REVIEW ARTICLE

Sac phosphatase domain proteins

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Advances in our understanding of the roles of phosphatidylinositol phosphates in controlling cellular functions such as endocytosis, exocytosis and the actin cytoskeleton have included new insights into the phosphatases that are responsible for the interconversion of these lipids. One of these is an entirely novel class of phosphatase domain found in a number of well characterized proteins. Proteins containing this Sac phosphatase domain include the yeast *Saccharomyces cerevisiae* proteins Sac1p and Fig4p. The Sac phosphatase domain is also found within the mammalian phosphoinositide 5-phosphatase synaptojanin and the yeast synaptojanin homologues Inp51p, Inp52p and Inp53p. These proteins therefore contain both Sac phosphatase and 5-phosphatase domains. This review describes the Sac phosphatase domain-containing proteins and their actions, with particular reference to the genetic and biochemical insights provided by study of the yeast *Saccharomyces cerevisiae*.

Key words: actin, endocytosis, exocytosis, phosphoinositide, trafficking.

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 to play 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 (PtdIns4*P* 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 PtdIns4*P* 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-phosphatidyltransferase, transfers a phosphatidyl group on to

Abbreviations used: ARF, ADP-ribosylation factor; ER, endoplasmic reticulum; GAP, GTPase-activating protein; PH, pleckstrin homology; PI, phosphoinositide; Type I PIPkin, PtdIns4*P* 5-kinase; PLD, phospholipase D; PtdCho, phosphatidylcholine; SCIP, Sac domain-containing inositide 5-phosphatase; SH2, Src homology 2; SNARE, soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor; t-SNARE, target-membrane SNARE.

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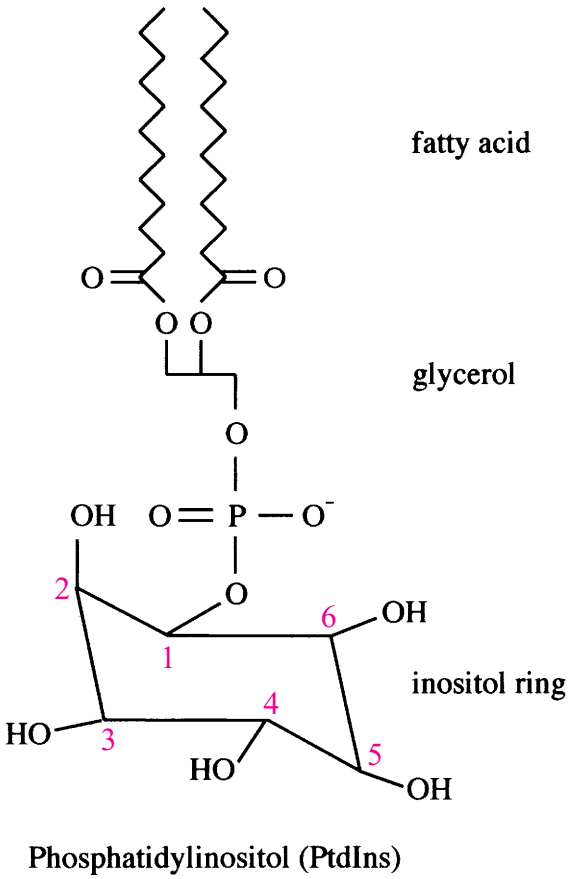

Figure 1 Representation of phosphatidylinositol

Phosphatidylinositol (PtdIns) is shown with the headgroup carbon atoms numbered. Physiologically in *S. cerevisiae* the fatty acids found on the *sn*-1 and *sn*-2 positions of glycerol are predominantly oleic acid ($C_{18:1}$) and palmitic acid ($C_{16:0}$), or palmitic acid and palmitoleic acid ($C_{16:1}$) [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.

inositol to form PtdIns. 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. PtdIns3*P*, PtdIns4*P*, PtdIns(3,5)P_2 and PtdIns(4,5)P_2. The kinases involved in the production of these lipids have been identified and characterized (Figure 2).

PtdIns4*P*

PtdIns4*P* 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*^{ts}, 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 phosphatidylserine 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 PtdIns4*P*, 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)P_2

PtdIns(4,5)P_2 is synthesized from PtdIns4*P* 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)P_2 was as a substrate for phospholipase C, to produce the second messengers diacylglycerol and Ins(1,4,5)P_3. 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)P_2 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)P_2 can also act as a cofactor for PLD [5,31] and is necessary for homotypic vacuolar fusion [32]. Thus PtdIns(4,5)P_2

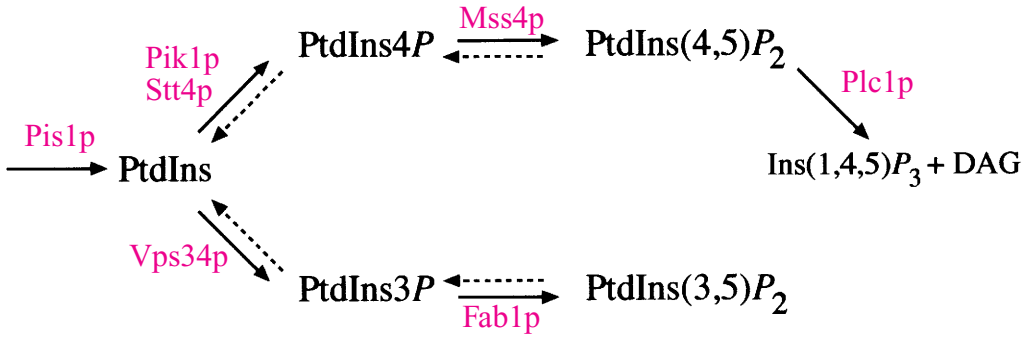

Figure 2 Phosphatidylinositol phosphates and phosphatidylinositol phosphate kinases in *S. cerevisiae*

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 PtdIns4*P* and PtdIns3*P* respectively. The products of these enzymes can, in turn, be phosphorylated by the PtdIns4*P* 5-kinase (Mss4p) or by the PtdIns3*P* 5-kinase (Fab1p), to form PtdIns(4,5)P_2 and PtdIns(3,5)P_2 respectively. Phospholipase C (Plc1p) can hydrolyse PtdIns(4,5)P_2, forming Ins(1,4,5)P_3 and diacylglycerol (DAG). There is no evidence that PtdIns(3,5)P_2 can be degraded by Plc1p. Phosphatases can dephosphorylate the phosphatidylinositol phosphate species, although the specific phosphatases involved remain unclear.

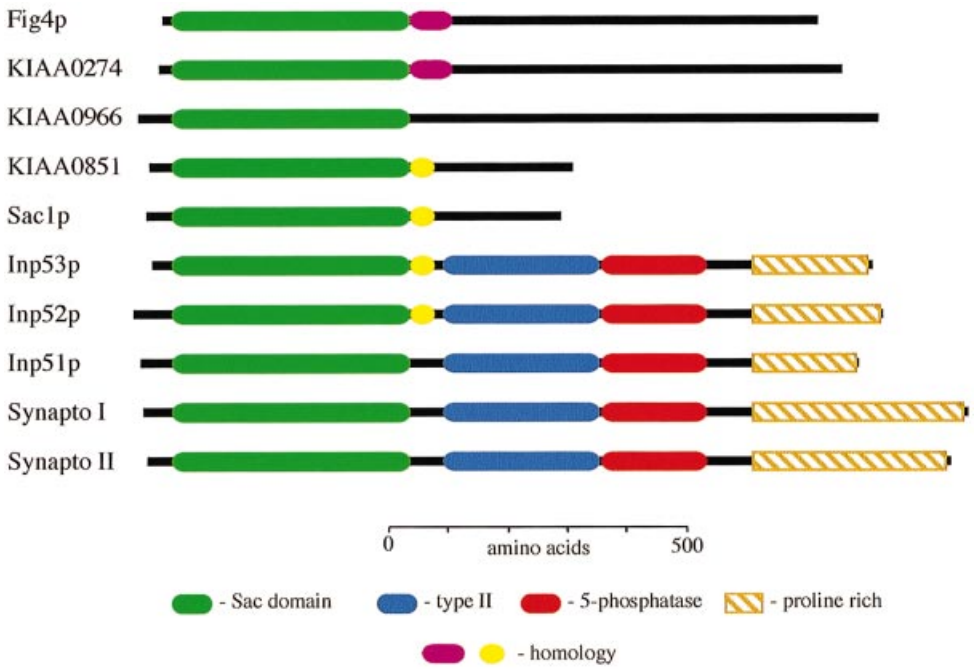

Figure 3 Sac domain-containing proteins

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.

