INTRODUCTION

The phosphatidylinositol phosphates are lipid derivatives of the phosphoinositides (PIs) which differ with regard to the presence or absence of phosphate groups on the available 3-, 4- and 5-hydroxy positions of the inositol head group (Figure 1). Phosphatidylinositol phosphates are classically associated with the production of the second messengers diacylglycerol and inositol trisphosphate [Ins(1,4,5)$P_3$], which are involved in the activation of protein kinase C and the release of intracellular stored calcium [1]. However, the phosphatidylinositol phosphates are now widely recognized as important regulators of a number of signal transduction processes which control a diverse range of cellular functions [2,3]. Particular phosphatidylinositol polyphosphates are essential activators of proteins such as phosphoinositide-dependent kinase 1 (PDK1) (reviewed in [4]) and phospholipase D (PLD) (reviewed in [5]). Recently, the phosphatidylinositol phosphates have been directly implicated in the regulation of membrane trafficking [6–8] and of cytoskeletal function [9,10], and can themselves be considered as second messengers [11,12]. A number of protein domains have been characterized, including PH (pleckstrin homology) domains [13,14] and FYVE domains (named after the first four proteins found with this domain) [15,16], that bind to specific phosphatidylinositol phosphates and hence confer sensitivity to these processes. In view of the pleiotropic functions of phosphatidylinositol phosphates, it is self evident that the kinases and phosphatases responsible for the metabolism of these lipids have an important role in cell regulation.

Recently a novel class of phosphatidylinositol phosphate phosphatase was identified in yeast. This, Sac-type, phosphatase appears to be found in mammalian, lower eukaryotic and plant cells; however, to date, the only detailed characterization of this phosphatase class has been carried out for proteins from the yeast Saccharomyces cerevisiae. In S. cerevisiae there are five proteins containing Sac phosphatase domains, and all of these proteins have been characterized, at least partially. Since the roles of the relevant phosphatidylinositol phosphates appear to be conserved from yeast to mammalian cells, this model organism provides an excellent basis from which to review the roles and the implications for cellular function of these novel Sac phosphatases. In this review we will consider first the other enzymes involved in the metabolism of phosphatidylinositol phosphates with particular reference to yeast.

PHOSPHATIDYLINOSITOL PHOSPHATE METABOLISM IN YEAST

Phosphatidylinositol phosphates are synthesized by kinases and phosphatases which respectively phosphorylate and dephosphorylate phosphatidylinositol (PtdIns). The phosphatidylinositol kinases have been relatively well characterized and fall into a number of classes, defined by their phosphorylation of specific inositol hydroxy groups. The PI 3-kinases phosphorylate the 3-position of PtdIns [2], whereas phosphatidylinositol 4-phosphate 5-kinases (PtdIns4P 5-kinases; Type I PIPkins) add phosphate at the 5-position to PtdIns that is already phosphorylated at the 4-position, i.e. they convert PtdIns4P into PtdIns(4,5)$P_2$ [17]. These and a number of other enzymes involved in the phosphorylation of phosphatidylinositol phosphates all contain a well conserved lipid kinase domain (see [18] and references therein), and their roles in the production of phosphatidylinositol phosphates are relatively well understood. In contrast with the PI kinases, the PI phosphatases are less well characterized, and fall into a number of phosphatase classes with little similarity to each other. How the phosphatidylinositol phosphate phosphatases contribute to the interconversion of the phosphatidylinositol phosphates is only starting to become clear.

In the yeast S. cerevisiae the synthesis of the phosphatidylinositol phosphates starts with the production of PtdIns, which is catalysed by the enzyme phosphatidylinositol synthase (Pis1p) (see [19]). This enzyme, CDP-diacylglycerol–inositol 3-phosphatidylinositoltransferase, transfers a phosphatidyl group on to
Figure 1 Representation of phosphatidylinositol

Physiologically in *S. cerevisiae* the fatty acids found on the sn-1 and sn-2 positions of glycerol are predominantly oleic acid (C18:1) and palmitic acid (C16:0), or palmitic acid and palmitoleic acid (C16:1) [148]. The inositol ring with five hydroxylated carbon atoms is attached to the sn-3 carbon of the glycerol via a phosphate group at position 1. This represents the only mechanism of PtdIns synthesis, and is a key regulatory step. PtdIns is utilized for glycosylphosphatidylinositol modification of proteins, sphingolipid synthesis or phosphorylation to form the four phosphatidylinositol phosphates found in yeast, i.e. PtdIns3P, PtdIns4P, PtdIns(3,5)P2, and PtdIns(4,5)P2. The kinases involved in the production of these lipids have been identified and characterized (Figure 2).

**PtdIns4P**

PtdIns4P is unique in *S. cerevisiae* because it can be synthesized by different kinases, Pik1p and Stt4p [20,21]. Both of these kinases are essential for growth [20,21], demonstrating that they discharge separate functions. Pik1p is involved in the mating-pheromone signalling cascade [21], secretion from the Golgi [22,23] and cytoskeletal function [23]. Furthermore, a proportion of Pik1p is localized in the nucleus [23,24], and a temperature-sensitive mutant of *PIK1*, *pik1-12*<sup>ts</sup>, displays a cytokinesis defect at the restrictive temperature [24]. These data indicate an additional nuclear role for Pik1p.

*STT4* was originally isolated as a staurosporine-sensitive mutation, and interacts genetically with the yeast *PKC1* pathway [20,25]. *STT4* mutants are also defective in phosphatidylinerine metabolism [26]. Unlike Pik1p, Stt4p is sensitive to the PI 3-kinase inhibitor wortmannin [27], and overexpression of Stt4p confers wortmannin resistance. Although it is likely that PtdIns4P, which represents approximately 2% of cellular PtdIns [28], will have a function in its own right, no proteins have been described to date that specifically interact with this lipid.

**PtdIns(4,5)P2**

PtdIns(4,5)P2 is synthesized from PtdIns4P by the essential type I PIPkin Mss4p [29,30]; this lipid represents approximately 0.8% of cellular PtdIns [28]. Temperature-sensitive mutations in *MSS4* cause growth arrest at the restrictive temperature, with an associated loss of organization of the actin cytoskeleton [29,30]. In mammalian cells the first function to be described for PtdIns(4,5)P2 was as a substrate for phospholipase C to produce the second messengers diacylglycerol and Ins(1,4,5)P3. However, although there is a phospholipase C in *S. cerevisiae*, it is unclear if either of these catabolites acts as a second messenger in yeast. More recently, PtdIns(4,5)P2 has been shown to bind to a number of proteins which bind actin and/or regulate the actin cytoskeleton, including cofilin and profilin (see below; [9,10]). PtdIns(4,5)P2 can also act as a cofactor for PLD [5,31] and is necessary for homotypic vacuolar fusion [32]. Thus PtdIns(4,5)P2

Figure 2 Phosphatidylinositol phosphates and phosphatidylinositol phosphate kinases in *S. cerevisiae*

The phosphatidylinositol phosphates in *S. cerevisiae* derive from PtdIns synthesized by the enzyme CDP-diacylglycerol–inositol 3-phosphatidyltransferase (Pis1p). This lipid can be phosphorylated by the PtdIns 4-kinases (Pik1p or Stt4p) and the PtdIns 3-kinase (Vps34p) to form PtdIns4P and PtdIns3P respectively. The products of these enzymes can, in turn, be phosphorylated by the PtdIns4P 5-kinase (Mss4p) or by the PtdIns3P 5-kinase (Fab1p), to form PtdIns(4,5)P2, PtdIns(3,5)P2, respectively. Phospholipase C (Plc1p) can hydrolyse PtdIns(4,5)P2, forming Ins(1,4,5)P3 and diacylglycerol (DAG). There is no evidence that PtdIns(3,5)P2 can be degraded by Plc1p. Phosphatases can dephosphorylate the phosphatidylinositol phosphate species, although the specific phosphatases involved remain unclear.

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Sac phosphatase domain proteins

Figure 3 Sac domain-containing proteins

The Sac domain is found in two classes of protein, one of which includes the Sac domain-containing phosphatidylinositol phosphate 5-phosphatases (SCIPs), such as human synaptojanins (Synapto) I and II and the related yeast proteins Inp51p, Inp52p and Inp53p. These proteins also have type II and phosphatase catalytic domains, and a proline-rich region at the C-terminus of the protein. The other class of protein with a Sac domain is represented by yeast Sac1p and Fig4p, and a variety of other sequences from numerous species, including human KIAA0274, KIAA0851 and KIAA0966; these do not contain the 5-phosphatase domains. Other regions of homology (identity) between these proteins are indicated.

is a functional PI in its own right, and not just a metabolic precursor for second messengers.

PtdIns3P

PtdIns3P is synthesized from PtdIns by Vps34p, and represents approximately 1.8% of PtdIns in yeast [28]. The VPS34 gene was originally identified from a genetic screen for vacuolar protein sorting mutants [33], and was subsequently found to have PtdIns 3-kinase activity [34]. Loss of VPS34 function causes a mis-sorting of the vacuolar hydrolase carboxypeptidase Y to the plasma membrane, implicating a role for PtdIns3P in vesicle trafficking [35]. PtdIns3P has been found to bind specifically to proteins containing an FYVE domain [16,36], a Zn-finger domain found in a wide variety of eukaryotic proteins, many of which are involved in vesicle trafficking/protein sorting. It has been postulated that the FYVE domain aids localization of proteins to appropriate membranes.

