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Phosphoinositide Phosphatases in Cell Biology and Disease

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Abstract

Phosphoinositides are essential signaling molecules linked to a diverse array of cellular processes in eukaryotic cells. The metabolic interconversions of these phospholipids are subject to exquisite spatial and temporal regulation executed by arrays of phosphatidylinositol (PtdIns) and phosphoinositide-metabolizing enzymes. These include PtdIns- and phosphoinositide-kinases that drive phosphoinositide synthesis, and phospholipases and phosphatases that regulate phosphoinositide degradation. In the past decade, phosphoinositide phosphatases have emerged as topics of particular interest. This interest is driven by the recent appreciation that these enzymes represent primary mechanisms for phosphoinositide degradation, and because of their ever-increasing connections with human diseases. Herein, we review the biochemical properties of six major phosphoinositide phosphatases, the functional involvements of these enzymes in regulating phosphoinositide metabolism, the pathologies that arise from functional derangements of individual phosphatases, and recent ideas concerning the involvements of phosphoinositide phosphatases in membrane traffic control.

Introduction

Phosphoinositides are phosphorylated derivatives of PtdIns (Fig. 1), and these lipid species represent quantitatively minor components of cell membranes. In eukaryotic cells, PtdIns generally constitutes less than 10% of the total cellular phospholipid with Phosphoinositides usually comprise only several percent of total cellular inositol lipids (Rameh *et al.*, 1997;Fruman *et al.*, 1998;Martin, 1998;Di Paolo and De Camilli, 2006). However, in spite of their low abundance, phosphoinositides regulate a host of fundamental cellular processes. These include signal transduction, intracellular membrane trafficking, cytoskeleton remodeling, nuclear events, control of cell growth and survival, etc. This functional diversity of function in part reflects the molecular diversity of these compounds. Mammalian cells produce seven chemically distinct, but interconvertible, phosphoinositide species: phosphatidylinositol 3-phosphate (PtdIns-3-P), PtdIns-4-P, PtdIns-5-P, phosphatidylinositol 3,5-bisphosphate (PtdIns-3,5-P_2), PtdIns-4,5-P_2, PtdIns-3,4-P_2, and phosphatidylinositol-3,4,5-trisphosphate (PtdIns-3,4,5-P_3) (Fig. 1). Phosphoinositides phosphorylated at the 3-OH position are not substrates for phospholipases C, so these phosphoinositides hold intrinsic signaling functions whose execution is not mediated through the action of derivative second messengers. It is through the action of phosphatases that 3-OH phosphoinositides are degraded.

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Yeast produce five phosphoinositide species (Fig. 2), and lack the capacity to generate the PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 species produced by mammals and other higher eukaryotes (Fig. 3). The 3-OH and 4-OH PtdIns-monophosphate species represent the major phosphoinositides in yeast, each constituting ca 1.5% of total inositol glycerophospholipid in this organism (ca. 0.3% of total glycerophospholipid). PtdIns-4,5-P_2 is present at approximately half the mass of PtdIns-3-P or PtdIns-4-P. Basal PtdIns-3,5-P_2 levels are vanishingly low, essentially at the level of detection, until yeast are subject to stress – particularly hyperosmotic stress (Dove *et al.*, 1997). Upon such challenge, PtdIns-3,5-P_2 levels rise rapidly. The 4-OH phosphoinositides are all essential in yeast as evidenced by demonstrations that functional ablation of either one of the two major PtdIns 4-OH kinases (Pik1 and Stt4), or of the single PtdIns-4-P 5-OH kinase (Mss4), represent lethal events. Although 3-OH phosphoinositides play important homeostatic functions in yeast, these are essential for cell viability only under stress conditions (Schu *et al.*, 1993;Fruman *et al.*, 1998;Martin, 1998).

Of the total inositol lipid content in mammalian cells, approximately 5% is invested in PtdIns-4-P and PtdIns-4,5-P_2, respectively – i.e. 0.5% of total cellular phospholipid in each case (Rameh and Cantley, 1999). These two 4-OH phosphorylated phosphoinositides represent by far the major phosphoinositide species in mammalian cells, constituting 90% of total cellular phosphoinositides (Roth, 2004; Di Paolo and De Camilli, 2006). By comparison, less than 0.25% of the total inositol phospholipid is phosphorylated on the D-3 position -- PtdIns-3-P represents ονλν σομε 0.04% of total membrane phospholipid (Fruman *et al.*, 1998; Martin, 1998; Whisstock *et al.*, 2002; Roth, 2004; Waselle *et al.*, 2005; Di Paolo and De Camilli, 2006).

The chemically distinct phosphoinositide species each execute unique functions in cells, and the mono-phosphorylated phosphoinositides are not simple intermediates in production of the higher poly-phosphorylated species. Translation of chemical diversity to functional diversity is in part determined by preferred interface of individual phosphoinositide species with regulatory effector proteins that harbor phosphoinositide - binding domains. Examples include the pleckstrin homology (PH) domains, phox homology (PX) domains, epsin N-terminal homology (ENTH) domains, band 4.1/ezrin/radixin/moesin (FERM) and Fab1p/YOTB/Vac1p/EEA1 (FYVE) domains and lysine–arginine patches (Di Paolo and De Camilli, 2006; Lemmon, 2008).

All phosphoinositides are restricted to the cytosolic leaflets of intracellular membranes, and these are not homogeneously distributed in the membranes that contain them (Roth, 2004). Moreover, the representation of individual phosphoinositide species varies between subcellular compartments and contributes to establishment and/or maintenance of organelle identity. For example, PtdIns-3-P is enriched on endocytic membranes, PtdIns-4-P on trans-Golgi (TGN) membranes, and PtdIns-4,5-P_2 is localized primarily on the plasma membrane -- although Golgi pools are detected. PtdIns-3,5-P_2 is most abundant in multivesicular bodies (MVBs) and late endosomes, on the yeast vacuole, and on mammalian lysosomes (Levine and Munro, 2002; De Matteis and Godi, 2004a; De Matteis and Godi, 2004b; Di Paolo and De Camilli, 2006). PtdIns-3,4,5-P_3 is produced almost exclusively on the inner leaflet of the plasma membrane, although this most highly modified phosphoinositide might also accumulate on membranes of intracellular organelles and in the nuclear matrix following growth factor receptor activation (Ellson *et al.*, 2001; Shisheva, 2001; Lee *et al.*, 2002; van Rheenen and Jalink, 2002; Watt *et al.*, 2002; Waselle *et al.*, 2005).

Steady-state phosphoinositide distribution is the manifestation of a highly dynamic program of production and turnover executed by the enzymes that regulate phosphoinositide synthesis, interconversion, and degradation. Under basal conditions, phosphoinositide metabolism is

tightly regulated by a set of specific kinases that are responsible for synthesis of phosphoinositides, and phosphatases that temporally and spatially catalyze phosphoinositide dephosphorylation. The yeast and mammalian phosphoinositide metabolic schemes are detailed in Fig. 2 and Fig. 3, respectively. In addition, under stimulated conditions, phospholipases C hydrolyze individual phosphoinositide species (excepting those phosphorylated on the D3 position) with the consequence of that a variety of soluble inositol phosphates are produced – each of which apparently manifest potent second messenger activities (Singer *et al.*, 1997;Liscovitch *et al.*, 2000;Rebecchi and Pentyala, 2000;Strahl and Thorner, 2007;Majerus *et al.*, 1999).

The anabolic arm of phosphoinositide metabolism is governed by lipid kinases that transfer the γ-phosphate of ATP to the 3-OH, 4-OH or 5-OH position of the inositol ring. PtdIns kinases are conserved enzymes found throughout the Eukaryota, and these enzymes are classified into three major subgroups based on their positional specificities: PtdIns 3-OH kinases ($PI3K_s$), PtdIns 4-OH kinases (PI4Ks), and phosphoinositide-kinases (PIPKs) (Loijens *et al.*, 1996; Zvelebil *et al.*, 1996; Domin and Waterfield, 1997). The PI3Ks constitute a large family of enzymes responsible for production of PtdIns-3-P, PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 (Fig. 2 and fig 3). PI4Ks and PtdIns 5-OH kinase (PI5K) convert PtdIns to PtdIns-4-P or PtdIns-5-P, respectively. We do note, however that while PI5K activity has been demonstrated in vitro, there is no available evidence to indicate that PtdIns-5-P is generated from PtdIns by such a reaction in vivo. Some mammalian enzymes also catalyze the higher order phosphorylation of PtdIns-5-P or PtdIns-4-P to PtdIns-4,5-P_2 (Fig. 3). Unlike most of the cognate mammalian PtdIns- and phosphoinositide-kinases, the yeast enzymes exhibit exquisite substrate specificities -- each kinase only phosphorylates a single inositol-phospholipid substrate. Yeast express one PI3K (Vps34), three PI4Ks (Lsb6, Pik1, and Stt4), one PtdIns-4-P 5-OH kinase (Mss4), and one PtdIns-3-P 5-OH kinase (Fab1) (Fig. 2) (Flanagan *et al.*, 1993; Schu *et al.*, 1993; Yoshida *et al.*, 1994a; Yoshida *et al.*, 1994b; Yamamoto *et al.*, 1995; Madania *et al.*, 1999; Han *et al.*, 2002; Strahl and Thorner, 2007). Of these enzymes, all but one executes unique biological functions linked to their respective lipid kinase activities. Lsb6 remains functionally enigmatic.