PtdIns3*P*

PtdIns3*P* 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 PtdIns3*P* in vesicle trafficking [35]. PtdIns3*P* 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)P_2

PtdIns(3,5)P_2 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 PtdIns3*P* 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)P_2 in some aspect of vacuolar homoeostasis [39]. PtdIns(3,5)P_2 levels rise rapidly and transiently in response to hyperosmotic stress, which would imply that PtdIns(3,5)P_2 has a role in stress responses [40]. Work done with the Fab1p regulatory protein Vac7p implies that Fab1p, and hence PtdIns(3,5)P_2, 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

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 inositide 5-phosphatases; SHIPs) or a C-terminal GAP (GTPase-activating protein) domain (GAP domain-containing inositide 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 inositide 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 domains was identified. The first indication for such an activity came during work characterizing inhibitors of mammalian PtdIns(4,5)P_2-dependent PLD. Synaptojanin was identified as one of several proteins able to inhibit the activity of PLD1, and this was attributed to the ability of synaptojanin to hydrolyse PtdIns(4,5)P_2 required for PLD1 activity [58]. Detailed analysis of the phosphatidylinositol phosphates hydrolysed by synaptojanin revealed that virtually no PtdIns4*P* 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 PtdIns3*P*, PtdIns4*P* and PtdIns(3,5)P_2 [59]. The Sac domain from Sac1p

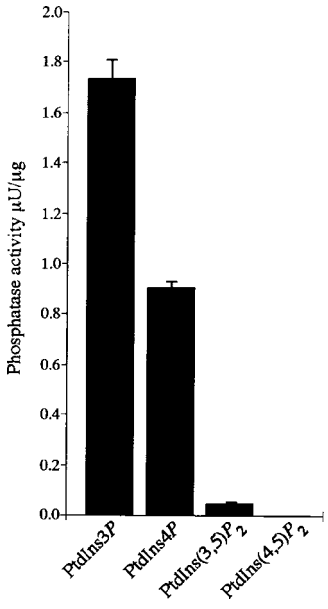

Figure 4 Phosphatidylinositol phosphate specificity of Sac-domain phosphatases

The figure represents data taken from the study of Hughes et al. [28], in which glutathione S-transferase-tagged Sac1p was expressed and purified from yeast and tested for phosphatase activity against ^{32}P-labelled PtdIns3*P*, PtdIns4*P*, PtdIns(3,5)P_2, PtdIns(4,5)P_2 and PtdIns(3,4)P_2. The protein shows activity principally against monophosphorylated phosphatidylinositides, and no activity could be detected for PtdIns(4,5)P_2 or PtdIns(3,4)P_2. Similar activity has been described for Sac domains from human synaptojanin and yeast Inp52p and Inp53 [59]. U, units.

Table 1 Sac domain identity/similarity matrix

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 118–481), human synaptojanin 2 (Syn2) (amino acids 66–433), Inp52p (Inp52) (amino acids 166–556), Inp53p (Inp53) (amino acids 141–531), KIAA0966 (0966) (amino acids 166–567) and Inp51p (Inp51) (amino acids 150–510). Values in the lower left portion of the matrix represent identity, and values in the upper right portion of the matrix 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].

	Identity/similarity (%)									
Sac domain	Sac1	0851	Fig4	0274	Syn1	Syn2	Inp52	Inp53	0966	Inp51
Sac1†	–	57.6	39.2	38.7	38.7	37.7	45.0	45.0	47.7	29.8
0851	43.4	–	40.2	38.9	44.3	42.6	43.6	42.6	50.0	30.6
Fig4	26.0	30.8	–	57.4	36.0	35.6	39.6	37.3	36.4	27.1
0274	25.7	26.8	44.2	–	36.3	33.6	34.6	35.5	35.9	26.5
Syn1*†	26.4	30.3	23.0	24.9	–	73.4	43.6	42.9	41.8	35.7
Syn2*	25.4	29.5	23.4	22.6	59.1	–	42.2	42.2	39.8	34.8
Inp52*†	29.7	30.8	26.3	22.1	33.4	31.0	–	84.1	40.1	39.6
Inp53*†	29.0	28.1	24.2	22.8	31.4	30.3	72.4	–	39.6	38.8
0966	34.2	36.2	24.6	22.6	28.0	28.5	27.2	27.9	–	27.8
Inp51*‡	18.5	17.5	16.9	14.2	22.0	21.7	25.6	22.6	15.1	–

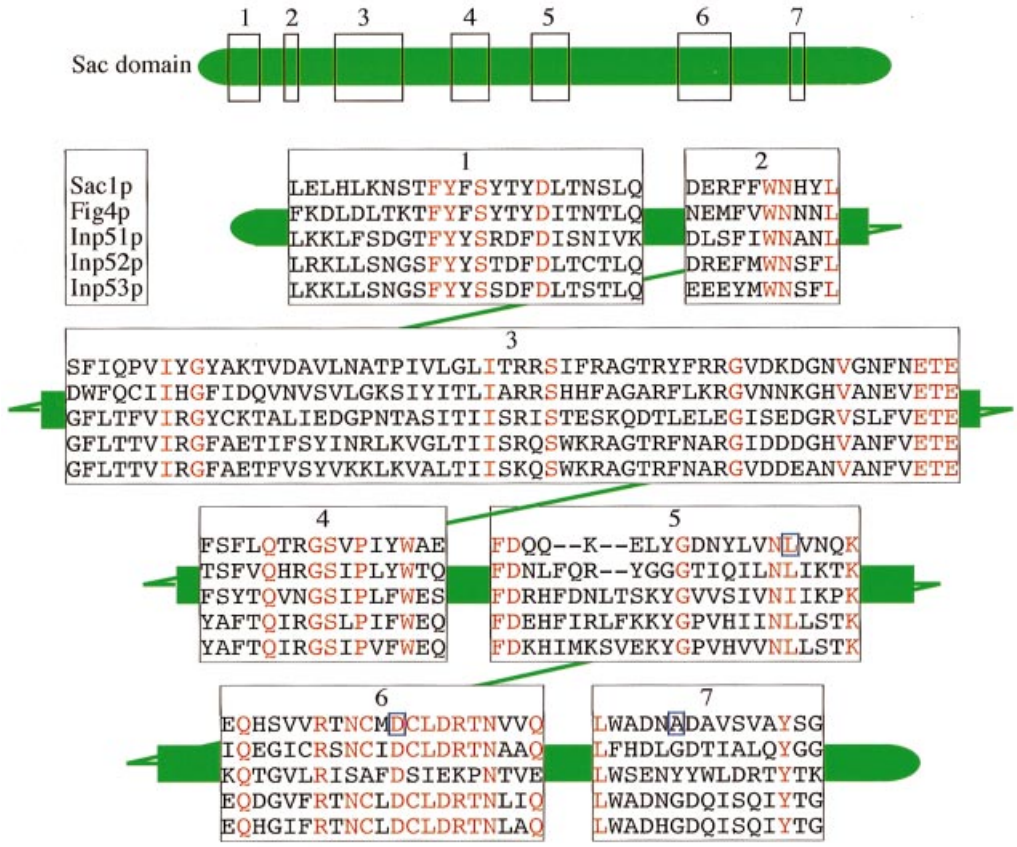

Figure 5 The Sac domain

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.

itself was also demonstrated to exhibit phosphatase activity directed against phosphate from the same lipids, i.e. PtdIns3*P*, PtdIns4*P* and PtdIns(3,5)P_2 [28]. Therefore the Sac domain found in synaptojanin and related proteins appears to represent a novel phosphatidylinositol phosphate phosphatase.

Phosphatase activity is contained within a region of the Sac domain, which retains activity when isolated from the parent protein either by proteolytic cleavage [59] or by expression as a recombinant fusion protein [28,59]. The phosphatase activity is Mg^{2+}-independent, is inhibited by 2 mM Cu^{2+}, Zn^{2+} or (in part) Mg^{2+}, and is activated by dithiothreitol [59]; it also has been demonstrated recently that Sac1p phosphatase is sensitive to *N*-ethylmaleimide [60]. Sac phosphatases are also sensitive to the manner in which substrate is presented. Total yeast lipids or purified lipid vesicles are substrates for the enzyme, but lipids presented in a 0.2% Triton X-100 micelle are not ([59]; W. E. Hughes and R. Woscholski, unpublished work). Substrate presented in 0.25% (w/v) octyl glucoside micelles is, however, readily hydrolysed by Sac-domain phosphatases, providing a convenient vehicle for substrate presentation [28]. Sac phosphatase domains (from Sac1p, Inp53p and synaptojanin) display activity principally against PtdIns3*P* and PtdIns4*P* (Figure 4) [28,59]. The proteins show much lower ($< 3\%$) activity towards PtdIns(3,5)P_2 and no detectable activity towards PtdIns(4,5)P_2 (Figure 4) [28,59]. The activity seen with the Sac1p Sac domain against PtdIns(3,5)P_2 is preferentially against phosphate in the *D*-3 position [28]; however, Sac-domain phosphatases from Inp53p and synaptojanin [59] and Sac1p [28] are able to dephosphorylate PtdIns(3,5)P_2 to PtdIns. Sac-domain phosphatases also show negligible activity towards soluble inositide polyphosphates [59] or PtdIns(3,4)P_2 (W. E. Hughes and R. Woscholski, unpublished work). The Sac phosphatases do not hydrolyse either PtdIns(3,4)P_2 or PtdIns(4,5)P_2, which contain adjacent phosphate groups (Figure 1), but retain reduced activity towards PtdIns(3,5)P_2, where the two phosphates are separated by a hydroxylated carbon. Therefore it appears that the Sac-domain phosphatase activity is predominantly a lipid-specific, phosphatidylinositol monophosphate phosphatase with 3-, 4- and 5-phosphatase activities (described as a PPIPase activity by Guo et al. [59]) (Figure 4).