PtdIns(3,5)P2

PtdIns(3,5)P2 is the least abundant phosphatidylinositol phosphate in S. cerevisiae under normal growth conditions, representing approximately 0.02% of cellular PtdIns [28]. It is synthesized from PtdIns3P by the type III PIPkin Fab1p, which is a FYVE domain-containing protein [37,38]. Disruptions in FAB1 cause S. cerevisiae cells to acquire large, poorly acidified vacuoles that fail to segregate properly, implicating PtdIns(3,5)P2 in some aspect of vacuolar homoeostasis [39]. PtdIns(3,5)P2 levels rise rapidly and transiently in response to hyperosmotic stress, which would imply that PtdIns(3,5)P2 has a role in stress responses [40]. Work done with the Fab1p regulatory protein Vac7p implies that Fab1p, and hence PtdIns(3,5)P2, controls retrograde transport from the vacuole [41]. It has also been demonstrated that the lipid is involved in regulation of protein sorting at the endosomal multivesicular body [42].

Phosphatidylinositol phosphate phosphatases

Compared with the kinases discussed above, the phosphatidylinositol phosphate phosphatases involved in the interconversion of the phosphatidylinositol phosphates are much less well characterized, and this is also true for the enzymes involved in phosphatidylinositol phosphate turnover in S. cerevisiae. The phosphoinositide phosphatases identified from a number of cellular sources fall into four major categories, hydrolysing the 1-, 3-, 4- or 5-phosphorylated inositol phosphates or phosphatidylinositol phosphates [43]. Since the 1-phosphate of the inositol headgroup is a diester linkage to the diacylglycerol moiety (Figure 1), only the 3-, 4- and 5-phosphatases are relevant to the interconversion of phosphatidylinositol phosphates.

A recent addition to the 3-phosphatase group of phosphatases is the product of the tumour suppressor gene PTEN, which was originally identified from a screen directed at a human genetic locus deleted in a variety of human cancers [44–46]. PTEN (phosphatase and tensin homologue), also known as MMAC (mutated in multiple advanced cancers), has a C2-like domain and an obvious tyrosine protein phosphatase motif [47]. Although some tyrosine phosphatase activity is detectable, PTEN has a higher turnover number for dephosphorylation of the 3-phosphate group of phosphatidylinositol phosphates and soluble inositol polyphosphates [48]. In yeast there are two putative PTEN homologues, Tep1p and Cdc14p. Tep1p shows 27% identity with mammalian PTEN, and although the gene has been

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shown to be induced 2–5 h after initiation of sporulation [49], disruption of the gene caused no discernible phenotypes [50]. The function or protein/lipid specificities of the protein are yet to be elucidated [50]. The other PTEN homologue, Cdc14p, is less related to PTEN and was originally isolated after analysis of mutants identified in a screen for cell division cycle mutants [51]. Cdc14p, a protein phosphatase, has a well characterized role in the cell cycle during the exit from mitosis [52] by a mechanism that involves the dephosphorylation of cell cycle proteins [53]. Therefore it is perhaps less likely that Cdc14p is involved in the regulation of the 3-phosphate position of phosphatidylinositol phosphates; however, this remains to be formally proven.

Of the few 4-phosphates identified from mammalian sources, there are no significant homologues in the S. cerevisiae genome to either rat or human type I or type II polyphosphate 4-phosphatases [54]. This indicates either that metabolism of 4-phosphorylated phosphatidylinositol phosphates is via phospholipase C (Figure 2) or that there exists a distinct class of monoesterases that will act on these lipids (see below).

The phosphatidylinositol phosphate 5-phosphatases consist of a number of well characterized protein families [43, 55–57]. The type I 5-phosphatases do not hydrolyse phosphatidylinositol phosphates, while the type II 5-phosphatases do. The latter share with the type I phosphatases a conserved catalytic domain, but additionally have an extended region N-terminal to the catalytic domain, termed the type II domain. The type II phosphatases are characterized by further regulatory domains each particular to subsets of enzymes: an N-terminal SH2 (Src homology 2) domain (SH2 domain-containing inositol 5-phosphatases; SHIPs) or a C-terminal GAP (GTPase-activating protein) domain (GAP domain-containing inositol 5-phosphatases; GIPs) [43]. The final subset of type II 5-phosphatases are characterized by the presence of an N-terminal Sac domain. These SCIPs (Sac domain-containing inositol 5-phosphatases) have a region of identity with the yeast protein Sac1p, in addition to the type II and 5-phosphatase domains associated with these proteins (Figure 3) [43]. There are six proteins coded for in the S. cerevisiae genome that show identity with the 5-phosphatase class of proteins. These include three proteins of the synaptojanin SCIP class of 5-phosphatases, namely Inp51p, Inp52p and Inp53p, which are discussed below (Figure 3). Inp54p also closely resembles the type II 5-phosphatases, as the protein consists of a partially truncated type II domain and the 5-phosphatase domain; however, this protein has no Sac, GAP or SH2 domains, lacks a proline-rich region and remains to be fully characterized. The two other proteins, Sac1p and Fig4p, only show identity with the 5-phosphatases in the Sac domain of these proteins.

**THE SAC-DOMAIN PHOSPHATASES**

It is only recently that the phosphatase activity associated with Sac domain was identified. The first indication for such an activity came during work characterizing inhibitors of mammalian PtdIns(4,5)P₂-dependent PLD. Synaptojanin was identified as one of several proteins able to inhibit the activity of PPLD₁, and this was attributed to the ability of synaptojanin to hydrolyse PtdIns(4,5)P₂ required for PPLD₁ activity [58]. Detailed analysis of the phosphatidylinositol phosphates hydrolysed by synaptojanin revealed that virtually no PtdIns₄P was produced, while PtdIns was readily detected [58]. Chung et al. concluded that the 5-phosphatase synaptojanin must also exhibit the ability to dephosphorylate 4-phosphate groups [58]. More recently, characterization of the yeast 5-phosphatases Inp52p and Inp53p and mammalian synaptojanin by Guo et al. [59] revealed a second phosphatase activity associated with these proteins. This was attributed to the N-terminal Sac domain (Figure 3), which was demonstrated to exhibit a broader-specificity phosphatase activity capable of hydrolysing phosphate from PtdIns3P, PtdIns4P and PtdIns(3,4)P₂ [59]. The Sac domain from Sac1p

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<th>Table 1 Sac domain identity/similarity matrix</th>
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<td>An identity and similarity matrix is given for the Sac domains of Sac1p (Sac1) (amino acids 114–503), KIAA0851 (0851) (amino acids 121–500), Fig4p (Fig4) (amino acids 157–577), KIAA0274 (0274), human synaptojanin 1 (Syn1) (amino acids 188–481), human synaptojanin 2 (Syn2) (amino acids 66–433), Inp52p (Inp52) (amino acids 166–556), Inp53p (Inp53) (amino acids 141–531), KIAA0968 (0968) (amino acids 166–567) and Inp51p (Inp51) (amino acids 150–510). Values in the upper right portion of the matrix represent identity, and values in the lower left portion represent similarity. The SCIP type II phosphatases are indicated by †. Sac domains with proven phosphatase activity are indicated by ‡, and inactive Inp51 Sac phosphatase is indicated by ‡. The sequences were compared using the Clustal X algorithm [147].</td>
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The Sac domain contains seven highly conserved motifs, including the putative catalytic RXNCLDCLDRTN motif (conserved motif 6). These are shown for the yeast Sac domain-containing proteins Sac1p, Fig4p, Inp51p, Inp52p, and Inp53p. Inp51p does not contain well conserved residues within a number of these motifs, which may account for the lack of phosphatase activity seen in this protein. Conserved residues are shown in red, and Sac1p mutations discussed in the text are boxed in blue.

Sac phosphatase domain proteins

Sac phosphatase domain proteins

Sac domain

Figure 5 The Sac domain

The Sac domain contains seven highly conserved motifs, including the putative catalytic RXNCLDCLDRTN motif (conserved motif 6). These are shown for the yeast Sac domain-containing proteins Sac1p, Fig4p, Inp51p, Inp52p, and Inp53p. Inp51p does not contain well conserved residues within a number of these motifs, which may account for the lack of phosphatase activity seen in this protein. Conserved residues are shown in red, and Sac1p mutations discussed in the text are boxed in blue.