Phosphoinositide phosphatases: classification and catalytic mechanisms

Phospholipase-independent mechanisms of phosphoinositide degradation are executed by dephosphorylation of phosphoinositides at the D-3, D-4 and/or D-5 positions of the inositol ring (Fig. 1), and the responsible phosphoinositide phosphatases are highly conserved throughout the eukaryotic kingdom. Unlike the case of PtdIns- and phosphoinositide-kinases, some yeast phosphoinositide phosphatases are promiscuous enzymes with regard to substrate specificity -- individual enzymes often dephosphorylate multiple phosphoinositide species (Strahl and Thorner, 2007). The seven known yeast phosphoinositide phosphatases are classified into three different subgroups on the basis of their catalytic domain properties. These include the SAC-domain phosphatases, the inositol polyphosphate 5-phosphatase domain enzymes, and the myotubularin ortholog Ymr1 (Fig. 4;Strahl and Thorner, 2007). Mammalian phosphatases are classified into two superfamilies: the protein tyrosine phosphatase superfamily, and the inositide polyphosphate phosphatase superfamily.

The phosphoinositide phosphatases of the PTP-superfamily fall into four primary classes. The phosphoinositide 4-phosphatases fall into types I and II that share 37% primary sequence identity, and primarily degrade PtdIns-3,4-P_2 to PtdIns-3-P (Norris et al., 1997; Nystuen *et al.*, 2001). The *weeble* ataxia mouse is deficient in the type 1 enzyme (Nystuen *et al.*, 2001). PTEN, the famous PtdIns-3,4,5-P_3 3-phosphatase, also belongs to the PTP-superfamily. The Sac1-like phosphoinositide phosphatases fall into two categories – the stand-alone SAC-domain enzymes that show relaxed positional specificity with regard to phosphoinositide

substrate, and the dual functional enzymes that exhibit tandem arrangements of SAC-domains appended to phosphoinositide 5-phosphatase domains (e.g. the synaptojanins). Finally, the myotubularins constitute an intriguing class of phosphoinositide 3-phosphatases.

Phosphoinositide phosphatases of the PTP superfamily are metal-independent enzymes that exhibit a signature Cys-X_5-Arg-Thr/Ser (CX_5RT/S) active site motif which physically cradles the various chemical steps of the reaction mechanism (Fig. 4A). First, the PO_3 moiety from the phospho-inositol headgroup is accepted by the nucleophilic cysteine residue within the CX_5RT/S motif to generate a phosphocysteine intermediate. The invariant arginine of the CX_5RT/S motif stabilizes the transition-state so that the PO_3 moiety is subsequently transferred to a water molecule. Finally, a conserved aspartic acid from an adjacent structural loop completes the catalytic cycle by protonating the leaving-group oxygen and reconstituting an uncharged hydroxy group at the position from which the phosphate moiety was originally removed (Guan and Dixon, 1991;Fauman and Saper, 1996;Hughes *et al.*, 2000a).

The phosphoinositide 5-phosphatases fall into four categories. The group I enzymes act only on soluble inositol polyphosphates (Ins-1,4,5-P_3 and Ins-1,3,4,5-P_4), the group II enzymes (e.g. OCRL1; discussed at length below) utilize both phosphoinositide RIPT and soluble inositol phosphate substrates, and the group III enzymes (e.g. SHIP1 and SHIP2; see below) utilize phosphoinositides as preferred substrates. Finally, the group IV enzymes act exclusively on PtdIns-3,4,5-P_3 and PtdIns-4,5-P_2.

The inositol polyphosphate 5-phosphatase exhibit structural folds similar to those of Mg^{2+}-dependent endonucleases, and all of these enzymes exhibit magnesium-dependent phosphomonoesterase activities (Mitchell *et al.*, 1996; Blero *et al.*, 2007; Majerus and York, 2009). Bioinformatic approaches, coupled with mutagenesis studies, further indicate the inositol polyphosphate 5-phosphatases share the same principle catalytic mechanism as the apurinic/apyrimidinic base excision repair endonucleases (Dlakić, 2000; Whisstock et al., 2000). The model catalytic mechanism employs a nucleophilic water molecule that is positioned and activated by an invariant Asp residue – a residue that is itself properly oriented by H-bonding to the side-chain of a conserved Asn (Fig. 4B). The target 5'-phosphate group of the inositol headgroup is configured for the nucleophilic attack by an invariant His/Asp pair and an invariant Asn. A metal ion (Mg^{2+}) stabilizes the transition state of the inversion of configuration of the phosphate with the result that the PO_3 moiety is transfered to water and the dephosphorylated inositol phospholipid product is released (Fig. 4B). It is not clear whether the proton donor that stabilizes the inositol leaving group is a water molecule or some other functional group of the enzyme (Whisstock et al., 2000).

Phosphatases of both the double-displacement class (i.e. members of the PTP superfamily that displace the inositol leaving group via an active-site nucleophile and phospho-enzyme intermediate; Fig. 4A), and the class of phosphatases that catalyze direct displacement of the inositol leaving group via nucleophilic water (i.e. members of the inositol polyphosphate 5-phosphatase family; Fig. 4B), are truly remarkable enzymes with regard to their catalytic proficiencies (Lad et al., 2003). Both classes of enzymes enhance reaction rates some 17- to 20-orders of magnitude over spontaneous rates. That is, the reactions catalyzed by phosphatases at subsecond time scales occur spontaneously with half-times of ca. 1×10^{12} years (Lad et al., 2003)!

This review is focused on the phosphoinositide phosphatases and their various involvements in membrane trafficking and cellular homeostasis. Linkage of individual enzymes to mammalian disease is also discussed. For purposes of clarity, the domain organizations of the relevant phosphoinositide phosphatases are illustrated in Fig. 5, while the subcellular distributions of the various phosphoinositide phosphatases are illustrated in Fig. 6.

Phosphoinositide 3-Phosphate Phosphatases

PTEN

The *PTEN/MMAC1/ TEP1* (**p**hosphatase and **ten**sin homolog/**m**utated in **m**ultiple **a**dvanced **c**ancers/**T**GFβ-regulated and **e**pithelial cell–enriched **p**hosphatase) gene was originally identified as a candidate tumor suppressor gene located on human chromosome 10q23 (Li and Sun, 1997; Li *et al.*, 1997; Myers *et al.*, 1997; Steck *et al.*, 1997; Ali *et al.*, 1999). PTEN harbors the CX_5RT/S phosphatase catalytic signature and shares primary sequence similarity with members of the protein tyrosine phosphatase superfamily – a circumstance that prompted the initial suggestion that PTEN is a protein phosphatase. This hypothesis is not supported by biochemical characterizations which indicate recombinant PTEN is a poor protein phosphatase in vitro, irrespective of whether the enzyme is presented with phospho-Ser, phospho-Thr or phospho-Tyr substrates (Li and Sun, 1997; Li et al., 1997; Myers et al., 1997). Rather, PTEN exhibits a significant phosphoinositide phosphatase activity that targets the D-3 phosphate of PtdIns-3-P, PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 substrates. PtdIns-3,4,5-P_3 is the preferred substrate in vitro and is a primary PTEN substrate in vivo (Myers *et al.*, 1997; Maehama and Dixon, 1998). The unimpressive activity of PTEN towards phosphopeptide substrates, while consistent with the idea it is a primarily lipid phosphatase, does not exclude the possibility that PTEN is also a protein phosphatase -- but one with an exquisite substrate specificity. This issue is further discussed below.

PTEN consists of a ca. 200 residue N-terminal phosphatase domain required for catalytic activity (Fig 6; Lee *et al.*, 1999;Maehama *et al.*, 2001). The PTEN $CX_5R(S/T)$ motif resides within an enlarged active site that surrounds the catalytic signature with three basic residues, and these residues are critical for PTEN lipid phosphatase activity (Maehama *et al.*, 2001). This structural arrangement endows PTEN with its preference for highly acidic phospholipid substrates such as PtdIns-3,4,5-P_3. Interestingly, PTEN protein- and phosphoinositide-phosphatase activities can be uncoupled. The $G_{129}E$ missense mutation alters the phosphoinositide binding pocket and selectively eliminates PTEN phosphoinositide-phosphatase activity in vitro (Myers *et al.*, 1998).