The Sac domain is approximately 400 residues in length, and proteins containing this domain show approximately 35% identity with other Sac domains throughout this region (Table 1). The domain consists of seven highly conserved motifs (Figure 5) which appear to define the catalytic and regulatory regions of the phosphatase. The sequence RXNCLDCLDRTN within the sixth highly conserved motif (Figure 5) is of particular interest. The $CX_5R(T/S)$ motif found within this sequence is also present in a variety of metal-independent protein and inositide polyphosphate phosphatases [59]. These include the family of protein tyrosine phosphatases and the dual-specificity serine/threonine phosphatases, for which the structure–function relationship has been

relatively well characterized [61–63]. The $CX_5R(T/S)$ motif in these proteins is thought to cradle the phosphate moiety, which is then transferred to the nucleophile cysteine, generating a phospho-cysteine intermediate. The PO_3 is transferred to a water molecule, and a conserved aspartic acid from an adjacent loop facilitates catalysis by protonating the leaving-group oxygen, to generate an uncharged hydroxy group [61]. It is possible that the 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 $CX_5R(T/S)$ 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 $CX_5R(T/S)$ 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 *sac1-8* and *sac1-22* alleles are mutated at the first conserved aspartate residue within the putative RXNCLDCL-DRTN catalytic motif [64] (Figure 5, motif 6), and in the *sac1-10* and *sac1-17* alleles mutations are seen at an alanine residue within the conserved WAD(N/H)(A/G)D sequence [64] (Figure 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 *mds1-sac1* allele of Sac1p [65] contains a leucine-to-proline mutation (L246P) within motif 5 (Figure 5) [28] which is responsible for the mutant phenotypes of this *sac1* 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 *sac1* gene and plasmid-borne Sac1p-L246P only display *sac1* mutant phenotypes, including drug-sensitivity and elevated levels of PtdIns4*P*, 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 phosphatylinositol 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 synaptojanins and yeast Inp51p, Inp52p and Inp53p (Table 1; Figure 3). The other class of Sac-containing phosphatases consists of proteins with an N-terminal Sac phosphatase domain and no other recognizable domains (Figure 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 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 *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-containing proteins in yeast and other organisms due to sequence identity in the Sac domains of these proteins (Table 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 3).

Sac1p was initially characterized in 1989 [67], but was only defined as an inositide phosphatase over a decade later [28]. Initially, mutations at the *sac1* allele were recovered as spontaneous heat-resistant revertants of yeast strains carrying a conditional-lethal temperature-sensitive actin mutation, *act1-1*^{ts} [68]. Novick and Botstein [68] isolated alleles of *sac1*, a recessive suppressor of actin mutations which had simultaneously acquired cold-sensitivity. The *sac1* alleles were tested for their ability to suppress the temperature-sensitivity displayed by other actin mutants (*act1-2*^{ts} and *act1-4*^{ts}); however, *sac1*/*act1-2*^{ts} mutants were inviable (synthetic lethality) and *sac1*/*act1-4*^{ts} mutants displayed both cold- and temperature-sensitivity (no suppression). Cleves et al. [69] showed that mutations in *SAC1* were also suppressors of the phenotypes seen in *sec14* mutant yeast. Sec14p is the yeast PtdIns/phosphatidylcholine (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 *SAC1* and deletion of the *sac1* open reading frame both suppressed the defects of either *sec14-1*^{ts} alleles or deletion mutants, unlike the suppression seen for *act1-1*^{ts} alleles [67,69]. Thus suppression of actin mutant phenotypes by *sac1* 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 *sac1* mutants, including cold-sensitivity, mating and sporulation defects, have been described, and mutants also display phenotypes reminiscent of the *act1-1* phenotype [65,67,69]. Sac1p mutants have disrupted actin deposition, resulting in almost imperceptible actin filaments at the permissive temperature (30 °C) which disappear completely 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 *sac1* mutants contained membrane-bound structures that resembled the Golgi-related structures seen in the Golgi secretory mutant *sec7-1* [71]. Although invertase secretion was largely unaffected, *sac1* mutants did show positive and negative synthetic interactions with some of the classically identified secretory mutants (see below; [69,71]).

Further characterization of *sac1* mutants showed that they also displayed phenotypes such as inositol auxotrophy [64–66,72] unrelated to defects in *de novo* inositol biosynthesis [66], multiple drug-sensitivity [65] and synthetic lethality with *trp1-1* mutants (interesting when compared with the phenotypes associated with

inp51 mutants; see below [65]). These phenotypes were displayed in different combinations by different mutants. For example, the *sac1-22* allele showed neither inositol auxotrophy nor drug sensitivity and could suppress *sec14-1* defects, but only in the presence of inositol. These confusing data did, however, indicate that Sac1p had functions relating to secretion, inositol metabolism and membrane permeability.

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) *sac1* 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 synaptojanin [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, *sac1* 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 *sac1*-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 mannosyldi-inositol diphosphorylceramide [64]. However, the identification of this lipid was clarified and it was correctly identified as PtdIns4*P* by Stock et al. [80] after deletion of the structural gene for mannosyldi-inositol diphosphorylceramide 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 PtdIns4*P* 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, secretory 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 PtdIns4*P* [28,59,72,80] and, although the enzyme has 3-phosphatase activity *in vitro*, the levels of PtdIns3*P* are only slightly raised (approximately 1.5-fold) [28,59]. This perhaps indicates that Sac1p functions primarily to regulate the levels of PtdIns4*P* in normal vegetative growth, and that there are other efficient mechanisms for the regulation of the levels of PtdIns3*P*. This selective role for Sac1p seems likely, given recent 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 PtdIns4*P* [64,72], tested whether overproduction of PtdIns*P* 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 PtdIns4*P* was required for Sec14p bypass. Moreover, Sec14p mutants were also shown to have greatly reduced levels of PtdIns4*P*, and *pik1* mutants were demonstrated to exhibit defective secretion, similar to that seen in *sec14* mutants [22,23]. At the non-permissive temperature *sec14-1*^{ts} mutants accumulate membrane structures called Berkeley bodies, which are readily visualized by electron microscopy. Cells containing a temperature-sensitive *pik1*^{ts} 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 PtdIns4*P* via Pik1p, a process essential for efficient secretion [22].

Cleves 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 *pik1* 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 PtdIns4*P* [or PtdIns(4,5)P_2] regulated by Sac1p.

Cleves 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 PtdIns4*P* or PtdIns(4,5)P_2 [91]. As Sac1p can regulate the levels of these lipids, Sac1p can control the binding of Sec13p to COPII vesicles.

In addition, Cleves 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

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 PtdIns4*P* [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)P_2 [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]. Sec23p 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 PtdIns4*P* 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 rRNA 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)P_2 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)P_2 levels [28]. This could well account for the actin defects seen in *sac1* mutants. A temperature-sensitive mutation in *mss4*, encoding the PtdIns4*P* 5-kinase, confers a 90% reduction in cellular PtdIns(4,5)P_2 levels after incubation at the restrictive temperature [29], resulting in a similar disorganization of the actin cytoskeleton [29,30]. Mutations within Pik1p 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 PtdIns4*P* 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 PtdIns4*P* 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 phosphatidylserine [72], and of the sphingolipid mannosyldiinositol 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 PtdIns4*P* [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 PtdIns4*P* 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 Fig4 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). 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-isotropic 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 phosphatidylinositol 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 PtdIns4*P* 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 polyphosphate 5-phosphatases [57].