Figure 5 The Sac domain

The Sac domain contains seven highly conserved motifs, including the putative catalytic RXNCLDCLDRTN motif (conserved motif 6). These are shown for the yeast Sac domain-containing proteins Sac1p, Fig4p, Inp51p, Inp52p, and Inp53p. Inp51p does not contain well conserved residues within a number of these motifs, which may account for the lack of phosphatase activity seen in this protein. Conserved residues are shown in red, and Sac1p mutations discussed in the text are boxed in blue.
and conserved aspartate residue within the putative RXNCLDCL-
\textit{DRTN} motif could well represent the catalytic
proteins. The residues conserved between the phosphatase-active Sac domain
consistent with loss of phosphatase activity; these also involve
phosphorylated species of PtdIns. Within yeast, an organism that probably only contains
four identified phosphatase domains, this phosphatase
activity of the Sac phosphatases is mediated in a
similar manner by this conserved catalytic motif. Evidence for
this is intriguing. The Inp51p Sac domain does not exhibit
phosphatase activity, and the cysteine, arginine and threonine/
serine residues are absent from C\textit{X}_{i}\text{R}(T/S) motif of this protein,
being replaced by alanine, lysine and proline respectively. As
these are among the few significant changes seen between the
inactive Inp51p Sac domain and the active domains of Sac1p,
Inp52p, Inp53p and synaptojanin, it is tempting to propose that the
\(\text{C}X_{i}\text{R}(T/S)\) residues are involved in catalysis. Further evidence
comes from the characterization of mutations within the
Sac domain of Sac1p, several of which confer phenotypes
consistent with loss of phosphatase activity; these also involve
residues conserved between the phosphatase-active Sac domain
proteins. The \textit{sac}1-\textit{R} and \textit{sac}1-\textit{L} alleles are mutated at the first
conserved aspartate residue within the putative RXNCLDCL-
\textit{DRTN} catalytic motif [64] (Figure 5, motif 6), and in the \textit{sac}1-
\textit{L} and \textit{sac}1-\textit{R} alleles mutations are seen at an alanine residue
within the conserved WAD(N/H)(A/G)D sequence [64] (Figure
\textit{5}, motif 7). Interestingly, the Sac domain of Inp51p is also
changed within this latter motif. This evidence indicates that the
RXNCLDCLDRTN motif could well represent the catalytic
core of the Sac phosphatases.

Characterization of another mutation within the Sac
domain of Sac1p has demonstrated regulation of the Sac-domain
phosphatases [28]. The \textit{mbs1-sac}1-\textit{R} allele of Sac1p [63] contains a
leucine-to-proline mutation (L246P) within motif 5 (Figure
\textit{5}) [28] which is responsible for the mutant phenotypes of this \textit{sac}1
allele. Interestingly, protein containing this mutation retained
phosphatase activity of the same specificity and specific activity
as wild-type protein [28]. Cells containing a completely deleted
\textit{sac}1 gene and plasmid-borne Sac1p-L246P only display \textit{sac}1
mutant phenotypes, including drug-sensitivity and elevated levels
of PtdIns4P, when grown on synthetic defined (SD) minimal
media and not on rich (YPD) media. Thus it appears that some
component within the rich media regulates the activity of the
Sac1p phosphatase.

It seems clear that the Sac domain indeed represents a novel,
regulated phosphatidylinositol 3-, 4-, 5-phosphate phosphatase,
and it is likely that the catalytic core of the enzyme has been
identified. This phosphatase domain is found in five proteins
within yeast, an organism that probably only contains four
phosphorylated species of PtdIns.

**PROTEINS CONTAINING THE SAC PHOSPHATASE DOMAIN**

Proteins containing the Sac phosphatase domain appear to fall
into two classes. The first comprises the SCIps mentioned above,
which, in addition to an N-terminal phosphatase Sac domain,
have all the domains associated with type II phosphatidylinositol
phosphate 5-phosphatases. These include mammalian synapto-
janins and yeast Inp51p, Inp52p and Inp53p (Table \textit{1}; Figure \textit{3}).
The other class of Sac-containing phosphatases consists of
proteins with an N-terminal Sac phosphatase domain and no
other recognizable domains (Figure \textit{3}). This class includes a
number of uncharacterized putative proteins from a variety of
species and two other yeast proteins: Fig4p and the archetype of
the Sac family of phosphatases, Sac1p (Figure \textit{3}). Therefore
yeast contains multiple proteins of both classes of Sac
phosphatases. Some of these have been characterized, in
particular Sac1p. The features associated with these proteins are
discussed below, and reassessed in light of the recent
demonstration of phosphatase activity for the Sac domain.

**Sac1p**

The \textit{SAC1} gene encodes Sac1p, which is a 67 kDa membrane
protein found in the endoplasmic reticulum (ER) and Golgi [66].
The protein shows similarity with the other Sac domain-contain-
ing proteins in yeast and other organisms due to sequence
identity in the Sac domains of these proteins (Table \textit{1}). These
include three uncharacterized human proteins (KIAA0851,
KIAA0966 and KIAA0274) and a variety of sequences from other
species. Sac1p displays extended identity with Inp52p,
Inp53p and the human sequence KIAA0851, in that a small
region at the C-terminal end of the Sac domain, of approx. 84
amino acids, also exhibits identity with these proteins (Figure \textit{3}).

Sac1p was initially characterized in 1989 [67], but was only
defined as an inositol phosphatase over a decade later [28].
Initially, mutations at the \textit{sac}1 allele were recovered as sp-
taneous heat-resistant revertants of yeast strains carrying a
conditional-lethal temperature-sensitive actin mutation, \textit{act}1-\textit{ts}
[68]. Novick and Botstein [68] isolated alleles of \textit{sac}1, a recessive
suppressor of actin mutations which had simultaneously acquired
cold-sensitivity. The \textit{sac}1 alleles were tested for their ability to
suppress the temperature-sensitivity displayed by other actin
mutants (\textit{act}1-\textit{ts} and \textit{act}1-\textit{Fr}); however, \textit{sac}1/\textit{act}1-\textit{ts}
mutants were inviable (synthetic lethality) and \textit{sac}1/\textit{act}1-\textit{Fr}
mutants displayed both cold- and temperature-sensitivity (no suppression).
Cleves et al. [69] showed that mutations in \textit{SAC1} were also
suppressors of the phenotypes seen in \textit{sec}14 mutant yeast. Sec14p
is the yeast PtdIns phosphatidylinositol (PtdCho) transfer
protein [70], and was identified as a mutant allele causing a block
in the secretory pathway (sec) within the Golgi apparatus [71].
Mutations within \textit{SAC1} and deletion of the \textit{sac}1 open reading
frame both suppressed the defects of either \textit{sec}14-\textit{1ts} alleles or
deletion mutants, unlike the suppression seen for \textit{act}1-\textit{1ts} alleles
[67,69]. Thus suppression of actin mutant phenotypes by \textit{sac}1
mutants is allele specific, and was thought unlikely to be a bypass
suppression, whereas it appeared that loss of Sac1p function did
bypass the requirement for Sec14p [67,69].

Other phenotypes associated with \textit{sac}1 mutants, including
cold-sensitivity, mating and sporulation defects, have been
described, and mutants also display phenotypes reminiscent of the
\textit{act}1-\textit{Fr} phenotype [65,67,69]. Sac1p mutants have disrupted
actin deposition, resulting in almost imperceptible actin filaments
at the permissive temperature (30 °C) which disappear com-
pletely at the restrictive temperature (14 °C); these mutants also
display mislocalized cortical actin distributed randomly over the
cell, rather than being concentrated in the budding daughter cell
[65,67,69]. Chitin, another cytoskeletal component, was also
aberrantly localized at the restrictive temperature [65,67,69].
Thin-section electron microscopy revealed that \textit{sac}1 mutants
contained membrane-bound structures that resembled the Golgi-
related structures seen in the Golgi secretory mutant \textit{sec}7-1 [71].
Although invertase secretion was largely unaffected, \textit{sac}1 mutants
did show positive and negative synthetic interactions with some
of the classically identified secretory mutants (see below; [67,71]).

Further characterization of \textit{sac}1 mutants showed that they
also displayed phenotypes such as inositol auxotrophy [64–66,72]
unrelated to defects in \textit{de novo} inositol biosynthesis [66], multiple
drug-sensitivity [65] and synthetic lethality with \textit{tep}1-\textit{1ts} mutants
(interesting when compared with the phenotypes associated with

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binding protein which is also another suppressor of bypass suppression of diphosphate–choline pathway [72], and PLD is also required for phenotypes [65]. However, one of the most compelling observations regarding the suppression of sec14 mutations. Hama et al. [22], observing that suppression of sec14 secretory defects by sac1 mutants was dependent on the increase in PtdIns4P [64,72], tested whether overproduction of PtdInsP via the PtdIns kinases Pik1p, Stt4p or Vps34p (Figure 2) had an ability to bypass the cellular requirement for Sec14p. Only Pik4p, one of the two PtdIns 4-kinases, could suppress sec14 defects, confirming that overproduction of PtdIns4P was required for Sec14p bypass. Moreover, Sec14p mutants were also shown to have greatly reduced levels of PtdIns4P, and pkl1 mutants were demonstrated to exhibit defective secretion, similar to that seen in sec14 mutants [22,23]. At the non-permissive temperature sec14-1* mutants accumulate membrane structures called Berkeley bodies, which are readily visualized by electron microscopy. Cells containing a temperature-sensitive pkl1* allele also accumulate Berkeley bodies at the restrictive temperature [23]. Additionally, Sac1p mutants that do not affect Sac1p phosphatase activity [28] were originally shown not to suppress the requirement for Sec14p function [65]. Therefore it seems likely that Sec14p may function, in addition to its role in the regulation of the levels of Golgi PtdCho [81,82], to regulate levels of PtdIns4P via Pik1p, a process essential for efficient secretion [22].