That PtdIns-3,4,5-P_3 is a PTEN substrate is of great interest as this phosphoinositide is the terminal lipid product of forward PI3K signaling and, as such, represents a potent promoter of cell growth and survival. PtdIns-3,4,5-P_3 exerts its proliferative effects by activating the Ser/Thr protein kinase PDK1. In turn, PDK1 activates the proto-oncogenic protein kinase Akt which contains a PtdIns-3,4,5-P_3 – responsive PH domain (Coffer *et al.*, 1998; Fruman *et al.*, 1998; Rameh and Cantley, 1999; Downes *et al.*, 2007). That PTEN is truly involved in PtdIns-3,4,5-P_3–mediated activation of Akt is supported by demonstrations that both cellular PtdIns-3,4,5-P_3 and activated Akt levels are constitutively elevated in PTEN-deficient mouse embryonic fibroblasts (Sun *et al.*, 1999; Stambolic *et al.*, 2000). Reciprocally, PTEN overexpression downregulates Akt activity and depresses cellular PtdIns-3,4,5-P_3 levels (Di Cristofano *et al.*, 1998; Cantley and Neel, 1999; Di Cristofano *et al.*, 1999). These data identify PTEN as an antagonist of proliferative signaling – i.e. a tumour suppressor. Consistent with a homeostatic function, PTEN is ubiquitously expressed throughout early embryogenesis in mammals (Di Cristofano *et al.*, 1998; Sun *et al.*, 1999; Suzuki *et al.*, 2008).

It is difficult to overstate the enormous significance of the PtdIns-3,4,5-P_3/AKT/PTEN regulatory circuit from the standpoint of human disease. Over the past two decades, PTEN has blossomed into one of the featured molecules in cancer biology. Hundreds of published reports focus on PTEN structure, function, and mutations in the context of various human diseases. After p53, PTEN is the most commonly mutated gene in human cancer, and the most deleted/mutated phosphatase in human sporadic and hereditary cancer syndromes such as Cowden disease, glioblastoma, prostate and endometrial cancers, Bannayan – Zonana syndrome, and

Lhermitte – Duclos disease, among others (Liaw *et al.*, 1997; Nelen *et al.*, 1997; Stiles, *et al.*, 2004; Suzuki *et al.*, 2008). As some of these syndromes are sensitive to inhibitors of the Target-of Rapamycin (TOR) pathway, and are at least superficially phenocopied by overexpression of downstream components of the TOR pathway, PTEN deficiencies evoke their homeostatic defects in part via deranged TOR signaling (Podsypanina *et al.*, 2001; Atkins et al., 2004; Wendel *et al.*, 2004; Chan *et al.*, 2005). Consistent with a role for PTEN as critical regulator of cellular homeostasis, *PTEN* nullizygosity in mice results in early embryonic lethality, and heterozygosity results in elevated incidence of various cancers and autoimmune diseases (reviewed in Stiles *et al.*, 2004; Suzuki *et al.*, 2008). PTEN and p53 also show a functional connection. PTEN transcriptional expression is activated by p53, and this circuit contributes to initiation of p53-dependent apoptotic responses and p53-dependent senescence programs as consequences of downregulated PtdIns 3-OH kinase signaling (Jin and Levine, 2001; Stambolic et al., 2001; Feng et al., 2005).

The dual activities of PTEN as a protein tyrosine- and a phosphoinositide-phosphatase pose the interesting question of which activity is the most directly relevant for PTEN action as tumour suppressor. This issue was initially brought to the fore by reports that PTEN protein tyrosine phosphatase activity is directed at key signaling proteins such as focal adhesion kinase and the adaptor protein Shc (Gu *et al.*, 1998; Gu *et al.*, 1999; Yamada and Araki, 2001). However, the available data fall most convincingly in favor of the phosphoinositide phosphatase activity representing the key anti-proliferative activity of PTEN. The $PTEN^{G129E}$ separation of function allele does not compromise PTEN tumour suppressor activity (Myers *et al.*, 1998), and studies in *D. melanogaster* demonstrate that PTEN-deficient flies are rescued by mutation of the PtdIns-3,4,5-P_3-specific PH domain of Akt (Stocker *et al.*, 2002).

Although PTEN protein tyrosine phosphatase activity does not play a major role in tumour suppressor function, several lines of evidence suggest the PTEN tyrosine protein phosphatase activity is of relevance in specific physiological contexts (Vasquez et al., 2000; Raftopolou *et al.*, 2004; Vogelmann et al., 2005; Leslie et al., 2007; Leslie et al.,2009). Although β-catenin is proposed as a direct target for PTEN protein tyrosine phosphatase activity (Vogelmann et al., 2005), the best data suggest PTEN catalyzes an auto-dephosphorylation reaction in order to promote the conformational transitions required for the priming of PTEN C-terminal domains for physiologically important protein binding functions (Vasquez et al., 2000; Vasquez et al., 2001; Raftopolou et al., 2004; Leslie et al., 2007; Leslie et al., 2009). A thorough test of these hypotheses will be greatly facilitated by PTEN mutations that selectively inactivate protein tyrosine phosphatase activity without compromising lipid phosphatase activity.

The central role for PTEN is attenuating proliferative signaling demands the enzyme be tightly regulated – both spatially and temporally. The domain structure of the phosphatase suggests this is indeed the case. A lipid-binding C2 domain is configured C-terminal to the phosphatase domain, and PTEN exhibits a PDZ-binding motif at its C-terminus (Fig 6). The latter motif is proposed to regulate PTEN subcellular localization via interactions with PDZ domain-containing scaffolding proteins typically associated with the sub-plasma membrane cytoskeleton -- such as the membrane associated guanylate kinase-like protein MAG1 (Lee et al., 1999;Wu et al., 2000;Vazquez *et al.*, 2001). With regard to localization, PTEN exhibits a rather broad distribution across intracellular compartments; having been reported to localize to the plasma membrane, Golgi complex and nucleus (Fig. 6;Myers *et al.*, 1998;Lee *et al.*, 1999;De Matteis and Godi, 2004a). In this regard, while PTEN is evolutionarily conserved throughout the eukaryotic kingdom (with homologs in yeast, *Drosophila, C. elegans* and mammals), the domain arrangements vary among the various forms. For instance, the C2 domain is absent from PTEN enzymes of simpler eukaryotes, and the *Drosophila and C. elegans* PTEN proteins exhibit longer C-terminal tails than does human PTEN. The PDZ-

binding domain is also missing from some *Drosophila* PTEN isoforms (Goberdhan *et al.*, 1999;Lee *et al.*, 1999;Maehama *et al.*, 2001;Goberdhan and Wilson, 2003).

In addition to the two lipid-binding signatures recognized by sequence homologies, PTEN also exhibits a conserved N-terminal polybasic motif. This motif is interrupted by a $K_{13}E$ missense substitution recovered from a sporadic human glioblastoma -- the consequences of which include diminished activation of PTEN phosphatase activity by PtdIns-4,5-P_2 and non-functionality of the mutant protein in a variety of in vivo contexts (Walker et al., 2004). Because the substitution does not compromise $PTEN^{K13E}$ activity against soluble Ins-1,3,4,5-P_4, i.e. the soluble cognate of the PtdIns-3,4,5-P_3 headgroup, or PtdIns-3,4,5-P_3 incorporated into neutral phosphatidylcholine liposomes, the data suggest this polybasic motif binds non-substrate acidic phospholipids to orient PTEN onto membrane surfaces for optimal activity against its lipid substrates. In support of this conclusion, myristoylation of the $PTEN^{K13E}$ N-terminus restores protein function (Walker et al., 2004). Further evidence for the functional importance of the PTEN N-terminus is provided by the $S_{10}N$ missense substitution identified from a non-Hodgkins malignant lymphoma (Bonneau and Longy, 2000). $PTENS_{10}N$ exhibits respectable catalytic activity in vitro, suggesting the mutant enzyme is defective in some regulatory aspect (Han et al., 2000). In that regard, the $S_{10}N$ substitution lies within the basic cluster interrupted by the $K_{13}E$ substitution.

PTEN activity is also regulated by interesting sets of binding partners. Recently, it was reported that transport of PTEN to the neuronal plasma membrane, presumably the primary site at which it encounters its PtdIns-3,4,5-P3 substrate, is controlled by the actin-based motor protein myosin Va (van Diepen et al., 2009). A combination of co-immunoprecipitation and Förster resonance energy transfer experiments suggest a direct interaction between PTEN and myosin Va, and indicate the interaction is promoted by phosphorylation of PTEN by casein kinase 2 and glycogen synthase kinase 3. Myosin Va deficiencies, as well as PTEN defects, lead to increased size of neuronal cells in a manner that is dependent on PtdIns 3-OK kinase and mTOR signaling (Mercer et al., 1991; van Diepen et al., 2009). Following a similar theme, the *Drosophila* PTEN associates directly with the Par-3 component of the PAR/atypical protein kinase C complex involved in establishment and maintenance of cell polarity (von Stein et al., 2005). In fly ovaries and embryos lacking PTEN, the actin cytoskeleton is disorganized. Failures in asymmetric distribution of specific mRNAs, in posterior movements and synchronous nuclear divisions, and in cellularization, are also observed (von Stein et al., 2005).