The open reading frame of Inp51p was first identified as a yeast homologue of synaptojanin [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)P_3 or PtdIns(4,5)P_2, 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 [^{3}H]inositol-labelled compounds in *inp51* mutants, where the levels of both PtdIns(4,5)P_2 and Ins(1,4,5)P_3 were elevated; in strains overexpressing Inp51p, PtdIns(4,5)P_2 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(1,4,5)P_3 levels were a consequence of an elevation of PtdIns(4,5)P_2 [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 FM4-64 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 $CaCl_2$ [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 $CaCl_2$, 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 $CaCl_2$ [112] 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 is required 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)P_2 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 by determining localization through interaction with lipid. Such a role is not unprecedented. The N-terminal PH domain of PI-specific phospholipase Cδ binds specifically to PtdIns(4,5)P_2 [120], and this lipid binding facilitates the ability of PI-specific phospholipase Cδ to hydrolyse PtdIns(4,5)P_2 [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)P_2 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, as Tor2p also participates in a phosphatidylinositol phosphate-dependent pathway controlling cytoskeletal reorganization [128], and since overexpression of Plc1p 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 endocytotic 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-CO_2H [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 Sjl2p) 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 $CaCl_2$, 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)P_2 [109], and *in vitro* analysis of the recombinant protein identified detergent-insensitive PtdIns(4,5)P_2-specific 5-phosphatase activity and detergent-sensitive activity capable of dephosphorylating PtdIns3*P*, PtdIns4*P* and PtdIns(3,5)P_2 [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 $MgCl_2$, but grew slowly on 1 M sorbitol and 0.5 M $CaCl_2$ [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)P_2 [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)P_2 5-phosphatase activity associated with the 5-phosphatase domain, and detergent-sensitive PtdIns4*P*, PtdIns3*P* and PtdIns(3,5)P_2 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)P_2 bound to the 5-phosphatase domain will be dephosphorylated, producing PtdIns4*P*, 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 cytoskeletally 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 *pma1*) [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 $CaCl_2$, 1.5 M NaCl or $MgCl_2$ [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 PtdIns4*P* from PtdIns(4,5)P_2 *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)P_2 and Ins(1,4,5)P_3 [109]. Recombinant Inp53p characterized *in vitro* exhibited similar activities to Inp52p, i.e. a detergent-insensitive PtdIns(4,5)P_2-specific 5-phosphatase activity and a detergent-sensitive activity capable of dephosphorylating phosphate from PtdIns3*P*, PtdIns4*P* and PtdIns(3,5)P_2 [59]. The 5-phosphatase domain showed no phosphatase activity towards PtdIns(3,5)P_2, while the Sac phosphatase domain could convert PtdIns(3,5)P_2 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-mating 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 PtdIns3*P*, PtdIns4*P* 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 PtdIns4*P* or PtdIns3*P* 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 PtdIns4*P* 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 PtdIns4*P* 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 PtdIns4*P*, suggesting that this pool of PtdIns4*P* 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, PtdIns4*P* and PtdIns(4,5)P_2 respectively, could be in a common compartment/pathway. However, Sac1p mutants with increased levels of PtdIns4*P* 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 PtdIns4*P* at a different cellular location, which could result in the observed cytoskeletal defects. These alternative interpretations suggest that the wild-type pool of PtdIns4*P* regulated by Sec14p/Sac1p is perhaps not phosphorylated

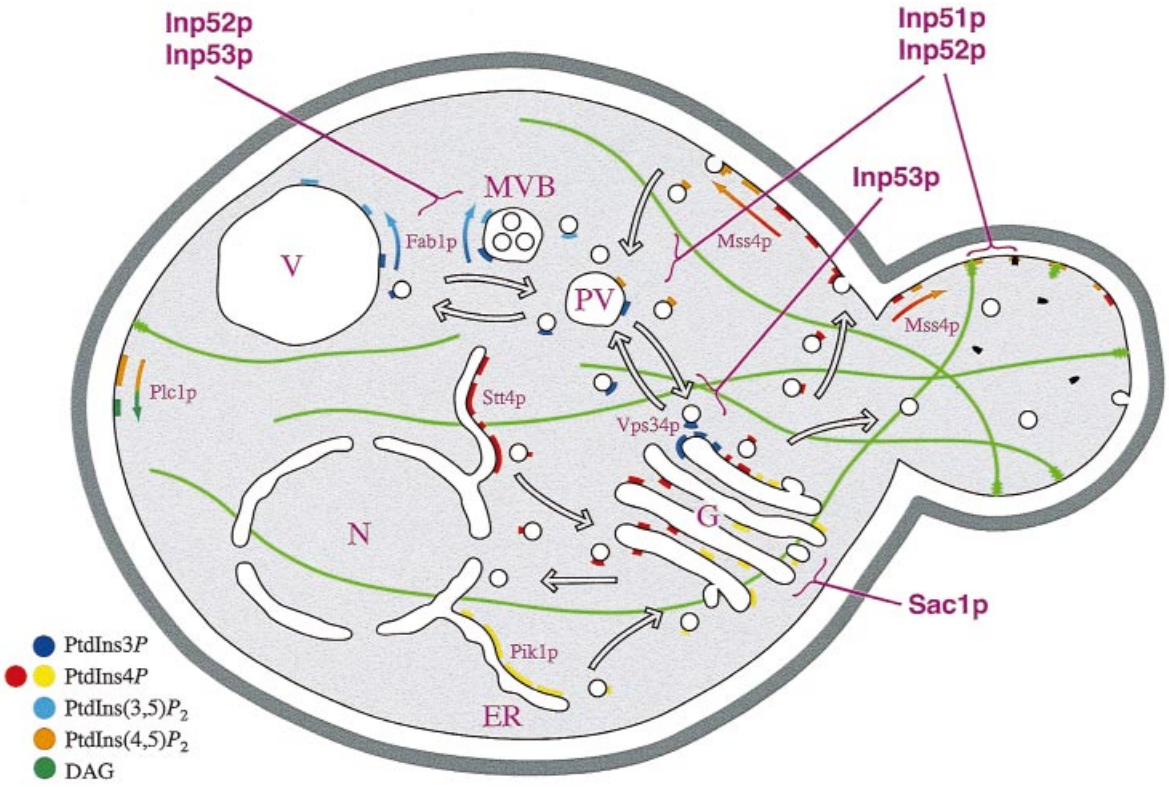

Figure 6 Potential roles for the *S. cerevisiae* phosphatidylinositol phosphate phosphatases

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 PtdIns4*P* is available for Mss4p to form PtdIns(4,5)P_2.

Stt4p, in contrast, clearly produces PtdIns4*P* that can be phosphorylated to form PtdIns(4,5)P_2. Overexpression of Mss4p suppresses the wortmannin-sensitivity of yeast (Stt4p is wortmannin-sensitive [27]), and Mss4p was initially identified as a multicopy suppressor of Stt4p defects [25]. However, the PtdIns4*P* 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 PtdIns4*P* has some specific function(s) yet to be identified. It is also unclear as to which of the other Sac-domain phosphatases could participate in the regulation of the Pik1p- or Stt4p-regulated pools of PtdIns4*P* 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)P_2 and may mediate its regulatory effects via the Stt4p-generated Ptdins4*P*.

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 PtdIns4*P* that is phosphorylated to form PtdIns(4,5)P_2 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)P_2 is involved in the regulation of the actin cytoskeleton. The actin-regulating protein profilin can bind either PtdIns(4,5)P_2 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)P_2 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)P_2 is also clearly involved in the regulation of vesicle trafficking in yeast. In mammalian systems a direct role for PtdIns(4,5)P_2 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)P_2 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)P_2 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)P_2 of the cytoskeleton could be a major regulatory factor in trafficking to, and from, the plasma membrane. PtdIns(4,5)P_2 has the potential to be metabolized by Plc1p, 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 endocytotic and cytoskeletal regulation, also function to dephosphorylate plasma membrane PtdIns(4,5)P_2 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)P_2 (see [11]). How this phosphatase could affect phosphatidylinositol phosphate metabolism has not yet been described.

PtdIns3*P* 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 PtdIns3*P* to form PtdIns(3,5)P_2 required for trafficking from the vacuole [41]. Sac-domain phosphatases can hydrolyse PtdIns3*P* 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 PtdIns3*P* 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)P_2 from the PtdIns3*P* 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 interrelationships, permits all forms to be produced independently? Perhaps not in a completely insulated 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.

We thank Rudiger Woscholski and members of the Protein Phosphorylation Laboratory for useful discussion, and Angela Hughes for help with the artwork.