Clevs et al. [69] demonstrated that sac1 mutants also displayed genetic interactions with other alleles identified as secretory mutants [69,71], which are now easier to interpret in light of the identification of Sac1p as a phosphatase. Sac1p mutants were able to significantly suppress the defects seen in sec6 and sec9 mutants. Sec6p is a component of the ‘exocyst’, a multiprotein complex required for targeting of secretion from the trans-Golgi to the plasma membrane in the bud tip [83,84]. Interestingly, mutations in sec6 cause a relocation of Pik1p, the PtdIns 4-kinase, from the nucleus and cytoplasm to the trans-Golgi [23]. Sec9p is a homologue of the mammalian t-SNARE (target-membrane soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor) [85] protein SNAP-25 (25 kDa synaptosome-associated protein), and is required for secretion between Golgi and plasma membrane [86]. Walch-Solimena and Novick [23] noted that pkl1 mutants also exhibit synthetic lethality with sec9 mutants. As both Sac1p and Pik4p function are implicated in Sec6p and Sec9p function, this perhaps indicates that these proteins may modulate secretion via interaction with PtdIns4P [or PtdIns(4,5)P2] regulated by Sac1p.

Clevs et al. also observed synthetic lethality between sac1 mutants and alleles of sec13 and sec20 [69]. As yet it is not clear how Sac1p may be involved in Sec20p function, as this protein forms a complex in the ER (with Tip20p and Sec22p) that is required for retrieval of ER proteins that are essential for secretion [87,88]. However, Sec13p is a component of the COPII coat protein required for vesicle formation and transport between the ER and the Golgi [89,90]. The COPII coat protein complex consists of the GTPase Sar1p, Sec23p–Sec24p dimers and Sec13p–Sec31p dimers [91]. The Sec13p–Sec31p protein complex has been demonstrated only to bind to membranes in the presence of both Sar1p GTPase and either PtdIns4P or PtdIns(4,5)P2 [91]. As Sac1p can regulate the levels of these lipids, Sac1p can control the binding of Sec13p to COPII vesicles.

In addition, Clevs et al. showed that mutation in Sac1p could exhibit negative genetic interactions with sec17, sec18, sec21 and sec23 [69]. Sec18p and Sec17p respectively represent the yeast phatase activity in vitro, the levels of PtdIns3P are only slightly raised (approximately 1.5-fold) [28,59]. This perhaps indicates that Sac1p functions primarily to regulate the levels of PtdIns4P in normal vegetative growth, and that there are other efficient mechanisms for the regulation of the levels of PtdIns3P. This selective role for Sac1p seems likely, given recent observations regarding the suppression of sec14 mutations. However, the identification of this lipid was clarified and it was emphasized that Sac1p is not required for ATP release into the extracellular medium [75], which is specific for ER transport, (2) ATP transport activity was directly proportional to the amount of cellular Sac1p, and (3) ATP transport activity could be immunoprecipitated with monoclonal antibodies generated to Sac1p [66,73]. However, Mayinger et al. [73] could be immunoprecipitated with monoclonal antibodies to the amount of cellular Sac1p, and (3) ATP transport activity was surprisingly convincing, as (1) sec1 deletion strains were shown to be defective in microsomal ATP transport, (2) ATP transport activity was directly proportional to the structural gene for mannosyl-di-inositol diphosphorylceramide synthase, (3) ATP transport activity was shown to have no effect on ceramide synthesis was shown to have no effect on synthesis of the cytidine diphosphate–choline pathway of PtdCho synthesis [77]. In addition, Sac1p was also identified in fractions enriched for ER ATP transport activity [73]. The evidence that Sac1p itself possessed ATP transport activity was surprisingly convincing, as (1) sec1 deletion strains were shown to be defective in microsomal ATP transport, (2) ATP transport activity was directly proportional to the amount of cellular Sac1p, and (3) ATP transport activity could be immunoprecipitated with monoclonal antibodies generated to Sac1p [66,73]. However, Mayinger et al. [73] emphasized that Sac1p could represent a regulatory factor, closely interacting with the ATP transporter, as Sac1p showed no identity with known solute carrier proteins. This was later confirmed, as purified recombinant glutathione S-transferase-tagged Sac1p was shown to be devoid of ATP transport activity [74]. This feature is specific for ER ATP transport, as Sac1p is not required for ATP release into the extracellular medium [75], and it was proposed that Sac1p probably interacts with an ATP transporter in this compartment. Thus it is clear that Sac1p also plays an important role in ATP transport specifically in the ER [74].

Numerous observations indicated that Sac1p is involved in lipid metabolism. Sac1p was seen to be analogous to the phosphatidylinositol phosphate 5-phosphatases, such as synaptosynadin [76]. Sac1p was also implicated in Sec14p PtdIns/PtdCho transfer protein function [69]. Other suppressors of sec14 mutations had been identified that included the structural genes for the proteins involved in the cytidine diphosphate–choline pathway of PtdCho synthesis [77]. In addition, sec1 mutants were demonstrated to exhibit greatly elevated flux through this pathway, while cellular PtdCho levels remained unchanged from those in wild-type cells [72]. PLD activity is required to maintain the sec1-induced high level of flux through the cytidine diphosphate–choline pathway [72], and PLD is also required for bypass suppression of sec14 mutants [72,78]. Kes1p, an oxysterol binding protein which is also another suppressor of sec14 defects [79], was identified as a multicopy suppressor of sac1 mutant phenotypes [65]. However, one of the most compelling observations linking Sac1p to lipid metabolism was the demonstration that sac1 mutants exhibit a dramatic increase in the levels of an inositol-containing lipid. This was initially identified as the sphingolipid mannosyl-di-inositol phosphorylceramide [64]. However, the identification of this lipid was clarified and it was correctly identified as PtdIns4P by Stock et al. [80] after deletion of the structural gene for mannosyl-di-inositol phosphophorylceramide synthesis was shown to have no effect on sac1 suppression of sec14 mutants. This observation was confirmed [28,59,72] when Sac1p was identified as a novel phosphatase. It remained clear, however, that overproduction of PtdIns4P was essential for the bypass of the requirement for Sec14p function in the Golgi.

So how does the identification of phosphatase activity within a domain of Sac1p affect the interpretation of the cellular role of Sac1p? Sac1p had been implicated in the functioning of the cytoskeleton, secretary traffic, lipid metabolism, membrane integrity and ER ATP transport – as discussed below, a role as a phosphatidylinositol 3-, 4-, 5-phosphate phosphatase is not inconsistent with many of these proposed functions.

Sac1p mutants have greatly (8–10 fold) elevated levels of PtdIns4P [28,59,72,80] and, although the enzyme has 3-phos-
homologues of NSF (N-ethylmaleimide-sensitive factor) and SNAP (soluble NSF attachment protein), which complex with target membrane receptors to allow vesicle fusion at all stages of the trafficking pathway [92,93]. The target receptor complexes, t-SNARES, identified in yeast include a Golgi-to-plasma-membrane t-SNARE complex consisting of Sec9p [94] which, as discussed above, may interact with Sac1p-regulated PtdIns4P [23]. Sec21p is a component of the COPI vesicle coating complex (analogous to the COPII coat consisting of Sec13p), which is essential for vesicular traffic [93,95]. Sec21p binding to membranes is dependent on the binding of the small GTPase ARF (ADP-ribosylation factor) to membranes, a process which occurs after ARF has bound GTP [96]. ARF–GTP is only able to bind efficiently to membranes rich in PtdIns(4,5)P2 [93,97], production of which could be regulated by Sac1p. It is likely that the phosphatidylinositol phosphates also regulate the binding of the COPI coat, as has been demonstrated for the COPII coat [91]. Sec32p is the GAP for Sar1p in the COPII coat complex. As discussed above, to allow binding of the Sec13p–Sec23p complex to vesicle membranes, both Sar1p- and Sac1p-regulated phosphatidylinositol phosphates were required [91]. Therefore, having assigned phosphatase activity to Sac1p, it is possible to see how the effect of Sac1p regulation of PtdIns4P metabolism can affect the secretory pathway and the proteins identified by genetic interaction with Sac1p.

Sac1p has also been demonstrated to have dramatic effects on the organization of the actin cytoskeleton. As noted above, Sac1p mutants exhibit allele-specific interactions with the actin gene ACT1 [65,67,69], and overexpression of Reb1p, an an mRNA enhancer binding protein which increases the transcription of actin [98,99], suppresses sac1 mutant phenotypes (W. E. Hughes, unpublished work). Regulation of the actin cytoskeleton, and thus cell wall chitin, has been shown to occur via the effects of PtdIns(4,5)P2 on some of the many actin binding proteins in yeast, such as profilin and cofilin [100–102]. The effects of sac1 mutation result in an up to 80% reduction in PtdIns(4,5)P2 levels [28]. This could well account for the actin defects seen in sac1 mutants. A temperature-sensitive mutation in mss4, encoding the PtdIns4P 5-kinase, confers a 90% reduction in cellular PtdIns(4,5)P2 levels after incubation at the restrictive temperature [29], resulting in a similar disorganization of the actin cytoskeleton [29,30]. Mutations within Pip1p also disrupt the actin cytoskeleton [23], and mutations in Stt4p, the other PtdIns 4-kinase in yeast, affect the function of another cytoskeletal protein, Arp5 [103]. Sac1p modulation of PtdIns4P levels could therefore affect cytoskeletal function, and it can be inferred that the specific mutations in actin that can be suppressed by sac1 mutations relate to the phosphatidylinositol phosphate regulation of actin function.