Finally, regulation of PTEN activity by a binding partner has been documented. Peroxiredoxin 1 (Prdx1), a low efficiency peroxidase scavenges reactive oxygen species binds PTEN through its C2 domain (Cao et al., 2009). The Prdx1-PTEN interaction prevents inactivation of PTEN by reactive oxygen species. This finding, when coupled with the rather unimpressive catalytic efficiency of Prdx1, suggests a privileged relationship between PTEN and Prdx1. Reductions in Prdx1 activity result in enhanced basal and peroxide- and growth factor-induced Akt signaling (Cao et al., 2009).

PTEN2, TPTE and TPIP

Humans express several other proteins with primary sequence similarity to PTEN. These include PTEN2, the **T**ransmembrane **P**hosphatase with **TE**nsin homology (TPTE), and the **T**PTE and **P**TEN homologous **I**nositol lipid **P**hosphatase (TPIP). Both humans and mice are reported to express PTEN2 proteins (Wu et al., 2001). Murine PTEN2 is a testes-specific protein expressed primarily in secondary spermatocytes and early spermatids – an expression pattern suggestive of a role for this protein in the terminal stages of spermatocyte differentiation. The protein localizes to the medial/trans-compartments of the Golgi complex, and is reported to have detectable phosphoinositide 3-phosphatase activity with a substrate

specificity resembling that described for PTEN (Wu et al., 2001). A signature feature of PTEN2 that distinguishes it from PTEN is presence of an extended N-terminal domain that contains four putative transmembrane domains. This extension is necessary and sufficient for localization of murine PTEN2 to Golgi membranes, and these transmembrane domains have been speculated (with no real evidence in support) as having the potential to form an ion channel (Wu et al., 2001).

Human TPTE and TPIP proteins were independently identified by several groups, and these species also harbor 2 or 3 putative transmembrane domains in their N-termini (Chen *et al*., 1999; Walker *et al.*, 2001). The mouse contains only a single TPTE gene from which various spliceoforms are generated, while humans express perhaps as many as three functional TPTE structural genes (Guipponi et al., 2001). Nothing is known whether these TPTE gene products are functionally distinct in any significant way. With regard to human TPTE, of which two isoforms (TPTEα and TPTEβ) have been characterized in some detail, Walker et al. (2001) find these proteins to be devoid of phosphoinositide phosphatase activities and to localize to the plasma membrane. The inactivity of these proteins as phosphoinositide phosphatases is consistent with the presence of missense substitutions that introduce acidic residues in the active site pocket, substitutions whose PTEN cognates compromise the phosphoinositide-3-phosphatase activities of this enzyme (Walker et al., 2001). Some confusion has arisen after the assignment of murine PTEN2 by Wu et al. (2001) as the orthologue of the human TPTEα described by Chen et al. (1999). The enzymatic and localization properties of human TPTEα reported by Walker et al. (2001) diverge significantly from those reported for murine PTEN2 by Wu et al. (2001). It is argued on the basis of these differences that murine PTEN2 and human TPTEα proteins are not orthologous (Walker et al., 2001).

TPIPs are characterized as novel PTEN homologs that are expressed as two predominant isoforms designated TPIPα and TPIPβ (Chen *et al.*, 1999; Walker *et al.*, 2001). TPIPα is homologous to PTEN within the phosphatase and C2 domains (Fig. 6), but lacks the C-terminal tail which harbors the PDZ-binding domain and phosphorylation sites proposed to regulate PTEN stability and activity (Maehama *et al.*, 2001; Simpson and Parsons, 2001; Walker *et al.*, 2001). This isoform exhibits clearly measurable phosphoinositide 3-phosphatase activity (but little of any Ins-1,3,4,5-P_4) phosphatase activity), is expressed most highly in testis, brain and stomach, exhibits two or three potential trans-membrane domains in its N-terminal region, and localizes to the ER (Walker *et al.*, 2001). By contrast, TPIPβ is expressed in a testis-specfic manner and is truncated at both the N- and C-termini relative to TPIPα. Thus, TPIPβ lacks both the putative trans-membrane domain region and the C2-domain and, consequently, does not bind stably to membranes. Moreover, recombinant TPIPβ presents no measurable phosphoinositide phosphatase activity (Walker *et al.*, 2001). The collective data indicate the minimal phosphatase requires both the catalytic- and C2-domains to generate a functional module. Although, TPIPα is a phosphoinositide 3-phosphatase, it does not appear to couple physiologically to the PTEN-sensitive PtdIns-3,4,5-P_3 pools involved in activation of PDK1 and Akt (Walker *et al.*, 2001).

The Myotubularins

Myotubularin (MTM1), the founding member of the myotubularin family, was discovered by isolation of a gene mutated in X-linked centromyotubular myopathy (Laporte *et al.*, 1996). MTM1 is a CX_5RT/S motif protein that exhibits potent PtdIns-3-P phosphatase activity (Blondeau et al., 2000; Taylor *et al.*, 2000). The large MTM-related (MTMR) protein family is highly conserved from yeast to man. Humans express 14 MTMR family members and, consistent with how these proteins were discovered, interest in MTMR proteins is stoked by their disease relevance. Mutations in several genes of this family are root causes of myotubular

myopathy and Charcot-Marie-Tooth peripheral neuropathies (Clague and Lorenzo, 2005; Robinson and Dixon, 2006).

The myotubularins are recognized as key components of the homeostatic strategy for regulation of PtdIns 3-phosphate and PtdIns-3,5-P_2 levels in eukaryotic cells (Maehama *et al.*, 2001; Tronchere *et al.*, 2003; Tronchere *et al.*, 2004). Human MTM1, and MTMR1,2,3,4,6,7 are phosphoinositide 3-phosphate phosphatases with substrate preferences restricted to PtdIns-3-P and PtdIns-3,5-P_2 (Tronchere *et al.*, 2003; Tronchere *et al.*, 2004). Curiously, nearly half of the metazoan MTM family members contain naturally-occurring missense substitutions that involve the Cys and Arg residues of the canonical $CX_5R(S/T)$ motif, thereby disqualifying these enigmatic proteins as catalytically-active phosphatases (Wishart *et al.*, 2001; Laporte *et al.*, 2003; Taylor and Dixon, 2003; Clague and Lorenzo, 2005; Robinson and Dixon, 2006). In at least several cases, the naturally inactive MTMs -- by virtue of their heterodimerization with catalytically competent members of the MTM family -- play important roles in regulation of mammalian phosphoinositide homeostasis (Clague and Lorenzo, 2005; Robinson and Dixon, 2006). Perhaps the inactive subunits are critical for appropriate presentation of the substrate phosphoinositide to the catalytically active subunit in cellular contexts. This interesting arrangement does not hold for all myotubularins, however. *S. cerevisiae* expresses a single active MTM1 homolog (yeast myotubularin-related 1; Ymr1), and the yeast genome does not display catalytically-inactive members of this family (Taylor *et al.*, 2000; De Matteis and Godi, 2004a; Robinson and Dixon, 2006).

Metazoan MTMRs are categorized into six subclasses, three of which define groups of naturally inactive phosphatases (Wishart *et al.*, 2001; Laporte *et al.*, 2003; Taylor and Dixon, 2003). The common MTM domain structures include a PHGRAM (**p**leckstrin **h**omology glucosyltransferases, **R**ablike GTPase **a**ctivators and **m**yotubularins) module and a ca. 370 residue central PTP domain (Fig. 5; Begley *et al.*, 2003). Coiled-coil regions that presumably organize interactions with other proteins reside C-terminal to the phosphatase domain in most MTMR proteins. The MTMR3/4 and MTMR5/13 subclasses contain additional conserved modules, including FYVE, DENN and PH domains (Begley *et al.*, 2003; Robinson and Dixon, 2006). Localization studies identify MTMs as peripheral membrane proteins of endosomal compartments (Zhao *et al.*, 2001; Laporte *et al.*, 2002; Mochizuki and Majerus, 2003) – i.e. profiles consistent with involvements of these proteins in control of the PtdIns-3-P and PtdIns-3,5-P_2 signaling that is intimately linked to protein trafficking through the endosomal system (Fig 6). PtdIns-3-P and PtdIns-3,5-P_2 are present on endosomal compartments -- PtdIns-3-P predominates on early endosomes, and PtdIns-3,5-P_2 on late endocytic organelle membranes. Insufficiencies in PtdIns-3-P and PtdIns-3,5-P_2 phosphatase activity compromise turnover of these endosomal phosphoinositides, derange membrane trafficking through this system, and it is this set of basic cellular malfunctions that manifests itself most strikingly in pathologies of post-mitotic cells (e.g. neurons).

SAC-Domain Phosphoinositide Phosphatases

The Sac1-like phosphoinositide phosphatases are characterized by a domain arrangement for which the yeast Sac1 protein serves as prototype (Cleves *et al.*, 1989; Chung *et al.*, 1997; Guo *et al.*, 1999). SAC-domain proteins are divided into two subgroups. One group is characterized by a stand-alone Sac domain. Examples include yeast Sac1 and Fig4, and other proteins expressed by various species – e.g. the human KIAA0274, KIAA0851 and KIAA0966 proteins. The second group presents a Sac1-like-phosphatase module configured adjacent to a 5-phosphatase domain. Examples include mammalian synaptojanins 1 and 2, and the cognate yeast phosphatases Inp51/Sjl1, Inp52/Sjl2, and Inp53/Sjl3 (Srinivasan *et al.*, 1997; Stolz *et al.*, 1998; Hughes *et al.*, 2000a).