REFERENCES

1 Berridge, M. J. and Irvine, R. F. (1989) Inositol phosphates and cell signalling. Nature (London) **341**, 197–205
2 Rameh, L. E. and Cantley, L. C. (1999) The role of phosphoinositide 3-kinase lipid products in cell function. J. Biol. Chem. **274**, 8347–8350
3 Toker, A. (1998) The synthesis and cellular roles of phosphatidylinositol 4,5-bisphosphate. Curr. Opin. Cell Biol. **10**, 254–261
4 Vanhaesebroeck, B. and Alessi, D. R. (2000) The PI3K–PDK1 connection: more than just a road to PKB. Biochem. J. **346**, 561–576
5 Liscovitch, M., Czarny, M., Fiucci, G. and Tang, X. (2000) Phospholipase D: molecular and cell biology of a novel gene family. Biochem. J. **345**, 401–415
6 De Camilli, P., Emr, S. D., McPherson, P. S. and Novick, P. (1996) Phosphoinositides as regulators in membrane traffic. Science **271**, 1533–1539

7 Corvera, S., D'Arrigo, A. and Stenmark, H. (1999) Phosphoinositides in membrane traffic. Curr. Opin. Cell Biol. **11**, 460–465
8 Martin, T. F. (1997) Phosphoinositides as spatial regulators of membrane traffic. Curr. Opin. Neurobiol. **7**, 331–338
9 Machesky, L. M. and Insall, R. H. (1999) Signaling to actin dynamics. J. Cell Biol. **146**, 267–272
10 Yin, H. L. and Stull, J. T. (1999) Proteins that regulate dynamic actin remodeling in response to membrane signaling. J. Biol. Chem. **274**, 32529–32530
11 Raucher, D., Stauffer, T., Chen, W., Shen, K., Guo, S., York, J. D., Sheetz, M. P. and Meyer, T. (2000) Phosphatidylinositol 4,5-bisphosphate functions as a second messenger that regulates cytoskeleton-plasma membrane adhesion. Cell **100**, 221–228
12 Hinchliffe, K. (2000) Intracellular signalling: Is PIP(2) a messenger too? Curr. Biol. **10**, R104–R105
13 Rebecchi, M. J. and Scarlata, S. (1998) Pleckstrin homology domains: a common fold with diverse functions. Annu. Rev. Biophys. Biomol. Struct. **27**, 503–528
14 Scaife, R. M. and Margolis, R. L. (1997) The role of the PH domain and SH3 binding domains in dynamin function. Cell. Signalling **9**, 395–401
15 Wurmser, A. E., Gary, J. D. and Emr, S. D. (1999) Phosphoinositide 3-kinases and their FYVE domain-containing effectors as regulators of vacuolar/lysosomal membrane trafficking pathways. J. Biol. Chem. **274**, 9129–9132
16 Stenmark, H. and Aasland, R. (1999) FYVE-finger proteins – effectors of an inositol lipid. J. Cell Sci. **112**, 4175–4183
17 Loijens, J. C. and Anderson, R. A. (1996) Type I phosphatidylinositol-4-phosphate 5-kinases are distinct members of this novel lipid kinase family. J. Biol. Chem. **271**, 32937–32943
18 Anderson, R. A., Boronenkov, I. V., Doughman, S. D., Kunz, J. and Loijens, J. C. (1999) Phosphatidylinositol phosphate kinases, a multifaceted family of signaling enzymes. J. Biol. Chem. **274**, 9907–9910
19 Paltauf, F., Kohlwein, S. D. and Henry, S. A. (1992) Regulation and compartmentalization of lipid synthesis in yeast. In The Molecular and Cellular Biology of the Yeast *Saccharomyces*, vol. II (Jones, E. W., Pringle, J. R. and Broach, J. R., eds), pp. 415–500, Cold Spring Harbor Laboratory Press, Cold Spring Harbor
20 Yoshida, S., Ohya, Y., Goebl, M., Nakano, A. and Anraku, Y. (1994) A novel gene, STT4, encodes a phosphatidylinositol 4-kinase in the PKC1 protein kinase pathway of Saccharomyces cerevisiae. J. Biol. Chem. **269**, 1166–1172
21 Flanagan, C. A., Schnieders, E. A., Emerick, A. W., Kunisawa, R., Admon, A. and Thorner, J. (1993) Phosphatidylinositol 4-kinase: gene structure and requirement for yeast cell viability. Science **262**, 1444–1448
22 Hama, H., Schnieders, E. A., Thorner, J., Takemoto, J. Y. and DeWald, D. B. (1999) Direct involvement of phosphatidylinositol 4-phosphate in secretion in the yeast Saccharomyces cerevisiae. J. Biol. Chem. **274**, 34294–34300
23 Walch-Solimena, C. and Novick, P. (1999) The yeast phosphatidylinositol-4-OH kinase Pik1 regulates secretion at the Golgi. Nat. Cell Biol. **1**, 523–525
24 Garcia-Bustos, J. F., Marini, F., Stevenson, I., Frei, C. and Hall, M. N. (1994) PIK1, an essential phosphatidylinositol 4-kinase associated with the yeast nucleus. EMBO J. **13**, 2352–2361
25 Yoshida, S., Ohya, Y., Nakano, A. and Anraku, Y. (1994) Genetic interactions among genes involved in the STT4-PKC1 pathway of Saccharomyces cerevisiae. Mol. Gen. Genet. **242**, 631–640
26 Trotter, P. J., Wu, W. I., Pedretti, J., Yates, R. and Voelker, D. R. (1998) A genetic screen for aminophospholipid transport mutants identifies the phosphatidylinositol 4-kinase, STT4p, as an essential component in phosphatidylserine metabolism. J. Biol. Chem. **273**, 13189–13196
27 Cutler, N. S., Heitman, J. and Cardenas, M. E. (1997) STT4 is an essential phosphatidylinositol 4-kinase that is a target of wortmannin in Saccharomyces cerevisiae. J. Biol. Chem. **272**, 27671–27677
28 Hughes, W. E., Woscholski, R., Cooke, F. T., Patrick, R. S., Dove, S. K., McDonald, N. Q. and Parker, P. J. (2000) SAC1 encodes a regulated lipid phosphoinositide phosphatase, defects in which can be suppressed by the homologous Inp52p and Inp53p phosphatases. J. Biol. Chem. **275**, 801–808
29 Desrivieres, S., Cooke, F. T., Parker, P. J. and Hall, M. N. (1998) MSS4, a phosphatidylinositol-4-phosphate 5-kinase required for organization of the actin cytoskeleton in Saccharomyces cerevisiae. J. Biol. Chem. **273**, 15787–15793
30 Homma, K., Terui, S., Minemura, M., Qadota, H., Anraku, Y., Kanaho, Y. and Ohya, Y. (1998) Phosphatidylinositol-4-phosphate 5-kinase localized on the plasma membrane is essential for yeast cell morphogenesis. J. Biol. Chem. **273**, 15779–15786
31 Rudge, S. A. and Engebrecht, J. (1999) Regulation and function of PLDs in yeast. Biochim. Biophys. Acta **1439**, 167–174
32 Mayer, A., Scheglmann, D., Dove, S., Glatz, A., Wickner, W. and Haas, A. (2000) Phosphatidylinositol 4,5-bisphosphate regulates two steps of homotypic vacuole fusion. Mol. Biol. Cell **11**, 807–817
33 Herman, P. K. and Emr, S. D. (1990) Characterization of VPS34, a gene required for vacuolar protein sorting and vacuole segregation in Saccharomyces cerevisiae. Mol. Cell. Biol. **10**, 6742–6754
34 Schu, P. V., Takegawa, K., Fry, M. J., Stack, J. H., Waterfield, M. D. and Emr, S. D. (1993) Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. Science **260**, 88–91
35 Stack, J. H. and Emr, S. D. (1994) Vps34p required for yeast vacuolar protein sorting is a multiple specificity kinase that exhibits both protein kinase and phosphatidylinositol-specific PI 3-kinase activities. J. Biol. Chem. **269**, 31552–31562
36 Driscoll, P. C. and Vuidepot, A. L. (1999) FYVE sticky fingers. Curr. Biol. **9**, R857–R860
37 Cooke, F. T., Dove, S. K., McEwen, R. K., Painter, G., Holmes, A. B., Hall, M. N., Michell, R. H. and Parker, P. J. (1998) The stress-activated phosphatidylinositol 3-phosphate 5-kinase Fab1p is essential for vacuole function in S. cerevisiae. Curr. Biol. **8**, 1219–1222
38 Gary, J. D., Wurmser, A. E., Bonangelino, C. J., Weisman, L. S. and Emr, S. D. (1998) Fab1p is essential for PtdIns(3)P 5-kinase activity and the maintenance of vacuolar size and membrane homeostasis. J. Cell Biol. **143**, 65–79
39 Yamamoto, A., DeWald, D. B., Boronenkov, I. V., Anderson, R. A., Emr, S. D. and Koshland, D. (1995) Novel PI(4)P 5-kinase homologue, Fab1p, essential for normal vacuole function and morphology in yeast. Mol. Biol. Cell **6**, 525–539
40 Dove, S. K., Cooke, F. T., Douglas, M. R., Sayers, L. G., Parker, P. J. and Michell, R. H. (1997) Osmotic stress activates phosphatidylinositol-3,5-bisphosphate synthesis. Nature (London) **390**, 187–192
41 Bryant, N. J., Piper, R. C., Weisman, L. S. and Stevens, T. H. (1998) Retrograde traffic out of the yeast vacuole to the TGN occurs via the prevacuolar/endosomal compartment. J. Cell Biol. **142**, 651–663
42 Odorizzi, G., Babst, M. and Emr, S. D. (1998) Fab1p PtdIns(3)P 5-kinase function essential for protein sorting in the multivesicular body. Cell **95**, 847–858
43 Woscholski, R. and Parker, P. J. (2000) Inositol phosphatases – constructive destruction of phosphoinositides and inositol phosphates. In Biology of Phosphoinositides, vol. 27 (Cockcroft, S., ed.), pp. 320–338, Oxford University Press, Oxford
44 Steck, P. A., Pershouse, M. A., Jasser, S. A., Yung, W. K., Lin, H., Ligon, A. H., Langford, L. A., Baumgard, M. L., Hattier, T., Davis, T. et al. (1997) Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet. **15**, 356–362
45 Maehama, T. and Dixon, J. E. (1999) PTEN: a tumour suppressor that functions as a phospholipid phosphatase. Trends Cell Biol. **9**, 125–128
46 Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S. I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R. et al. (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science **275**, 1943–1947
47 Essen, L. O., Perisic, O., Cheung, R., Katan, M. and Williams, R. L. (1996) Crystal structure of a mammalian phosphoinositide-specific phospholipase C delta. Nature (London) **380**, 595–602
48 Maehama, T. and Dixon, J. E. (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J. Biol. Chem. **273**, 13375–13378
49 Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P. O. and Herskowitz, I. (1998) The transcriptional program of sporulation in budding yeast. Science **282**, 699–705
50 Li, L., Ernsting, B. R., Wishart, M. J., Lohse, D. L. and Dixon, J. E. (1997) A family of putative tumor suppressors is structurally and functionally conserved in humans and yeast. J. Biol. Chem. **272**, 29403–29406
51 Pringle, J. R. and Hartwell, L. H. (1981) The Saccharomyces cerevisiae cell cycle. In The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance (Strathern, J. N., Jones, E. W. and Broach, J. R., eds.), pp. 97–142, Cold Spring Harbor Laboratory, Cold Spring Harbor
52 Shou, W., Seol, J. H., Shevchenko, A., Baskerville, C., Moazed, D., Chen, Z. W., Jang, J., Charbonneau, H. and Deshaies, R. J. (1999) Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. Cell **97**, 233–244
53 Visintin, R., Craig, K., Hwang, E. S., Prinz, S., Tyers, M. and Amon, A. (1998) The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. Mol. Cell **2**, 709–718
54 Norris, F. A., Atkins, R. C. and Majerus, P. W. (1997) The cDNA cloning and characterization of inositol polyphosphate 4-phosphatase type II. Evidence for conserved alternative splicing in the 4-phosphatase family. J. Biol. Chem. **272**, 23859–23864
55 Erneux, C., Govaerts, C., Communi, D. and Pesesse, X. (1998) The diversity and possible functions of the inositol polyphosphate 5-phosphatases. Biochim. Biophys. Acta. **1436**, 185–199