The phosphatase activity of Sac1p is also clearly responsible for the multiple drug-sensitive phenotype seen in some sac1 mutants [65], as the phenotype could be partially suppressed by the active Sac phosphatases of Inp52p and Inp53p, but not by the inactive Inp51p Sac domain [28]. It is unlikely that drug sensitivity is caused directly by the physical consequence of PtdIns4P overproduction, as this lipid is likely to be predominantly present in Golgi and post-Golgi vesicles and probably represents less than 0.5% of total cellular phospholipid in the sac1 mutant [28]. Sac1p mutants may also display reduced levels of PtdIns and phosphatidyserine [72], and of the sphingolipid mannosylinositol phosphorylceramide [80] which, in combination, could alter cell permeability to cause drug sensitivity. However, a selective effect on the trafficking of drug transporters cannot be excluded.

Such a role is also possible for efficient transport of ATP into the ER. Sac1p plays an important role in ATP transport into the ER; however, the protein itself is not a transporter protein [74,104]. It is likely that the phosphatase activity of Sac1p is not required for this function, as sac1-22 mutants, which display elevated levels of PtdIns4P [64,72] and are therefore likely to be phosphatase-deficient, retain wild-type levels of ER ATP transport [74]. ATP is required in the ER lumen by Sec61p, Sec63p and Kar2p (yeast BiP), which form complexes to ensure that nascent polypeptides are translocated across the ER membrane into the lumen [105,106]. Sac1p deletion strains show synthetic lethality with Sec61p, Sec63p and Kar2p mutations, confirming the role of Sac1p in ATP transport. Although it remains to be demonstrated that protein with the sac1-22 mutation lacks phosphatase activity, as sac1 synthetic lethality with sec61, sec63 or kar2 could be rescued by plasmid-borne sac1-22 [74], this does again suggest that Sac phosphatase function is not required to ensure ATP transport into the ER lumen [74]. Perhaps a region outside the Sac phosphatase domain of Sac1p is required for this function. Whether this second function of Sac1p also interacts with Sec20p, with which sac1 mutants show synthetic interactions (see above; [69]), is an interesting possibility. The Sec20p–Tip20p–Sec22p complex is required for retrieval of ER proteins from the Golgi, and sec22 mutants have been shown to secrete (i.e. fail to retrieve) Kar2p [107]. These observations indicate that Sac1p plays an important role in the maintenance of PtdIns4P levels to regulate secretory and cytoskeletal competence, while also carrying out ER functions associated with ATP transport and protein localization.

Fig4p

Fig4p, a predicted 101 kDa protein encoded by the gene FIG4, is the other yeast Sac domain-containing protein that does not resemble synaptojanin (Figure 3). As with Sac1p, the protein shows identity with the other Sac domain-containing proteins in the Sac domain (Table 1). In addition, a 220-amino-acid region immediately C-terminal to the Sac domain of Fig4p shows unique, additional identity with the Sac domain-containing protein KIAA0274 (Figure 3). This motif is also found at the C-terminal end of the Sac domain of the protein C34B7.3 in Caenorhabditis elegans.

Fig4p was identified in a detailed and comprehensive screen for pheromone-regulated or induced genes (FIG) [108]. Using a plasmid-borne genomic library containing random lacZ insertions, Erdman et al. [108] screened yeast for enhanced or reduced β-galactosidase production in response to α-factor mating pheromone. Of the 54 genes identified that were regulated by pheromone, FIG4 was characterized and transcription of the gene was assessed to be enhanced 44.7-fold by pheromone induction (by quantification of β-galactosidase production). Using a plasmid-borne genomic library containing random lacZ insertions, Erdman et al. [108] screened yeast for enhanced or reduced β-galactosidase production in response to α-factor mating pheromone. Of the 54 genes identified that were regulated by pheromone, FIG4 was characterized and transcription of the gene was assessed to be enhanced 44.7-fold by pheromone induction (by quantification of β-galactosidase production). Genes such as STE6 and FUS2, which were already known to be pheromone-induced, exhibited 6.5- and 798.9-fold induction respectively in the same assay [108]. This induction was thought to be due to the ‘PRE’ upstream regulator element consensus sequence (TGAAACA) identified upstream of the FIG4 open reading frame and also found in many pheromone-induced genes (FUS2, STE6, STE2) [108]. Complete disruption of the FIG4 open reading frame causes no defects in growth rate at 16, 25, 30 or 37 °C, or in cell cycle arrest, recovery, pheromone sensitivity [108], drug sensitivity or inositol auxotrophy [64,65]. However, FIG4 deletion and insertion mutants displayed significant mating defects, perhaps as a result of several morphological defects observed during production of the mating projection, the shmoo. In the presence of non-isotopic mating pheromone, fig4 mutant yeast produce a less distinct, broader shmoo than wild-type yeast,
often failing to polarize mating at all, resulting in production of multiple bumps around a larger cell. The actin within these projections is also more dispersed than the distinct cables directed towards shmoo production seen in wild-type cells. These observations, combined with the identity seen with Sac1p, led Erdman et al. [108] to speculate that Fig4p may function to regulate effector molecules of the actin cytoskeleton.

The Sac phosphatase domain of Fig4p has yet to be characterized, and the phosphatidylinositols phosphate content of fig4 mutants has not been analysed. Fig4p was shown to be unable to suppress the phosphatase defects seen in some Sac1p mutants [28] (although this is not proof that the domain lacks phosphatase activity). It has been reported that Fig4p can act as a multiple-copy suppressor of some other Sac1p mutant phenotypes [108]; however, the details of this are not clear. As Fig4p mutants display actin defects only during mating, it is likely that its predicted phosphatase activity could play an important role in the regulation of PtdIns4P required for actin organization and cellular morphogenesis during mating. Sac1p mutants also display mating defects, although the actin cytoskeleton is disrupted during all phases of the cell cycle in Sac1p mutants.

Inp51p

The INP51 gene encodes Inp51p, a 108 kDa membrane protein [109]. As with the other members of the SCIP class of Sac phosphatase-containing proteins, the enzyme contains a Sac domain (Table 1), the 5-phosphatase domains and a proline-rich C-terminal region (Figure 3). The protein shows identity over its entire length with the other SCIPs from yeast and mammalian sources. Within the 5-phosphatase domain the protein is also similar to all other 5-phosphatases, including yeast Inp54p and the mammalian ‘oculocerebrorenal syndrome of Lowe’ gene product, 75 kDa and 145 kDa polyphosphatase 5-phosphatases [57].

The open reading frame of Inp51p was first identified as a yeast homologue of synaptotagmin [76,110–113]. Due to the similarity seen with Sac1p, deletions of the open reading frame were tested for suppression of the phenotypes associated with mutations in Sec14p; however, no such interaction was detected [64]. To characterize the function of the gene, deletion strains were engineered; these were viable, indicating that the function of the protein was not essential for normal vegetative growth [112,113]. Wild-type and Inp51p deletion strains were tested for phosphatase activity against either Ins(1,4,5)P3 or PtdIns(4,5)P2, and reduced activity was seen in extracts prepared from mutant strains [109,112,113]. The function of Inp51p was also assessed in vivo by examination of [3H]inositol-labelled compounds in inp51 mutants, where the levels of both PtdIns(4,5)P2 and Ins(4,5)P2 were elevated; in strains overexpressing Inp51p, PtdIns(4,5)P2 was found to be reduced [113]. Thus Inp51p indeed represents a lipid-specific phosphatidylinositol phosphate 5-phosphatase, and it seemed likely that changes in Ins(4,5)P2 levels were a consequence of an elevation of PtdIns(4,5)P2 [113].

Phenotypic analysis of Inp51p deletion strains indicated that they were able to grow at a range of temperatures [112,113] and also displayed cold tolerance, in that inp51 mutants were able to grow at 12 °C and 14 °C, unlike the parent strain [113]. This phenotype was attributed to a lack of 5-phosphatase function, as Stolz et al. [113] noted that mutation of the conserved 5-phosphatase catalytic motif PAWCDRILW within Inp51p, which abolished 5-phosphatase activity, was sufficient to confer cold tolerance. This phenotype also showed increased tryptophan uptake, suggesting that tryptophan uptake may be a rate-limiting step in growth at low temperatures. This is interesting when compared with the cold-sensitivity and synthetic lethality phenotypes seen with some trp and sac1 mutants (see above and below; [65]).

Other phenotypic characteristics of inp51 deletion were tested; however, no defects in endocytosis (monitored by FM-64 labelling) or in carboxypeptidase Y processing through the Golgi to the yeast vacuole were reported [109,112]. The deletion strain did not show defects in Kex2p Golgi localization [114], and also did not show inositol auxotrophy [112] or drug sensitivity [65]. It was also reported that inp51 deletions show no growth defects on a variety of osmolytes, although growth was slightly impaired when supported in the presence of 1 M sorbitol or 0.5 M CaCl2 [112].