Yeast and Mammalian Sac1 Phosphatases

The founding member of the SAC-domain phosphoinositide phosphatase family was identified in yeast by two independent genetic screens searching for novel actin-regulatory proteins and novel regulators of membrane trafficking through the yeast *trans*-Golgi/endosomal networks, respectively (Cleves *et al.*, 1989; Novick *et al.*, 1989; Whitters *et al.*, 1993). While it was explicitly postulated that Sac1 (**s**uppressor of **a**ctin) is an antagonist of 4-phosphoinositide signaling, and that it is this function that accounts for the effects of *sac1* mutations on actin organization and membrane trafficking (Cleves et al., 1991), the demonstration that Sac1 is a phosphoinositide phosphatase came only eight years later (Guo et al., 1999). Sac1 is a type II integral membrane protein that disposes the N-terminal and C-terminal Sac1 domains to the cytosol (Fig. 5), it localizes to endoplasmic reticulum (ER) and Golgi membranes in yeast and in animal cells (Fig. 6), and the protein is anchored to membranes by two C-terminal transmembrane domains (Cleves et al., 1989; Whitters *et al.*, 1993; Nemoto *et al.*, 2000; Liu *et al.*, 2008; Mayinger, 2009). Sac1 and its orthologs are the only integral membrane proteins of the SAC1-domain family of phosphatases, and this property is essential for optimal catalytic activity in cells (Rivas et al., 1999; Foti et al., 2001). Genetic ablation of Sac1 function in yeast is not a lethal event, and a chromosomal *sac1Δ* allele recapitulates the genetic interactions exhibited by *sac1* alleles recovered from the actin- and TGN-directed genetic screens (Cleves et al., 1989). Thus, the two genetic screens through which Sac1 was identified uncovered genuine loss-of-function mutations. Other phenotypes include cold-sensitivity for growth, cell wall abnormalities, and assorted ER-associated defects (Cleves et al., 1989; Novick et al., 1989; Kochendörfer et al., 1999).

Biochemical and in vivo inositol-labeling experiments demonstrate yeast Sac1 and its mammalian homologs are promiscuous phosphoinositide phosphatases that show little positional specificity with regard to phosphoinositide substrate -- PtdIns-3-P, PtdIns-4-P, PtdIns-5-P, and PtdIns-3,5-P_2 are all acceptable substrates (Guo et al., 1999). The exceptions to the rule are PtdIns-3,4-P_2 and PtdIns-4,5-P_2. Thus, Sac1 domains cannot degrade poly-phosphoinositides with vicinal phosphates. In yeast, it is clear the major in vivo substrate for Sac1 is PtdIns-4-P; a conclusion rigorously established by headgroup chemical analysis of the predominant phosphoinositide species that accumulates in Sac1-deficient yeast (Guo et al., 1999; Rivas et al., 1999). Indeed, genetic ablation of Sac1 activity effects 8- to 10-fold increases in PtdIns-4-P mass in yeast with little effect on the other phosphoinositides (Guo *et al.*, 1999; Rivas *et al.*, 1999; Hughes *et al.*, 2000a; Hughes *et al.*, 2000b; Nemoto *et al.*, 2000). The massive accumulation of PtdIns-4-P is a large part of the mechanism, but not the entire mechanism, for why Sac1 defects alter membrane trafficking from the yeast TGN (Xie *et al.*, 1998; Rivas *et al.*, 1999; Li *et al.*, 2002). Thus, the in vitro promiscuity of the enzyme notwithstanding, Sac1 exhibits impressive substrate specificity in vivo. This substrate specificity is likely a revealing manifestation of how the enzyme encounters its substrate phosphoinositides.

The Sac1 substrate specificity is all the more intriguing in that not all PtdIns-4-P pools are accessed by Sac1. The source of PtdIns-4-P matters as, at least under normal growth conditions, yeast Sac1 specifically degrades the PtdIns-4-P generated by one of the two essential PtdIns 4-OH kinases in yeast – i.e. the plasma membrane isoform Stt4 – with insignificant access to the PtdIns-4-P pool generated by the other essential yeast PtdIns 4-OH kinase Pik1 (Nemoto et al., 2000; Foti *et al.*, 2001; Routt *et al.*, 2005). This intriguing functional relationship is not well understood given the localization of Stt4 to the plasma membrane and Sac1 residence in intracellular membrane compartments. Ideas for how to reconcile this apparent physical paradox are emerging (see below). The effects of Sac1 dysfunction are not limited to PtdIns-4-P. Larger derangements of lipid metabolism are apparent in Sac1-deficient yeast – particularly in neutral lipid metabolism (Kearns et al., 1997; Rivas et al., 1999; Foti et al., 2001).

Sac1 cycles between the ER and Golgi – indicating cells impose regulatory oversight on the mechanisms by which Sac1 is retained in, and retrieved from, distinct intracellular compartments. As discussed below, interesting cell biology is associated with such a trafficking itinerary. Retention of Sac1 in the yeast ER is reported to be mediated by an interaction of the Sac1 C-terminal tail with Dpm1, the highly abundant ER dolicholphosphate-mannose synthase. Functional ablation of this interaction disrupts ER localization of the enzyme (Faulhammer *et al.*, 2005). How yeast Sac1 is recognized as a retrograde cargo in Golgi membranes is not understood.

A different strategy for Sac1 traffic control is in evidence in mammalian cells. Whereas Sac1 also cycles between the mammalian ER and Golgi, its competence for exit from the ER compartment requires oligomerization of the enzyme via a leucine-zipper motif – a motif not present in yeast Sac1 (Blagoveshchenskaya et al., 2008). How the enzyme is retrieved from Golgi compartments back to the ER is known, however. Mammalian Sac1 presents a C-terminal KXKXX motif that serves as binding site for the coatomer (COPI) complex, the presumptive coat for retrograde trafficking vesicles, and is obligately required for retrieval of the enzyme from the Golgi back to the ER (Fig. 5; Rohde *et al.*, 2003; Liu *et al.*, 2008). Interaction of Sac1 with the COP1 machinery requires disassembly of the Sac1 oligomers. Functional integrity of the Sac1/COPI complex also demands that Sac1 be an active phosphatase, and assembly of this complex is dynamically regulated by cell-growth conditions (Rohde et al., 2003; Blagoveshchenskaya *et al.*, 2008; Liu *et al.*, 2008). How Sac1 phosphatase activity regulates this interaction is unclear, but some phosphoinositide species (PtdIns-4-P?) likely inhibit the interaction – directly or indirectly. Such a mechanism promises a different paradigm for how phosphoinositides regulate cargo::vesicle coat protein interactions. In the late secretory and endocytic pathways, phosphoinositides are positive regulators that faciltitate cargo interactions with coat proteins (De Matteis and Godi, 2004a; Mayinger, 2009). At any rate, it is abundantly clear that endogenous Sac1 levels (at least in yeast) are saturating -- given that 20-fold overproduction of Sac1 protein has no effect on cell viability, phosphoinositide homeostasis, or ER localization of the enzyme (Cleves et al., 1989; Rivas et al., 1999; Liu et al., 2008).

Unlike the case in yeast, the single mammalian Sac1 isoform executes essential cellular functions (Liu *et al.*, 2008). Nullizygous embryos exhibit pre-implantation lethality obvious by the blastocyst stage, and siRNA experiments report a loss of viability upon acute Sac1 depletion in cultured mammalian cells. One obvious intracellular phenotype associated with Sac1-depletion is a manifest disorganization of cis-, medial-and trans-Golgi compartments in the absence of obvious morphological derangements of other compartments of the secretory pathway. The structurally disturbed Golgi membranes show no overt membrane trafficking defects, however (Liu *et al.*, 2008). Instead, Sac1-depleted cells fail to exit mitosis and present a high incidence of spindle disorganization highlighted by ectopic and mechanically-active spindle asters. These deviant spindle asters are marked with γ-tubulin but, as is normally the case, only two of the asters harbor centrosomal Centrin-2 landmarks. Thus, licensing of centrosome division is unperturbed in Sac1-deficient cells. The mechanical activity of ectopic spindle asters, when coupled with the failure of multipolar spindles to efficiently resolve, promotes catastrophic defects in chromosomal segregation (Liu *et al.*, 2008). Complementation experiments show Sac1 phosphoinositide phosphatase activity, and recycling from the Golgi back to the ER, are functional properties required for Sac1 activity in vivo (Liu *et al.*, 2008).