56 Mitchell, C. A., Brown, S., Campbell, J. K., Munday, A. D. and Speed, C. J. (1996) Regulation of second messengers by the inositol polyphosphate 5-phosphatases. Biochem. Soc. Trans. **24**, 994–1000

57 Woscholski, R. and Parker, P. J. (1997) Inositol lipid 5-phosphatases – traffic signals and signal traffic. Trends Biochem. Sci. **22**, 427–431

58 Chung, J. K., Sekiya, F., Kang, H. S., Lee, C., Han, J. S., Kim, S. R., Bae, Y. S., Morris, A. J. and Rhee, S. G. (1997) Synaptojanin inhibition of phospholipase D activity by hydrolysis of phosphatidylinositol 4,5-bisphosphate. J. Biol. Chem. **272**, 15980–15985

59 Guo, S., Stolz, L. E., Lemrow, S. M. and York, J. D. (1999) SAC1-like domains of yeast SAC1, INP52, and INP53 and of human synaptojanin encode polyphosphoinositide phosphatases. J. Biol. Chem. **274**, 12990–12995

60 Maehama, T., Taylor, G. S., Slama, J. T. and Dixon, J. E. (2000) A sensitive assay for phosphoinositide phosphatases. Anal. Biochem. **279**, 248–250

61 Fauman, E. B. and Saper, M. A. (1996) Structure and function of the protein tyrosine phosphatases. Trends Biochem. Sci. **21**, 413–417

62 Fauman, E. B., Yuvaniyama, C., Schubert, H. L., Stuckey, J. A. and Saper, M. A. (1996) The X-ray crystal structures of Yersinia tyrosine phosphatase with bound tungstate and nitrate. Mechanistic implications. J. Biol. Chem. **271**, 18780–18788

63 Fauman, E. B., Cogswell, J. P., Lovejoy, B., Rocque, W. J., Holmes, W., Montana, V. G., Piwnica-Worms, H., Rink, M. J. and Saper, M. A. (1998) Crystal structure of the catalytic domain of the human cell cycle control phosphatase, Cdc25A. Cell **93**, 617–625

64 Kearns, B. G., McGee, T. P., Mayinger, P., Gedvilaite, A., Philips, S. E., Kagiwada, S. and Bankaitis, V. A. (1997) Essential role for diacylglycerol in protein transport from the yeast Golgi complex. Nature (London) **387**, 101–104

65 Hughes, W. E., Pocklington, M. J., Orr, E. and Paddon, C. J. (1999) Mutations in the *Saccharomyces cerevisiae* gene *SAC1* cause multiple drug sensitivity. Yeast **15**, 1111–1124

66 Whitters, E. A., Cleves, A. E., McGee, T. P., Skinner, H. B. and Bankaitis, V. A. (1993) SAC1p is an integral membrane protein that influences the cellular requirement for phospholipid transfer protein function and inositol in yeast. J. Cell Biol. **122**, 79–94

67 Novick, P., Osmond, B. C. and Botstein, D. (1989) Suppressors of yeast actin mutations. Genetics **121**, 659–674

68 Novick, P. and Botstein, D. (1985) Phenotypic analysis of temperature-sensitive yeast actin mutants. Cell **40**, 405–416

69 Cleves, A. E., Novick, P. J. and Bankaitis, V. A. (1989) Mutations in the *SAC1* gene suppress defects in yeast Golgi and yeast actin functions. J. Cell Biol. **109**, 2939–2950

70 Bankaitis, V. A., Aitken, J. R., Cleves, A. E. and Dowhan, W. (1990) An essential role for a phospholipid transfer protein in yeast Golgi function. Nature (London) **347**, 561–562

71 Novick, P., Field, C. and Schekman, R. (1980) Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell **21**, 205–215

72 Rivas, M. P., Kearns, B. G., Xie, Z., Guo, S., Sekar, M. C., Hosaka, K., Kagiwada, S., York, J. D. and Bankaitis, V. A. (1999) Pleiotropic alterations in lipid metabolism in yeast sac1 mutants: relationship to 'Bypass Sec14p' and inositol auxotrophy. Mol. Biol. Cell **10**, 2235–2250

73 Mayinger, P., Bankaitis, V. A. and Meyer, D. I. (1995) Sac1p mediates the adenosine triphosphate transport into yeast endoplasmic reticulum that is required for protein translocation. J. Cell Biol. **131**, 1377–1386

74 Kochendörfer, K. U., Then, A. R., Kearns, B. G., Bankaitis, V. A. and Mayinger, P. (1999) Sac1p plays a crucial role in microsomal ATP transport, which is distinct from its function in Golgi phospholipid metabolism. EMBO J. **18**, 1506–1515

75 Boyum, R. and Guidotti, G. (1997) Sac1p of Saccharomyces cerevisiae is not involved in ATP release to the extracellular fluid. Biochem. Biophys. Res. Commun. **236**, 50–53

76 McPherson, P. S., Garcia, E. P., Slepnev, V. I., David, C., Zhang, X., Grabs, D., Sossin, W. S., Bauerfeind, R., Nemoto, Y. and De Camilli, P. (1996) A presynaptic inositol-5-phosphatase. Nature (London) **379**, 353–357

77 Cleves, A. E., McGee, T. P., Whitters, E. A., Champion, K. M., Aitken, J. R., Dowhan, W., Goebl, M. and Bankaitis, V. A. (1991) Mutations in the CDP-choline pathway for phospholipid biosynthesis bypass the requirement for an essential phospholipid transfer protein. Cell **64**, 789–800

78 Xie, Z., Fang, M., Rivas, M. P., Faulkner, A. J., Sternweis, P. C., Engebrecht, J. A. and Bankaitis, V. A. (1998) Phospholipase D activity is required for suppression of yeast phosphatidylinositol transfer protein defects. Proc. Natl. Acad. Sci. U.S.A. **95**, 12346–12351

79 Fang, M., Kearns, B. G., Gedvilaite, A., Kagiwada, S., Kearns, M., Fung, M. K. and Bankaitis, V. A. (1996) Kes1p shares homology with human oxysterol binding protein and participates in a novel regulatory pathway for yeast Golgi-derived transport vesicle biogenesis. EMBO J. **15**, 6447–6459