Several reports on the combination of deletion mutations in INP51, INP52 and INP53 by Srinivasan et al. [112] and Stolz et al. [109] generally identified the same features, and suggested that Inp51p may act on a different pathway to Inp52 and Inp53. Morphological studies have revealed some of the more interesting features associated with these mutants [109,112,115]. All inp51/inp52 double mutants tested show thickened cell walls, especially in the mother cell [109,112], extended plasma membrane invaginations [109,112] and a highly distorted and fragmented vacuolar structure [109,112]. Cytoskeletal and mitochondrial organization in the inp51/inp52 double mutants was also disrupted, but it was proposed that the mitochondrial disorganization could be a consequence of the plasma membrane invaginations [115]. The cytoskeletal disorganization phenotype was seen to include actin disruption and budding defects resulting from a loss of cell polarity [115]. Fluid-phase uptake (measured by Lucifer Yellow uptake) and receptor-mediated endocytosis (measured by uptake of radiolabelled mating pheromone) were also seen to be defective in inp51/inp52 double mutants, although the initial rate of uptake of mating pheromone was similar to that of wild-type yeast, and invertase secretion was slightly defective [115]. The mutants continued to show impaired growth when supported in the presence of 1 M sorbitol or 0.5 M CaCl2, but also became sensitive to 1.5 M NaCl [112] and lost the cold-tolerance phenotype seen in the single inp51 mutant [109]. Total cellular 5-phosphatase activity was unchanged from that in the single inp51 mutant [109].

The inp51/inp53 double deletion mutant also had thickened cell walls, again especially in the mother cell [109,112]; however, this was not as severe as in the inp51/inp52 double mutant. The plasma membrane was only slightly distorted and the vacuole showed slight fragmentation and an irregular shape [109,112]. Fluid-phase and receptor-mediated endocytosis and invertase secretion were all unaffected, unlike in the inp51/inp52 double mutants [115], and the actin cytoskeleton was also completely unaffected [112,115]. The inp51/inp53 double mutant also continued to exhibit sensitivity to growth on 1 M sorbitol or 0.5 M CaCl2 and cold sensitivity [109] and, as with the inp51/inp52 double mutant, total cellular 5-phosphatase activity was unchanged from that in the single inp51 mutant [109].

The triple inp51/inp52/inp53 deletion mutant was not viable [109,112,115]; however, it could be rescued and grown on 1 M sorbitol [109]. The mutant exhibits many of the features seen in the inp51/inp52 and inp51/inp53 double mutants, having a highly distorted membrane and excessive cell wall thickening [109].

Two interesting genetic interactions have also been characterized for inp51 mutants. Overexpression of the cytoskeletal proteins actin (ACT1) and fimbrin (SAC6 [116] or Rvs167p, an amphiphysin homologue required for endocytosis [117]) had no effect on single inp51 deletions or the inp51/inp52 and inp51/inp53 double mutants. However, deletion of the inp51 gene was seen to partially suppress the defects seen in temperature-sensitive sac6
mutants [115]. Deletion of the inp51 gene also confers synthetic lethality with pan1-20 [118]. Pan1p is a yeast eps15 homologue [119] which requires to co-ordinate several proteins essential for endocytosis [118].

Thus, taken together, these data suggest that the function(s) of Inp51p appears to overlap with those of the other Inp5–phosphatases, while retaining some unique functions. The protein is clearly involved in endocytosis and regulation of the actin cytoskeleton under conditions of normal vegetative growth.

The Inp51p Sac domain exhibits no phosphatase activity [59], and also is unable to suppress the phenotypes of phosphatase-deficient Sac1p mutants [28]. As discussed above, the Sac phosphatase family contain the putative catalytic motif RXNCLDCLDRTN. However, in Inp51p, the motif contains several significant mutations, reading RisafDsiekpN (Figure 5), which may account for the inactivity of the Inp51p Sac domain [59].

Although the Sac phosphatase domain of Inp51p may be catalytically inactive, the domain may retain other functions. Deletion of amino acids 2–490 of Inp51p, comprising the Sac domain, results in an increase in PtdIns(4,5)P2 levels within this mutant [113], indicating that the 5-phosphatase activity of the protein is impaired. This deletion does not affect the 5-phosphatase catalytic domains of the enzyme, and the evidence from in vitro studies with the core 5-phosphatase domains of Inp53p [59] indicate that the truncated Inp51p should retain catalytic activity. Investigation of the ability of the Sac domain of Sac1p to bind various phosphatidylinositol phosphates has indicated that the Sac domain has reasonable potential to act as a lipid binding motif, showing a preference for PtdIns (I. Grunwald, W. E. Hughes and R. Woscholski, unpublished work). Whether the RXNCLDCLDRTN motif is required for this interaction is unclear; however, these observations do suggest a role for the Sac domain in determining localization through interaction with lipid. Such a role is not unprecedented. The N-terminal PH domain of PI-specific phospholipase Cο binds specifically to PtdIns(4,5)P2 [120], and this lipid binding facilitates the ability of PI-specific phospholipase Cο to hydrolyse PtdIns(4,5)P2 [13,47]. Similarly, the C2-like domain of PTEN has recently been demonstrated to bind lipids to facilitate catalytic activity, both by recruiting PTEN to the membrane and also by positioning the catalytic domain optimally with respect to membrane-bound substrate [121]. The Sac domain of Inp51p could function in a similar manner. Thus it is clear that Inp51p encodes an enzyme with only PtdIns(4,5)P2, 5-phosphatase activity, and that the phenotypes discussed above can be primarily attributed to this function.

One of these phenotypes, cold tolerance, is of particular interest in relation to Sac1p mutations that confer cold-sensitivity (see above). Sac1p mutants also confer synthetic lethality with mutations in a gene involved in the synthesis of tryptophan, while Inp51p mutants exhibit increases in tryptophan uptake. The tryptophan transporter Tat2p [122,123] is thought to function inefficiently at low temperatures [113], and it is possible that sac1 mutants lacking Sac1p required for growth at low temperatures have compromised tryptophan uptake. These observations perhaps indicate that a signalling pathway involving phosphatidylinositol phosphates and regulated by Sac1p and Inp51p participates in the control of Tat2p. Tat2p is one of a number of specific amino acid permeases, including the phosphatidylinositol kinase homologues Tor1p and Tor2p [124], that are thought to be regulated by a nutrient signalling pathway. Tor proteins control the phosphorylation of Npr1p (via Tap42p, a regulatory component of the protein phosphatase 2A complex [125,126]), which regulates the internalization and degradation of plasma membrane Tat2p [127]. As yet, a requirement for phosphatidylinositol phosphate signalling has not been identified within this pathway. However, Tor2p also participates in a phosphatidylinositol phosphate-dependent pathway controlling cytoskeletal reorganization [128], and since overexpression of Pkl1p suppresses defects associated with mutations in either Tor1p or Tor2p [129,130], this is perhaps not unlikely. Cdc55p, another regulatory component of the protein phosphatase 2A complex, was also identified in a screen for multi-copy suppressors of sac1 mutations (65; W. E. Hughes, unpublished work), further implicating these proteins in phosphatidylinositol phosphate metabolism. How the phosphatidylinositol phosphates participate in regulation of Tat2p is not clear, although as Tat2p down-regulation from the plasma membrane involves endocytic trafficking, and as Tat2p is also regulated by control of trafficking from the Golgi to either the plasma membrane or the vacuole [127,131], it is possible that trafficking processes represent the mode of phosphatidylinositol phosphate regulation of tryptophan uptake.

Inp52p

The INP52 gene encodes Inp52p, a 133 kDa membrane protein [109] which shows identity particularly with Inp53p (> 60 % identity; > 70 %, similarity). As with the other members of the SCIP class of Sac phosphatase-containing proteins, the enzyme contains a Sac domain (Table 1), the 5-phosphatase domains and a proline-rich C-terminal region (Figure 3). The protein shows sequence similarity with other 5-phosphatases and, in addition, the extreme C-terminus of the protein contains the isoprenylation motif CDPN-CO2H [132], a feature seen in some mammalian 5-phosphatases (see [55]). A small region between the Sac domain and the 5-phosphatase domain shows additional identity with Sac1p (see above; Figure 3).

Inp52p (also known as SJ2p) was characterized extensively by Srinivasan et al. [112] and Stolz et al. [109] in studies looking at the phenotypes and consequences of deletion of each of the three yeast Sac-containing 5-phosphatases, Inp51p, Inp52p and Inp53p. The deletion strain displayed no growth defects at any temperature tested [109,112] and, in particular, only exhibits a very modest cold-resistance phenotype at 14 °C [109]. Unlike inp51 deletions, the mutants display sensitivity to growth on 0.5 M CaCl2, but, as with the inp51 mutants, exhibit sensitivity to growth on 1 M sorbitol [112]. The deletion strain did not show defects in Kex2p Golgi localization [114], or the inositol auxotrophy [112] or drug-sensitivity phenotypes associated with sac1 mutants [65]. Morphologically the mutants appear normal [109], except for some slight vacuolar defects [112]. Receptor-mediated and fluid-phase endocytosis were unaffected [115], and carboxypeptidase Y processing through the Golgi to the vacuole appeared normal [112]. As with inp51, an inp52 deletion strain was tested for suppression of sec14 mutant phenotypes, but none was seen [64].