Why is Sac1 ER-localization a conserved and functionally important feature for this phosphoinositide phosphatase? The ER is not a compartment generally associated with phosphoinositide signaling. One possibility is Sac1 scavenges ‘wandering’ phosphoinositides in the ER for the purpose of degrading mislocalized phosphoinositide molecules that would otherwise confuse phosphoinositide-centric mechanisms for establishing and maintaining

organelle identity. This model confronts two issues. First, Sac1 is particularly tuned to activity of the plasma membrane Stt4 (mammalian PtdIns kinase α), yet inactivation of this circuit is not lethal in yeast nor is the secretory pathway particularly confused regarding organelle identities in *sac1Δ* cells that accumulate dramatic amounts of PtdIns-4-P. While Sac1-deficient mammalian cells do not thrive, these nonetheless traffic proteins quite normally – even though Golgi structure is deranged (Liu et al., 2008). On balance, it does not seem likely that Sac1 plays dedicated phosphoinositide cleanup duty with the ER as repository for mislocalized phosphoinositides. It would seem surface dilution of phosphoinositide across a large ER area would confound such cleanup strategies anyway.

Alternatively, Sac1 may coordinate plasma membrane and ER functions and, by orienting itself on the ER surface at points of apposition with the plasma membrane (membrane contact sites?), degrade excess phosphoinositide on the plasma membrane. If that were the simple case, one would expect significant and specific PtdIns-4-P accumulation in the plasma membrane in Sac1-deficient cells. Although there is one report to suggest this is the case (Roy and Levine, 2004), other experiments report PtdIns-4-P accumulation in ER-like compartments of Sac1-deficient yeast cells (Li et al., 2002; Woods et al., 2009). These phosphoinositide localization data yeast amply demonstrate the reporter-dependence of the visualized phosphoinositide profile, emphasizing yet again that caution must be exercised in interpreting the results obtained with such biosensors. The idea that Sac1 is either a component of, or takes advantage of, membrane contact sites to access specific PtdIns-4-P pools remains an open question. More expansive discussions of putative membrane contact sites are found in several recent treatments of this topic (Pichler *et al.*, 2001; Levine and Loewen, 2006; Peretti *et al.*, 2008).

The involvement of mammalian Sac1 in cell cycle progression and mitotic spindle organization, suggests the simplest possibility may hold the most merit. That is, Sac1 localizes to the ER because it regulates phosphoinositide-mediated signal transduction from cytosolic compartments, perhaps even the ER, to the nucleus or nuclear envelope. The nuclear matrix is an active compartment for phosphoinositide-signaling (Cocco *et al.*, 1987; Divecha *et al.*, 1991; Irvine and Divecha, 1992; Irvine, 2003; Martelli *et al.*, 2005; Gonzales and Anderson, 2006), and one can imagine a dedicated role for an ER-localized phosphatase in survey of phosphoinositide status on the nuclear envelope (which is contiguous with the ER). Nuclear pores are attractive sites for such surveillance. It is around these structures that continuities occur between the cytosolic membrane leaflet of the ER/nuclear envelope and the leaflet that faces the nuclear matrix.

Alternatively, Sac1 may regulate nuclear envelope dynamics in specific physiological contexts. Nuclear envelope assembly and disassembly occurs during the mammalian mitotic cycle, and the process is also highly regulated in non-somatic cells such as fertilized oocytes (Larijani and Poccia, 2009). Evidence for a direct role for phosphoinositides in regulating nuclear envelope assembly is provided by Larijani and colleagues who demonstrated sea urchin nuclear envelope precursor vesicles (i.e. vesicles that must fuse for completion of envelope assembly) exhibit a highly unusual lipid composition -- one strikingly enriched in sterols and phosphoinositides (Byrne et al., 2007; Garnier-Lhomme et al., 2009).

Other possibilities for why Sac1 localizes to the ER include compartment-specific involvements for Sac1 in integration of nutrient/growth factor status with activity of membrane trafficking through the late stages of the Golgi complex. Evidence to support such integrator roles for Sac1 has been obtained from both the yeast and mammalian systems, and the principle is based on differential control of the ER-Golgi-ER cycling itinerary of the Sac1 phosphatase (Fig. 7). When yeast are cultured in nutrient-replete conditions, Sac1 is retained in the ER where it stimulates Dpm activity in ER oligosaccharide biosynthesis (Faulhammer et al., 2005). Efficient glycosylation of proteins is a critical component of ER quality control

mechanisms that survey cargo incorporation into the secretory pathway (Hebert et al., 2005;Meusser et al., 2005). Upon glucose starvation, Sac1 redistributes from the yeast ER to the Golgi system where it is suggested to depress Golgi secretory activity by depleting Golgi membrane PtdIns-4-P pools. In this manner, Sac1 is proposed to function as a pro-secretory molecule when growth conditions are favorable, and as a brake when conditions are poor (Fig. 7;Faulhammer et al., 2005;Blagoveshchenskaya et al., 2008;Mayinger, 2009).

Analogous Sac1-mediated mechanisms for coupling membrane trafficking to growth factor status also operate in mammalian cells. In this case, homo-oligomerization of Sac1 via its leucine zipper motif must occur in a regulated manner to support timely COPII-mediated exit of the phosphatase from the ER, and the complexes must be disassembled on demand in the Golgi complex to promote timely COP1-mediated retrieval of the phosphatase back to the ER (Fig. 7; Blagoveshchenskaya et al., 2008;Mayinger, 2009). The p38 mitogen-activated protein kinase and extracellular signal-regulated kinases 1/2 regulate these events (Blagoveshchenskaya et al., 2008).

How physiologically significant is the complex Sac1 trafficking itinerary in yeast and mammals? As the bulk of the experiments that describe the linkage between Sac1 trafficking and nutrient/mitogen signaling report correlative relationships, this remains an open question. In support of views that assign high physiological significance to Sac1 traffic control are demonstrations that yeast Sac1 expression cannot rescue Sac1 deficiencies mammalian cells (Liu et al., 2008) -- presumably because yeast Sac1 is not outfitted to heed the various mitogenic signals that normally control the dynamic cycling of mammalian Sac1 between ER and Golgi. In opposition, tethering of the Sac1 catalytic domain to an ER-localized integral membrane protein Sec61 generates an ER-localized chimera whose expression is sufficient to fully complement all known phenotypes associated with Sac1 loss-of-function in yeast (Li et al., 2002). Presumably, this chimera is uncoupled from the principle regulatory mechanisms that couple yeast nutrient sensing to Sac1 trafficking. Moreover, expression of mammalian Sac1 in yeast, a protein also expected to be indifferent to the mechanisms controlling the yeast Sac1 trafficking itinerary, demonstrates it to be an effective surrogate for endogenous Sac1 (Liu et al., 2008). One possibility for reconciling these various data is that yeast might be less sensitive to subversion of dynamic Sac1 trafficking than are mammalian cells. Another possibility is that normal laboratory conditions are inappropriate contexts in which to examine this question. Nevertheless, the concept of Sac1 as integrator of nutrient/mitogen-sensing with membrane trafficking is an enticing one that will enjoy its share of future experimental scrutiny. While, to our knowledge, there is as yet no direct linkage between Sac1 dysfunction and disease, establishment of such a linkage will drive further interest in this under-investigated phosphoinositide phosphatase. In that regard, work in *Drosophila* demonstrates that Sac1 hypomorphism results in embryonic lethality associated with defects in dorsal closure of the mutant embryo and deranged activation of the Jun N-terminal kinase MAPK signaling cascade (Wei et al., 2003).

Yeast and Mammalian Fig4 Phosphatases

Another important member of the SAC-domain phosphatase family is Fig4 (Factor induced gene; Fig. 5). First identified in yeast through a large-scale enhancer trap screen designed to identify genes whose expression is regulated by mating pheromone (Erdman *et al.*, 1998), Fig4 does not discharge essential functions in yeast. It is, however, required for normal mating projection formation and actin polarization in response to mating pheromone (Erdman *et al.*, 1998). Fig4 harbors intrinsic polyphosphoinositide phosphatase activity with positional specificity for the D-5 phosphoester bond of PtdIns-3,5-P_2, is localized to the vacuolar membrane, and insufficiencies cause defects in vacuole fission with the result that vacuoles become enlarged and retrograde trafficking through the endosomal system is also impaired

(Bonangelino *et al.*, 2002;Gary *et al.*, 2002;Michell *et al.*, 2006;Michell and Dove, 2009). Paradoxically, Fig4 is required for activation of the Fab1 PtdIns-3-P 5-OH kinase that produces the PtdIns-3,5-P_2 which, in turn, is degraded by Fig4 (Gary *et al.*, 2002;Rudge *et al.*, 2004;Duex et al., 2006a;Duex *et al.*, 2006b). It is for this reason that Fig4 loss-of-function mutations evoke reduced, rather than elevated, intracellular PtdIns-3,5-P_2. The physiological rationale for such a paradoxical coupling between a phosphatase and a kinase remains obscure.