80 Stock, S. D., Hama, H., DeWald, D. B. and Takemoto, J. Y. (1999) SEC14-dependent secretion in Saccharomyces cerevisiae. Nondependence on sphingolipid synthesis-coupled diacylglycerol production. J. Biol. Chem. **274**, 12979–12983

81 Phillips, S. E., Sha, B., Topalof, L., Xie, Z., Alb, J. G., Klenchin, V. A., Swigart, P., Cockcroft, S., Martin, T. F., Luo, M. and Bankaitis, V. A. (1999) Yeast Sec14p deficient in phosphatidylinositol transfer activity is functional in vivo. Mol. Cell **4**, 187–197

82 Skinner, H. B., McGee, T. P., McMaster, C. R., Fry, M. R., Bell, R. M. and Bankaitis, V. A. (1995) The Saccharomyces cerevisiae phosphatidylinositol-transfer protein effects a ligand-dependent inhibition of choline-phosphate cytidylyltransferase activity. Proc. Natl. Acad. Sci. U.S.A. **92**, 112–116

83 TerBush, D. R., Maurice, T., Roth, D. and Novick, P. (1996) The Exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. EMBO J. **15**, 6483–6494

84 Hsu, S. C., Hazuka, C. D., Foletti, D. L. and Scheller, R. H. (1999) Targeting vesicles to specific sites on the plasma membrane: the role of the sec6/8 complex. Trends Cell Biol. **9**, 150–153

85 Rothman, J. E. and Warren, G. (1994) Implications of the SNARE hypothesis for intracellular membrane topology and dynamics. Curr. Biol. **4**, 220–233

86 Brenwald, P., Kearns, B., Champion, K., Keränen, S., Bankaitis, V. A. and Novick, P. J. (1994) Sec9 is a SNAP-25-like component of a yeast SNARE complex that may be the effector of Sec4 function in exocytosis. Cell **79**, 245–258

87 Lewis, M. J., Rayner, J. C. and Pelham, H. R. (1997) A novel SNARE complex implicated in vesicle fusion with the endoplasmic reticulum. EMBO J. **16**, 3017–3024

88 Cosson, P., Schroder-Kohne, S., Sweet, D. S., Demolliere, C., Hennecke, S., Frigerio, G. and Letourneur, F. (1997) The Sec20/Tip20p complex is involved in ER retrieval of dilysine-tagged proteins. Eur. J. Cell Biol. **73**, 93–97

89 Salama, N. R., Yeung, T. and Schekman, R. W. (1993) The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins. EMBO J. **12**, 4073–4082

90 Barlowe, C., Orci, L., Yeung, T., Hosobuchi, M., Hamamoto, S., Salama, N., Rexach, M. F., Ravazzola, M., Amherdt, M. and Schekman, R. (1994) COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. Cell **77**, 895–907

91 Matsuoka, K., Orci, L., Amherdt, M., Bednarek, S. Y., Hamamoto, S., Schekman, R. and Yeung, T. (1998) COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes. Cell **93**, 263–275

92 Haas, A. (1998) NSF – fusion and beyond. Trends Cell Biol. **8**, 471–473

93 Rothman, J. E. (1994) Mechanisms of intracellular protein transport. Nature (London) **372**, 55–63

94 Rossi, G., Salminen, A., Rice, L. M., Brunger, A. T. and Brennwald, P. (1997) Analysis of a yeast SNARE complex reveals remarkable similarity to the neuronal SNARE complex and a novel function for the C terminus of the SNAP-25 homolog, Sec9. J. Biol. Chem. **272**, 16610–16617

95 Hosobuchi, M., Kreis, T. and Schekman, R. (1992) SEC21 is a gene required for ER to Golgi protein transport that encodes a subunit of a yeast coatomer. Nature (London) **360**, 603–605

96 Walker, M. W., Bobak, D. A., Tsai, S. C., Moss, J. and Vaughan, M. (1992) GTP but not GDP analogues promote association of ADP-ribosylation factors, 20-kDa protein activators of cholera toxin, with phospholipids and PC-12 cell membranes. J. Biol. Chem. **267**, 3230–3235

97 Terui, T., Kahn, R. A. and Randazzo, P. A. (1994) Effects of acid phospholipids on nucleotide exchange properties of ADP-ribosylation factor 1. Evidence for specific interaction with phosphatidylinositol 4,5-bisphosphate. J. Biol. Chem. **269**, 28130–28135

98 Svetlov, V. V. and Cooper, T. G. (1995) Review: compilation and characteristics of dedicated transcription factors in Saccharomyces cerevisiae. Yeast **11**, 1439–1484

99 McLean, M., Hubberstey, A. V., Bouman, D. J., Pece, N., Mastrangelo, P. and Wildeman, A. G. (1995) Organization of the Saccharomyces cerevisiae actin gene UAS: functional significance of reiterated REB1 binding sites and AT-rich elements. Mol. Microbiol. **18**, 605–614

100 Moon, A. L., Janmey, P. A., Louie, K. A. and Drubin, D. G. (1993) Cofilin is an essential component of the yeast cortical cytoskeleton. J. Cell Biol. **120**, 421–435

101 Iida, K., Moriyama, K., Matsumoto, S., Kawasaki, H., Nishida, E. and Yahara, I. (1993) Isolation of a yeast essential gene, COF1, that encodes a homologue of mammalian cofilin, a low-M(r) actin-binding and depolymerizing protein. Gene **124**, 115–120

102 Haarer, B. K., Lillie, S. H., Adams, A. E., Magdolen, V., Bandlow, W. and Brown, S. S. (1990) Purification of profilin from Saccharomyces cerevisiae and analysis of profilin-deficient cells. J. Cell Biol. **110**, 105–114

103 Muhua, L., Adames, N. R., Murphy, M. D., Shields, C. R. and Cooper, J. A. (1998) A cytokinesis checkpoint requiring the yeast homologue of an APC-binding protein. Nature (London) **393**, 487–491