Analysis of the phosphatase activity of the protein revealed that the deletion strain exhibited only slightly altered levels of PtdIns(4,5)P2 [109], and in vitro analysis of the recombinant protein identified detergent-insensitive PtdIns(4,5)P2-specific 5-phosphatase activity and detergent-sensitive activity capable of dephosphorylating PtdIns3P, PtdIns4P and PtdIns3,5P2 [59].

Studies on the consequences of double mutation combinations were also analysed. The phenotypes associated with the inp52/inp51 double mutant, as discussed above, include extended plasma membrane invaginations, cell wall thickening, and defects in actin deposition and in fluid and receptor-mediated endocytosis [109,112,115]. The inp52/inp53 double mutant is also viable, but
shows reduced growth rates on synthetic defined (SD) minimal medium [112], while growing normally at 22 °C, 30 °C and 37 °C on rich (YPD) medium [112]. As with the inp51 single deletion mutant, this double mutant was cold-tolerant at 14 °C [109]. The double deletion strain exhibited no sensitivity to 0.5 M KCl, 1.5 M NaCl or MgCl₂, but grew slowly on 1 M sorbitol and 0.5 M CaCl₂ [112]. The double deletion exhibited defects in fluid-phase endocytosis, invertase trafficking (secretion) and receptor-mediated endocytosis [115]. Morphologically the inp52/inp53 mutant displays thickened cell wall, plasma membrane and vacuolar defects [109,112]; in addition, the actin cytoskeleton is completely disorganized [112,115], resulting in the pattern of budding and process of cell polarization being defective [115]. The double mutant exhibited only slightly altered levels of PtdIns(4,5)P₂ [109].

Inp52p was recently identified in a screen for mutants for elevated levels of unequal sister chromatid recombination [133]. Mutations in Inp52p were identified which showed a dominant meiotic lethal phenotype similar to that seen in dmc1 mutants [133]. DMC1 encodes a meiosis-specific recombinase [134], and it was proposed that Inp52p could regulate the Dmc1p-promoted interhomologue recombination pathway.

Thus, taken together, these data suggest that the function(s) of Inp52p appear to overlap with those of the other Inp5-phosphatases, while retaining some unique functions. The function of the protein is more able to be complemented by other Inp5-phosphatases, but appears to be involved in both endocytosis and regulation of the actin cytoskeleton under conditions of normal vegetative growth.

The Inp52p Sac domain contains none of the alterations within the proposed catalytic motif RXNCLDCLDRTN that are seen in the Inp51p Sac domain (Figure 5), and would therefore be predicted to encode an active enzyme. Analysis of recombinant Inp52p indeed confirmed this, as the protein exhibited detergent-insensitive PtdIns(4,5)P₂ 5-phosphatase activity associated with the 5-phosphatase domain, and detergent-sensitive PtdIns4P, PtdIns3P and PtdIns(3,5)P₃ 3-, 4- and 5-phosphatase activities respectively associated with the Sac domain [59]. Additionally, Inp52p was seen to be able to suppress the phosphatase-deficient phenotypes associated with sac1 mutants, and this activity could be attributed to the Sac phosphatase domain of Inp52p [28]. Therefore this single enzyme is in a position to be able to convert all of the phosphatidylinositol phosphates found in yeast into PtdIns. This process is likely to occur efficiently as, for example, PtdIns(4,5)P₂ bound to the 5-phosphatase domain will be dephosphorylated, producing PtdIns4P, which would in turn be available immediately for hydrolysis to PtdIns catalysed by the proximal Sac domain, without the need for the enzyme to dissociate from the lipid bilayer.

Inp53p

The INP53 gene encodes Inp53p, a 124 kDa membrane protein [109]. As with the other members of the SCIP class of Sac phosphatase-containing proteins, the enzyme contains Sac (Table 1) and 5-phosphatase domains and a proline-rich C-terminal region, and is particularly similar to Inp52p (Figure 3). In addition, a motif within the C-terminal proline-rich region of the protein exhibits identity (31 %, identity; 52 %, similarity, over 95 residues) with the yeast protein Gin4p, a cytoskeletal located protein kinase required for cell cycle control [135,136], and a small region between the Sac domain and the 5-phosphatase domain shows additional identity with Sac1p (Figure 3).

Inp53p was characterized after its identification in a screen for genes involved in regulating yeast endosomal traffic [110]. The plasma membrane ATPase, Pma1p, is essential for growth, and one mutant can be mis-targeted to the endosome on shift to the restrictive temperature. Characterization of mutants that delivered the newly synthesized mutant Pma1p correctly to the plasma membrane at the restrictive temperature led to the isolation of sop2 (a suppressor of gnn1) [110]. Analysis of the sop2 mutant confirmed that this represented mutation within the open reading frame later identified as INP53. The sop2/inp53 cells showed reduced steady-state levels of Kex2p, a Golgi resident protein required for α-factor maturation. A decrease in Golgi Kex2p levels often occurs in trafficking mutants where recycling between late Golgi and the vacuole is defective and Golgi resident proteins are degraded in the vacuole; therefore Luo and Chang [110] proposed that Inp53p may function in Golgi-to-vacuolar trafficking. This possibility was strengthened by the observation that inp53 mutants also secreted an immature unprocessed form of α-factor.

Further characterization of the inp53 deletion strain has identified other features associated with lack of Inp53p function [109,112,115]. Deletion strains appeared to grow slightly more slowly than wild-type strains at 22, 30 and 37 °C [112], and did not show inp51-type cold tolerance at 14 °C [109]. The deletion strain showed no sensitivity to 1 M sorbitol. 0.5 M KCl, 0.5 M CaCl₂, 1.5 M NaCl or MgCl₂ [112], and did not display inositol auxotrophy [112] or multiple drug-sensitivity [65]. Mutants did not exhibit defects in fluid-phase or receptor-mediated endocytosis [115], or carboxypeptidase Y trafficking [112]. Morphological assessment of the consequences of the deletion revealed that the cell wall was thickened and that there was an increase in plasma membrane invagination and irregularities to the vacuole [109,112,115]. As with inp51 and inp52 deletion strains, the inp53 deletion was unable to suppress sec14 mutant phenotypes [64]. The consequences of double deletion mutants are discussed above and indicate that, as with Inp51p and Inp52p, these phosphatases have many similar and some distinct properties.}

Extracts prepared from inp53 deletion strains exhibited a considerably reduced ability to produce PtdIns4P from PtdIns(4,5)P₂ in vitro compared with wild-type or inp51 or inp52 mutant yeast [112]; however, deletion of inp53 was demonstrated to have little effect on the cellular levels of PtdIns(4,5)P₂ and Ins(1,4,5)P₃ [109]. Recombinant Inp53p characterized in vitro exhibited similar activities to Inp52p, i.e. a detergent-insensitive PtdIns(4,5)P₂-specific 5-phosphatase activity and a detergent-sensitive activity capable of dephosphorylating phosphate from PtdIns3P, PtdIns4P and PtdIns(3,5)P₃ [59]. The 5-phosphatase domain showed no phosphatase activity towards PtdIns(3,5)P₃; while the Sac phosphatase domain could convert PtdIns(3,5)P₃ into PtdIns [59].

Inp53p was also recently identified in a screen to identify mutations that have synthetic interactions with a temperature-sensitive clathrin mutant [114]. Clathrin is involved in endocytosis [137], the retention of Golgi proteins and protein sorting in the trans-Golgi network [138]. Mutations in inp53 were found to cause severe growth defects in combination with the clathrin mutant, which prompted a close assessment of the α-factor maturation properties of the strain, which is a measure of the proper localization of Kex2p, the Golgi resident protein responsible for maturation of α-factor [114]. At 30 °C the inp53 deletion exhibits minor defects in the maturation of α-factor, which at 20 °C become much more pronounced [114]. These phenotypes seen with inp53 mutants were specific for Inp53p, as mutation of inp51 or inp52 did not have any effect on viability with clathrin mutants or maturation of α-factor [114].
The Sac domain of Inp53 appears to retain all of the features seen in the Inp52p Sac domain discussed above. Inp53p exhibits detergent-insensitive PtdIns(4,5)P_2 5-phosphatase activity associated with the 5-phosphatase domain and detergent-sensitive PtdIns3P, PtdIns4P and PtdIns(3,5)P_2 3-, 4- and 5-phosphatase activities associated with the Sac domain [59]. Inp53p was also able to suppress the phosphatase-deficient phenotypes associated with sac1 mutants, a feature that, as with Inp52p, could be attributed to the Sac phosphatase domain of Inp53p [28]. Therefore this enzyme is also in a position to be able to convert all of the phosphatidylinositol phosphates found in yeast into PtdIns. As Inp53p appears to function in the process of Golgi-to-endosomal trafficking, the role of the Sac domain of this protein may be to ensure that there is no PtdIns4P or PtdIns3P present on these trafficking membranes.