The Sac3 PtdIns-3,5-P_2 5-phosphatase is the mammalian ortholog of yeast Fig4 (Fig. 5), and defects in this enzyme are associated with Charcot–Marie–Tooth peripheral neuropathies (CMT) that affect motor and sensory nerves and cause progressive distal muscle weakness and atrophy (Quattrone *et al.*, 1996;Berger *et al.*, 2006;Nicot and Laporte, 2008). Haplo-insufficiencies for the human *SAC3* gene are also associated with autosomal recessive CMT4J neuropathies (Chow *et al.*, 2007). Insertional inactivation of murine *SAC3* results in the “pale tremor mouse” syndrome characterized by degeneration of the central nervous system, peripheral neurophathy, and diluted pigmentation. Fibroblasts derived from pale tremor mice exhibit enlarged late endosomal and lysosomal compartments, and reduced levels of PtdIns-3,5-P_2 (Chow *et al.*, 2007). Thus, the paradoxical functional relationship between a Fig4 phosphoinositide phosphatase and a Fab1 phosphoinositide kinase is preserved in the cognate mammalian enzymes.

Phosphoinositide 5-Phosphate Phosphatases

Enzymes of the phosphoinositide 5-phosphatase family dephosphorylate the D-5 phosphoester linkage of PtdIns-3,5-P_2, PtdIns-4,5-P_2 and PtdIns-3,4,5-P_3 -- generating PtdIns-3-P, PtdIns-4-P, and PtdIns-3,4-P_2, respectively. This enzyme family consists of ten mammalian and four yeast members whose activities intersect with a variety of cellular events such as synaptic vesicle recycling, hematopoietic cell proliferation, insulin signaling, and actin organization (Astle *et al.*, 2006; Ooms *et al.*, 2009). A typical feature of this enzyme family is a ca. 300 residue central catalytic polyphosphate 5-phosphatase domain highlighted by two signature motifs -- WXGDXN(F/Y)R and P(A/S)W(C/T)DRIL -- spaced some 60–75 residues apart (Majerus *et al.*, 1999). Crystallographic studies reveal these 5-phosphatases belong to the AP endonuclease family highlighted by a catalytic mechanism which employs a His/Asp active site pair (Dyson *et al.*, 2005).

Oculocerebrorenal Lowe Syndrome (OCRL) Phosphatase

OCRL is a devastating human X-linked developmental disorder that occurs in approximately 1 in 200,000 births, and is characterized by a variety of ocular, neurological, cognitive, renal, and musculoskeletal abnormalities (Lowe *et al.*, 1952; Leahey et al., 1993; Hoopes et al., 2005). Ocular syndromes include bilateral congenital cataracts, glaucoma, and microphthalmos. Neonatal hypotonia, mental retardation, araflexia, and behavioral abnormalities represent neurological manifestations. Renal defects include kidney absorbtion insufficiencies associated with Fanconi syndrome of kidney proximal tubules and type 2 Dent disease. The kidney malabsorbtion phenotypes contribute to the musculoskeletal defects that are manifested in joint hypermobility and in fracture-prone bones.

The molecular etiology of Lowe’s syndrome is loss-of-function for the OCRL1 inositol polyphosphate 5-phosphatase (Attree *et al.*, 1992; Leahey *et al.*, 1993). OCRL1 exhibits three conserved domains -- the central inositol polyphosphate 5-phosphatase domain, an ASH domain, and a C-terminal catalytically inactive Rho guanosine triphosphatase-activating protein (GAP)-like domain (Fig. 5; Lowe, 2005; Erdmann et al., 2007). OCRL1 shares 51% primary sequence identity with INPP5B, the only other inositol polyphosphate 5-phosphatase with a GAP-like domain in humans and mice (Jefferson and Majerus, 1995; Speed *et al.*, 1995; Matzaris *et al.*, 1998). Both OCRL1 and INPP5B hydrolyze PdIns-4,5-P_2 and

PtdIns-3,4,5-P_3, as well as the soluble inositol polyphosphates Ins-1,4,5-P_3 and Ins-1,3,4,5-P_4, but with distinct substrate preferences. While PdIns-4,5-P_2 is preferred to PtdIns-3,4,5-P_3 by both OCRL1 and INPP5B, the latter does not discriminate between molecular species of these phosphoinositides and does not dephosphorylate PtdIns-3,5-P_2 (Zhang *et al.*, 1995; Zhang *et al.*, 1998; Schmid *et al.*, 2004). Available evidence suggests INPP5B systematically dephosphorylates PtdIns-3,4,5-P_3 to PtdIns-3-P, a key phosphoinositide required for endosome homeostasis (Shin et al., 2005). Some measure of functional redundancy likely exists between OCRL1 and INPP5B in mice. Combinatorial ablation of OCRL1 and INPP5B structural genes results in embryonic lethality, while OCRL1 nullizygosity does not result in murine Lowe syndrome disease (Janne *et al.*, 1998). Lower eukaryotes (flies, worms and amoeba) express only one of those two proteins, and no GAP-like domain-containing 5-phosphatases are expressed in yeast (Lowe, 2005).

The OCRL1 protein localizes to the trans-Golgi network (TGN) and early endosomes (Fig. 6; Olivos-Glander *et al.*, 1995;Ungewickell *et al.*, 2004;Choudhury *et al.*, 2005;Faucherre *et al.*, 2005). OCRL1 also interacts with the small GTPase Rac and the TGN/endosomal adaptor protein APPL1 via its Rho-GAP domain. These interactions target the enzyme to the TGN/endosomal system (Faucherre *et al.*, 2003;Erdmann et al., 2007;McCrea et al., 2008). The presence of OCRL1 in clathrin-decorated budding profiles at the TGN, and as cargo in clathrin-coated vesicles (CCV) shuttling between the TGN and endosomes, suggests this phosphatase plays a role in vesicular transport between these compartments (Ungewickell *et al.*, 2004;Choudhury *et al.*, 2005). The redistribution of cargo that cycles between TGN and endosomes (e.g. TGN46) to early endosomal compartments (accompanied by endosome enlargement) upon siRNA-mediated OCRL1 depletion supports this view. Thus, retrograde trafficking from endosomes to the TGN is compromised by OCRL1-insufficiencies, and the 5-phosphatase domain is essential for OCRL1-mediated control of this trafficking step (Choudhury *et al.*, 2005). Morever, mutations that reside in non-catalytic domains of OCRL1 (i.e. the RhoGAP-like and ASH domains) all result in mislocalization of the enzyme by compromising its ability to bind APPL1 (Erdmann et al., 2007;McCrea et al., 2008).

The mechanism for how OCRL1 controls clathrin-mediated membrane trafficking through the TGN/endosome system involves regulation of PtdIns-4-P and/or PtdIns-4,5-P_2 levels. PtdIns-4-P, together with ARF GTPases, facilitates membrane recruitment of effector proteins (e.g. clathrin adaptors AP1, an AP1/clathrin interacting protein epsinR, and lipid-binding proteins such as oxysterol-binding proteins) that regulate the biogenesis and dynamics of the TGN/endosomal system (Kalthoff *et al.*, 2002; Li et al, 2002; Mills *et al.*, 2003; Wang *et al.*, 2003; Godi *et al.*, 2004). Loss of OCRL1-mediated degradation of PtdIns-4,5-P_2 to PtdIns-4-P in TGN/endosomes might upset balanced recruitment of these effector proteins to membranes due to PtdIns-4-P insufficiency. Alternatively, given PtdIns-4,5-P_2 plays an essential role in clathrin-mediated endocytosis, by facilitating recruitment of clathrin accessory proteins to the plasma membrane, OCRL1 inactivation may provoke PtdIns-4,5-P_2 derangements which influence the TGN/endosomal system indirectly (Wang *et al.*, 2003; Legendre-Guillemin *et al.*, 2004).

OCRL1 is suggested to regulate actin dynamics on the basis that actin organization is defective in Lowe syndrome fibroblasts (Suchy and Nussbaum, 2002). This effect likely reflects an indirect relationship between ORCL1 and actin dynamics, however. Even subtle derangements in phosphoinositide levels, particularly those of PtdIns-4,5-P_2, can upset the delicate homeostatic balance between actin and actin binding proteins -- as occurs in yeast mutants deficient for the Sac1 phosphoinositide phosphatase (Novick et al., 1989; Cleves et al., 1989; Cleves et al., 1991; Whitters *et al.*, 1993). Abnormal Ca^{2+} signaling may also confuse actin dynamics (Suchy et al., 2009).

Synaptojanins

The synaptojanins are dual function Sac-domain phosphatases also evolutionarily conserved from yeast to humans. Synaptojanin 1 was discovered by Pietro De Camilli and colleagues as a nerve terminal protein that functions in synaptic vesicle endocytosis and recycling (McPherson *et al.*, 1994a; McPherson *et al.*, 1994b). This protein is characterized by an N-terminal Sac1-like polyphosphate phosphatase domain which converts PtdIns-3-P, PtdIns-4-P, PtdIns-5-P and PtdIns-3,5-P_2 to PtdIns, and a central inositol polyphosphate 5-phosphatase domain that hydrolyze phosphoinositides at the D-5 position of the inositol ring (Fig. 5; McPherson *et al.*, 1996; Haffner *et al.*, 1997). The synaptojanin 1 C-terminus is subject to modification by alternative splicing programs in adult vs developing neurons, and exhibits a proline-rich region which mediates of synaptojanin 1 interaction with the Src homology 3 (SH3) domains of Grb2 (McPherson *et al.*, 1994a; McPherson *et al.*, 1994b). Grb2 is a 25-kDa adaptor protein with two SH3 domains that flank a Src homology 2 (SH2) domain, and this adaptor is a regulatory component of the synaptic vesicle cycle and neurotransmitter release. Candidate Grb2 effectors include dynamin (a GTPase required for synaptic vesicle endocytosis) and synapsin I -- a synaptic vesicle-associated protein that mediates interaction of synaptic vesicles with the presynaptic cytomatrix (McPherson *et al.*, 1994a).

