan owing to neurological abnormalities and a leukaemia-like syndrome respectively. In addition, *Ship2*$^{-/-}$ mice display resistance to weight gain when fed on a high-fat diet. Knockout of specific 5-ptases such as PIPP and 72-5ptase/Type IV/Inpp5e in mice as well as tissue-specific knockout mice for *Skip* and *Ship2* will aid us in elucidating the specific roles of these 5-ptases in cellular function and disease. Further studies are required to determine whether the expression of specific 5-ptases can be used as diagnostic/prognostic markers of disease such as cancer. Also, whether the 5-ptases will prove to be drug targets for treatment of diseases such as diabetes or obesity will be evaluated in the next few years.

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