**Perspectives**

The identification and characterization of the Sac domain phosphatidylinositol phosphate phosphatases and recent advances in understanding of the cellular role of the other enzymes involved in phosphatidylinositol phosphate regulation indicates potential functions for individual phosphatidylinositol phosphates. A model indicating potential sites of action for the phosphatidylinositol phosphates and the phosphatidylinositol phosphate phosphatases is shown in Figure 6.

The observation that Pik1p and Stt4p are both essential PtdIns 4-kinases indicates that the enzymes have specific functions, producing the same phosphatidylinositol phosphate probably in distinct cellular compartments. The evidence discussed above suggests that the Pik1p pool of PtdIns4P is required for secretion through the Golgi, and is regulated in this respect by Sec14p and Sac1p. Pik1p is regulated and localized by the essential protein Frq1p, a homologue of neuronal frequenin, which is involved in vesicle-mediated neurotransmitter release [139], again indicating that Pik1p produces a phosphatidylinositol phosphate required for secretion. Stt4p is unable to substitute for Sec14/Sac1p or Frq1p-related Pik1p functions, confirming that Pik1p-generated PtdIns4P alone participates in these roles (Figure 6).

Both Sac1p and Pik1p mutants show cytoskeletal defects, probably as a result of changes in the levels of PtdIns(4,5)P_2 rather than PtdIns4P, suggesting that this pool of PtdIns4P is also phosphorylated to form PtdIns(4,5)P_2. Additionally, both Sac1p and Inp51p appear to affect tryptophan uptake, confirming that the lipids regulated by these proteins, PtdIns4P and PtdIns(4,5)P_2 respectively, could be in a common compartment/pathway. However, Sac1p mutants with increased levels of PtdIns4P unexpectedly show a decrease in cellular PtdIns(4,5)P_2 levels, a phenomenon proposed to result from overexpression or mislocalization of another Sac-domain protein, such as Inp52p or Inp53p. In addition, the temperature-sensitive mutant Pik1p is incorrectly localized at the restrictive temperature and therefore is able to produce PtdIns4P at a different cellular location, which could result in the observed cytoskeletal defects. These alternative interpretations suggest that the wild-type pool of PtdIns4P regulated by Sec14p/Sac1p is perhaps not phosphorylated.

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**Figure 6** Potential roles for the *S. cerevisiae* phosphatidylinositol phosphate phosphatases

Shown is a representation of a budding yeast, containing the nucleus (N), ER, Golgi apparatus (G), prevacuolar compartment (PV) [149], multivesicular body (MVB) and vacuole (V) [150]. Actin filaments and cortical patches [151] are represented in green, and actin/phosphatidylinositol phosphate interacting proteins, such as profilin, are shown in black. The potential sites of action of the phosphatidylinositol phosphate phosphatases Sac1p, Inp51p, Inp52p and Inp53p are indicated. See the text for discussion.
further. Therefore it remains unclear whether the Pik1p pool of PtdIns4P is available for Mss4p to form PtdIns(4,5)P2.

Stt4p, in contrast, clearly produces PtdIns4P that can be phosphorylated to form PtdIns(4,5)P2. Overexpression of Mss4p suppresses the wortmannin-sensitivity of yeast (Stt4p is wortmannin-sensitive [27]), and Mss4p was initially identified as a multicyclop suppressor of Stt4p defects [25]. However, the PtdIns4P produced by Stt4p does not function simply to be phosphorylated by Mss4p, as analysis of stt4 (pstB) mutants indicated that the lipid is an essential component involved in the transport of phosphatidylserine from the ER to the Golgi [26] (Figure 6). Whether this is a function specific to Stt4p remains to be established, but it is probable that Stt4p-derived PtdIns4P has some specific function(s) yet to be identified. It is also unclear as to whether the other Sac-domain phosphatases could participate in the regulation of the Pik1p- or Stt4p-regulated pools of PtdIns4P lipid. Fig4p has a role in controlling the cytoskeleton during mating. Having only a Sac domain, this enzyme cannot directly affect the cytoskeleton via PtdIns(4,5)P2 and may mediate its regulatory effects via the Stt4p-generated PtdIns4P.

Mss4p is located primarily in the plasma membrane [30], and as Pik1p and Stt4p do not appear to be similarly located [23,26], it is possible that the PtdIns4P that is phosphorylated to form PtdIns(4,5)P2 is placed at the plasma membrane by trafficking processes. It is noteworthy, however, that the location of Pik1p appears to be regulated by an associated factor (Frq1p [139]), and it has been proposed that a regulatory component is involved in the function of Stt4p [26]; therefore it remains possible that, with the appropriate regulatory component, either of these enzymes could phosphorylate lipids at the plasma membrane. Many observations indicate that PtdIns(4,5)P2 is involved in the regulation of the actin cytoskeleton. The actin-regulating protein profilin can bind either PtdIns(4,5)P2 or actin, inhibiting filament polymerization [140,141]. Lipid-bound profilin is found predominantly in the plasma membrane [141], presumably at the site of PtdIns(4,5)P2 production and actin polymerization. This regulation is essential, as profilin-deficient cells exhibit dramatically altered actin distributions [102], and the essential nature of this regulation is confirmed by the observation that profilin/Mss4p double mutants are synthetically lethal [30]. PtdIns(4,5)P2 is also clearly involved in the regulation of vesicle trafficking in yeast. In mammalian systems a direct role for PtdIns(4,5)P2 in trafficking is clear, through interactions with proteins such as ARF, clathrin adaptor complexes, dynamin and synaptotagmin (see [6.7] and references therein). Although there is evidence that PtdIns(4,5)P2 could be involved in the association of COPI and COPII coats and in ARF association with the membrane, these interactions remain to be demonstrated in yeast. Additionally, the involvement of Inp52p and Inp53p in trafficking processes indicates a role for the lipid in yeast; however, the nature of this is also unclear. Currently there is significant evidence for an indirect role of PtdIns(4,5)P2 in yeast trafficking processes. Mutations in actin and many other actin regulatory proteins cause perturbations of the secretory pathway [142]. Analysis of some of these mutants (act1, sla2/end4) has indicated that the vesicles accumulated in these mutants represent a late post-Golgi vesicle containing the Ypt1p Rab GTPase [142], indicating an essential role for actin late in the exocytotic process. Actin and the associated protein fimbrin (Sac6p) have also been shown to be essential for the internalization step of endocytosis [143]; thus it appears that regulation by PtdIns(4,5)P2 of the cytoskeleton could be a major regulatory factor in trafficking to, and from, the plasma membrane. PtdIns(4,5)P2 has the potential to be metabolized by Plcl1p, Inp51p, Inp52p, Inp53p and Inp54p, which therefore could play a role in the regulation of secretion and actin deposition. Inp51p, which interacts with both Sac6p (fimbrin) and Pan1p (eps15), is clearly involved in metabolism of this lipid to regulate actin structure and endocytosis, probably at the plasma membrane. Whether Inp52p and Inp53p, which are also implicated in endocytic and cytoskeletal regulation, also function to dephosphorylate plasma membrane PtdIns(4,5)P2 is less clear. Inp53p in particular appears to have a role in intra-Golgi and Golgi-to-endosomal trafficking (Figure 6). Inp54p is a type II-like 5-phosphatase, and has recently been suggested to specifically hydrolyse PtdIns(4,5)P2 (see [11]). How this phosphatase could affect phosphatidylinositol phosphate metabolism has not yet been described.

PtdIns3P has a well established role in regulating Golgi-to-endosomal trafficking, and is produced by Vps34p in the late Golgi compartments before trafficking to the vacuole [144,145]. Fab1p kinase is proposed to be found on vacuolar membranes [42], where it phosphorylates PtdIns3P to form PtdIns(3,5)P2 required for trafficking from the vacuole [41]. Sac-domain phosphatases can hydrolyse PtdIns3P efficiently, although which of the Sac-domain phosphatases are involved in the degradation of these lipids has not been established. It has been proposed that PtdIns3P is degraded in the vacuole by hydrolases such as PrA and PrB [145], and this remains a possibility, as no Sac-domain phosphatase has yet been localized to this compartment. As trafficking from the vacuolar compartments is dependent on the production of PtdIns(3,5)P2 from the PtdIns3P delivered to the vacuole [145], this offers an alternative route for the removal of the lipid. OCRL 5-phosphatase has been identified on the mammalian lysosome, which may suggest that yeast Inp5 phosphatases could function on the yeast vacuolar membrane [146]. Inp52p and Inp53p have both been implicated in vacuolar function and therefore are likely candidates for Sac-domain phosphatases involved in the regulation of the level of these lipids.

As is evident in the brief discussion above, the compartmentalization of phosphatidylinositol phosphates appears to have profound effects upon cellular behaviour. Since each of these lipids has potential downstream targets, there is an absolute need to control their individual production and destruction. Can this be achieved in a manner that, despite the metabolic inter-relationships, permits all forms to be produced independently? Perhaps not in a completely isolated fashion; however, the broad specificity of the Sac domains for phosphatidylinositol phosphates and their juxtaposition to domains encoding 5-phosphatase activities does mean that, for particular compartments, the metabolism of polyphosphorylated phosphatidylinositol phosphates can proceed to PtdIns in a manner that may well bypass the accumulation of intermediates.

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