104 Mayinger, P. and Meyer, D. I. (1993) An ATP transporter is required for protein translocation into the yeast endoplasmic reticulum. EMBO J. **12**, 659–666
105 Lyman, S. K. and Schekman, R. (1997) Binding of secretory precursor polypeptides to a translocon subcomplex is regulated by BiP. Cell **88**, 85–96
106 Pilon, M. and Schekman, R. (1999) Protein translocation: how Hsp70 pulls it off. Cell **97**, 679–682
107 Semenza, J. C., Hardwick, K. G., Dean, N. and Pelham, H. R. (1990) ERD2, a yeast gene required for the receptor-mediated retrieval of luminal ER proteins from the secretory pathway. Cell **61**, 1349–1357
108 Erdman, S., Lin, L., Malczynski, M. and Snyder, M. (1998) Pheromone-regulated genes required for yeast mating differentiation. J. Cell Biol. **140**, 461–483
109 Stolz, L. E., Huynh, C. V., Thorner, J. and York, J. D. (1998) Identification and characterisation of an essential family of inositol polyphosphate 5-phosphatases (*INP51*, *INP52* and *INP53* gene products) in the yeast *Saccharomyces cerevisiae*. Genetics **148**, 1715–1729
110 Luo, W. and Chang, A. (1997) Novel genes involved in endosomal traffic in yeast revealed by suppression of a targeting-defective plasma membrane ATPase mutant. J. Cell Biol. **138**, 731–746
111 Bassett, Jr, D. E., Boguski, M. S. and Hieter, P. (1996) Yeast genes and human disease. Nature (London) **379**, 589–590
112 Srinivasan, S., Seaman, M., Nemoto, Y., Daniell, L., Suchy, S. F., Emr, S., DeCamilli, P. and Nussbaum, R. (1997) Disruption of three phosphatidylinositol polyphosphate 5-phosphatase genes from *S. cerevisiae* results in pleiotropic abnormalities of vacuole morphology, cell shape and osmohomeostasis. Eur. J. Cell Biol. **74**, 350–360
113 Stolz, L. E., Kuo, W. J., Longchamps, J., Sekhon, M. K. and York, J. D. (1998) *INP51*, a yeast inositol polyphosphate 5-phosphatase required for phosphatidylinositol-4,5-bisphosphate homeostasis and whose absence confers a cold-resistant phenotype. J. Biol. Chem. **273**, 11852–11861
114 Bensen, E. S., Costaguta, G. and Payne, G. S. (2000) Synthetic genetic interactions with temperature-sensitive clathrin in Saccharomyces cerevisiae. Roles for synaptojanin-like Inp53p and dynamin-related Vps1p in clathrin-dependent protein sorting at the trans-Golgi network. Genetics **154**, 83–97
115 Singer-Kruger, B., Nemoto, Y., Daniell, L., Ferro-Novick, S. and De Camilli, P. (1998) Synaptojanin family members are implicated in endocytic membrane traffic in yeast. J. Cell Sci. **111**, 3347–3356
116 Adams, A. E. M., Botstein, D. and Drubin, D. G. (1989) A yeast actin-binding protein is encoded by *SAC6*, a gene found by suppression of an actin mutation. Science **243**, 231–233
117 Munn, A. L., Stevenson, B. J., Geli, M. I. and Riezman, H. (1995) end5, end6, and end7: mutations that cause actin delocalization and block the internalization step of endocytosis in Saccharomyces cerevisiae. Mol. Biol. Cell **6**, 1721–1742
118 Wendland, B. and Emr, S. D. (1998) Pan1p, yeast eps15, functions as a multivalent adaptor that coordinates protein-protein interactions essential for endocytosis. J. Cell Biol. **141**, 71–84
119 Salcini, A. E., Chen, H., Iannolo, G., De Camilli, P. and Di Fiore, P. P. (1999) Epidermal growth factor pathway substrate 15, Eps15. Int. J. Biochem. Cell Biol. **31**, 805–809
120 Lemmon, M. A., Ferguson, K. M., O'Brien, R., Sigler, P. B. and Schlessinger, J. (1995) Specific and high-affinity binding of inositol phosphates to an isolated pleckstrin homology domain. Proc. Natl. Acad. Sci. U.S.A. **92**, 10472–10476
121 Lee, J. O., Yang, H., Georgescu, M. M., Di Cristofano, A., Maehama, T., Shi, Y., Dixon, J. E., Pandolfi, P. and Pavletich, N. P. (1999) Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. Cell **99**, 323–334
122 Schmidt, A., Hall, M. N. and Koller, A. (1994) Two FK506 resistance-conferring genes in Saccharomyces cerevisiae, TAT1 and TAT2, encode amino acid permeases mediating tyrosine and tryptophan uptake. Mol. Cell. Biol. **14**, 6597–6606
123 Chen, X. H., Xiao, Z. and Fitzgerald-Hayes, M. (1994) SCM2, a tryptophan permease in Saccharomyces cerevisiae, is important for cell growth. Mol. Gen. Genet. **244**, 260–268
124 Heitman, J., Movva, N. R. and Hall, M. N. (1991) Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science **253**, 905–909
125 Jiang, Y. and Broach, J. R. (1999) Tor proteins and protein phosphatase 2A reciprocally regulate Tap42 in controlling cell growth in yeast. EMBO J. **18**, 2782–2792
126 Di Como, C. J. and Arndt, K. T. (1996) Nutrients, via the Tor proteins, stimulate the association of Tap42 with type 2A phosphatases. Genes Dev. **10**, 1904–1916
127 Schmidt, A., Beck, T., Koller, A., Kunz, J. and Hall, M. N. (1998) The TOR nutrient signalling pathway phosphorylates NPR1 and inhibits turnover of the tryptophan permease. EMBO J. **17**, 6924–6931
128 Schmidt, A., Bickle, M., Beck, T. and Hall, M. N. (1997) The yeast phosphatidylinositol kinase homolog TOR2 activates RHO1 and RHO2 via the exchange factor ROM2. Cell **88**, 531–542
129 Helliwell, S. B., Howald, I., Barbet, N. and Hall, M. N. (1998) TOR2 is part of two related signaling pathways coordinating cell growth in Saccharomyces cerevisiae. Genetics **148**, 99–112
130 Alarcon, C. M., Cardenas, M. E. and Heitman, J. (1996) Mammalian RAFT1 kinase domain provides rapamycin-sensitive TOR function in yeast. Genes Dev. **10**, 279–288
131 Beck, T., Schmidt, A. and Hall, M. N. (1999) Starvation induces vacuolar targeting and degradation of the tryptophan permease in yeast. J. Cell Biol. **146**, 1227–1238
132 Zhang, F. L. and Casey, P. J. (1996) Protein prenylation: molecular mechanisms and functional consequences. Annu. Rev. Biochem. **65**, 241–269
133 Thompson, D. A. and Stahl, F. W. (1999) Genetic control of recombination partner preference in yeast meiosis. Isolation and characterization of mutants elevated for meiotic unequal sister-chromatid recombination. Genetics **153**, 621–641
134 Bishop, D. K., Park, D., Xu, L. and Kleckner, N. (1992) DMC1: a meiosis-specific yeast homolog of E. coli recA required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69**, 439–456
135 Altman, R. and Kellogg, D. (1997) Control of mitotic events by Nap1 and the Gin4 kinase. J. Cell Biol. **138**, 119–130
136 Longtine, M. S., Fares, H. and Pringle, J. R. (1998) Role of the yeast Gin4p protein kinase in septin assembly and the relationship between septin assembly and septin function. J. Cell Biol. **143**, 719–736
137 Tan, P. K., Davis, N. G., Sprague, G. F. and Payne, G. S. (1993) Clathrin facilitates the internalization of seven transmembrane segment receptors for mating pheromones in yeast. J. Cell Biol. **123**, 1707–1716
138 Redding, K., Seeger, M., Payne, G. S. and Fuller, R. S. (1996) The effects of clathrin inactivation on localization of Kex2 protease are independent of the TGN localization signal in the cytosolic tail of Kex2p. Mol. Biol. Cell **7**, 1667–1677
139 Hendricks, K. B., Wang, B. Q., Schnieders, E. A. and Thorner, J. (1999) Yeast homologue of neuronal frequenin is a regulator of phosphatidylinositol-4-OH kinase. Nat. Cell Biol. **1**, 234–241
140 Amberg, D. C., Basart, E. and Botstein, D. (1995) Defining protein interactions with yeast actin in vivo. Nat. Struct. Biol. **2**, 28–35
141 Ostrander, D. B., Gorman, J. A. and Carman, G. M. (1995) Regulation of profilin localization in Saccharomyces cerevisiae by phosphoinositide metabolism. J. Biol. Chem. **270**, 27045–27050
142 Mulholland, J., Wesp, A., Riezman, H. and Botstein, D. (1997) Yeast actin cytoskeleton mutants accumulate a new class of Golgi-derived secretory vesicle. Mol. Biol. Cell **8**, 1481–1499
143 Kubler, E. and Riezman, H. (1993) Actin and fimbrin are required for the internalization step of endocytosis in yeast. EMBO J. **12**, 2855–2862
144 Tall, G. G., Hama, H., DeWald, D. B. and Horazdovsky, B. F. (1999) The phosphatidylinositol 3-phosphate binding protein Vac1p interacts with a Rab GTPase and a Sec1p homologue to facilitate vesicle-mediated vacuolar protein sorting. Mol. Biol. Cell **10**, 1873–1889
145 Wurmser, A. E. and Emr, S. D. (1998) Phosphoinositide signaling and turnover: PtdIns(3)P, a regulator of membrane traffic, is transported to the vacuole and degraded by a process that requires lumenal vacuolar hydrolase activities. EMBO J. **17**, 4930–4942
146 Zhang, X., Hartz, P. A., Philip, E., Racusen, L. C. and Majerus, P. W. (1998) Cell lines from kidney proximal tubules of a patient with Lowe syndrome lack OCRL inositol polyphosphate 5-phosphatase and accumulate phosphatidylinositol 4,5-bisphosphate. J. Biol. Chem. **273**, 1574–1582
147 Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**, 4673–4680
148 Schneiter, R., Brugger, B., Sandhoff, R., Zellnig, G., Leber, A., Lampl, M., Athenstaedt, K., Hrastnik, C., Eder, S., Daum, G. et al. (1999) Electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis of the lipid molecular species composition of yeast subcellular membranes reveals acyl chain-based sorting/remodeling of distinct molecular species en route to the plasma membrane. J. Cell Biol. **146**, 741–754
149 Piper, R. C., Cooper, A. A., Yang, H. and Stevens, T. H. (1995) VPS27 controls vacuolar and endocytic traffic through a prevacuolar compartment in Saccharomyces cerevisiae. J. Cell Biol. **131**, 603–617
150 Kaiser, C. A., Gimeno R. E. and Shaywitz, D. A. (1997) Protein secretion, membrane biogenesis, and endocytosis. In The Molecular and Cellular Biology of the Yeast Saccharomyces, vol. 3 (Pringle, J. R., Broach, J. R. and Jones, E. W., eds), pp. 91–228, Cold Spring Harbor Laboratory Press, Cold Spring Harbor
151 Botstein, D., Amberg, D., Mulholland, J., Huffaker, T., Adams, A., Drubin, D. and Stearns, T. (1997) The yeast cytoskeleton. In The Molecular and Cellular Biology of the Yeast Saccharomyces, vol. 3 (Pringle, J. R., Broach, J. R. and Jones, E. W., eds), pp. 1–90, Cold Spring Harbor Laboratory Press, Cold Spring Harbor