The synaptojanin 2 catalytic domain is closely related to that of synaptojanin 1 at the primary sequence level, is also a phosphoinositide 5-phosphatase, and displays a broader tissue distribution than synaptojanin 1. The two synaptojanins exhibit unrelated C-terminal regions, and this property manifests itself in the differential protein-protein interaction spectra of these enzymes (Nemoto *et al.*, 1997; Khvotchev and Südhof, 1998). Whereas synaptojanin 2 binds the SH3 domain containing protein, Grb2, synaptojanin 1 not only binds Grb2, but also amphiphysin and members of SH3p4/8/13 protein family via its C-terminal proline-rich domain (Nemoto *et al.*, 1997). The two synaptojanin isoforms are directed to distinct subcellular locations as a result of their unique sets of protein-protein interactions (Nemoto *et al.*, 1997).

Synaptojanins 1 and 2 exhibit distinct biological functions. Synaptojanin 1 nullizygous mice survive to term but die shortly after birth. The neonates present neurological defects and accumulate clathrin-coated vesicles at nerve endings, a consequence of impaired turnover of a plasma membrane PtdIns-4,5-P_2 pool whose persistence interferes with disassembly of clathrin coats and results in defective synaptic transmission (Cremona *et al.*, 1999; Kim *et al.*, 2002). This regulatory function may have other interesting neurological manifestations. The synaptojanin 1 structural gene resides in a genomic region present in trisomic arrangement in Ts65Dn mice, a model for Down's syndrome, and PtdIns-4,5-P_2 homeostasis is altered in brains of these mice (Voronov et al., 2008). The neurological and PtdIns-4,5-P_2 homeostatic defects that characterize Ts65Dn mice are corrected by restoring synaptojanin 1 structural gene to disomy, and the trisomy of the synaptojanin 1 structural gene alone is sufficient to recapitulate Down's syndrome in mice. Given the human synaptojanin 1 structural gene is located on the trisomic chromosome 21 of human Down's syndrome patients, it is an attractive proposition that increased synaptojanin 1 activity is directly associated with the brain dysfunction and cognitive defects of this disease (Voronov *et al.*, 2008).

Synaptojanin 2 functions in non-neuronal cells by regulating an early step in the clathrin-mediated endocytic pathway (Hill *et al.*, 2001; Rusk *et al.*, 2003). Synaptojanin 2 specifically binds the GTP-bound form of the small GTPase Rac1, and overexpression of a membrane-targeted version of synaptojanin 2 inhibits endocytosis of epidermal growth factor (EGF) and transferrin receptors (Malecz *et al.*, 2000). Moreover, siRNA experiments demonstrate depletion of synaptojanin 2 (but not synaptojanin 1) compromises clathrin-mediated internalization of EGF receptor and antagonizes formation of clathrin-coated pits and CCVs in lung carcinoma cells (Rusk *et al.*, 2003). The synaptojanin execution point at late stages of

the secretory pathway, and in endocytosis, is conserved. Dysfunction of the yeast synaptojanin homolog Inp53/Sjl3 comprises protein transport from the TGN to endosomes (Ha *et al.*, 2003).

SHIP Polyphosphate 5-Phosphatases

SHIP1, the prototype for the SH2 domain-containing inositol polyphosphate-5-phosphatases, was discovered in murine hematopoietic cells as a component of a Grb2- and Shc-containing complex (Damen *et al.*, 1996; Kavanaugh *et al.*, 1996; Lioubin *et al.*, 1996). SHIP1 is a 145-KDa protein with an N-terminal SH2 domain, a central catalytic 5-phosphatase domain that contains the two highly conserved signatures of inositol polyphosphate 5-phosphatases, a C-terminal proline-rich region with consensus sites for SH3 domain interactions, and two potential phosphotyrosine binding (PTB) domain-binding sites (NPXY) at the C-terminus (Fig. 5; Damen *et al.*, 1996; Backers *et al.*, 2003). Both humans and rodents also express a related 142-KDa protein (SHIP2) that is widely expressed in different tissues in human with particularly high levels in heart, skeletal muscle and placenta (Pesesse *et al.*, 1997; Pesesse *et al.*, 1998; Ishihara *et al.*, 1999). The domain structure of SHIP2 is similar to that of SHIP1 with the exception that SHIP2 harbors a unique C-terminal sterile alpha motif (SAM) domain which mediates homotypic SAM-domain protein interactions (Fig. 5). Such interactions modulate EphrinA2 receptor internalization and degradation, a process that may also dampen growth factor signaling (Zhuang *et al.*, 2007). SHIP1 and 2 utilize both PtdIns-3,4,5-P_3 and Ins-1,3,4,5-P_4 as substrates and dephosphorylate these compounds at the D-5 position of the inositol ring (Damen *et al.*, 1996; Pesesse *et al.*, 1998). Kinetic studies report the substrate hierarchy for SHIP2 as Ins-1,2,3,4,5-P_5 > Ins-1,3,4,5-P_4 > PtdIns-3,4,5-P_3. PtdIns-3,5-P_2, and the soluble Ins-1,4,5,6-P_4, and Ins-2,4,5,6-P_4, are utilized as substrates with approximately the same efficiency as PtdIns-3,4,5-P_3 (Chi *et al.*, 2004).

With regard to biological functions, SHIP1 is involved in regulation of the immune response and in survival of myeloid cells while SHIP2 is involved in downregulation of insulin signaling (Huber *et al.*, 1999; Liu *et al.*, 1999; Clement *et al.*, 2001; Sasaoka *et al.*, 2004). Upon growth factor stimulation, SHIP1 translocates from the cytosol to the plasma membrane, and this redistribution is associated with downregulation of signaling via the MAP kinase pathway (Rohrschneider *et al.*, 2000).Similarly, upon insulin stimulation, SHIP2 is redistributed to the plasma membrane with consequent depression of PI3K-dependent insulin signaling resulting from SHIP2-mediated dephosphorylation of PtdIns-3,4,5-P_3 to PtdIns-3,4-P_2 (Sasaoka et al., 2004). These data indicate the SHIP1 and SHIP2 enzymes primarily survey agonist-stimulated PtdIns-3,4,5-P_3 pools. This is in contrast to PTEN, which accesses both signaling and basal pools of PtdIns-3,4,5-P_3 and remains active after long-term stimulation (Stambolic *et al.*, 1998; Liu *et al.*, 1999; Leslie and Downes, 2002; Blero *et al.*, 2005).

Summary

The evolution of inositol as a compact, yet versatile, signaling scaffold, one subject to position-specific phosphorylation, has allowed eukaryotes to multiplex the channels of signaling that pass through this simple six-carbon chemical backbone. The intricacies of inositol signaling are accompanied by a correspondingly complex enzymology that governs phosphoinositide synthesis and degradation in a spatially and temporally controlled manner. Degradation of phosphoinositides by phosphatases that do not produce soluble second messenger products is a major pathway for phosphoinositide homeostatic control, as evidenced not only by the number of phosphatases dedicated to phosphoinositide catabolism, but also by the direct linkage of phosphoinositide phosphatase deficiencies to an array of human diseases. From the basic science perspective, the domain arrangements of some phosphoinositide phosphatases are intriguing. For example, why are Sac-domains often physically appended to phosphoinositide 5-phosphate phosphatase catalytic domains? Is this arrangement analogous

to the interface between phosphoinositide 3-phosphate phosphatases and their catalytically-dead subunits, or does it describe an intimate arrangement for metabolic channeling of substrates? These curious modularities must reflect some fundamental property of these enzymes. Although emerging evidence favors metabolic channeling models (Mani et al., 2007), the nature of that property remains uncertain.

Do phosphoinositide phosphatases play unappreciated roles in promoting signaling? These enzymes degrade phosphoinositides species to other phosphoinositide products that have their own signaling potential after all. How are the phosphatase reactions channeled to prevent precocious activation of signaling? How determining is the mode of phosphoinositide production in terms of signaling outcome (e.g. PtdIns-4-P via action of a PtdIns 4-OH kinase as opposed to degradation of PtdIns-4,5-P_2 by a phosphoinositide 5-phosphate phosphatases)? These remain questions for future study.

From the perspective of human disease, the relevance of phosphoinositide phosphatases is established. These disease connections, when coupled with a thorough mechanistic understanding of how phosphoinositide phosphatases function in cells, will further reinforce interest in these enzymes as attractive druggable targets. When the pharmacological encyclopedia of treatment for human diseases is written, we expect that phosphoinositide phosphatases will occupy a significant place in the roster of targets.

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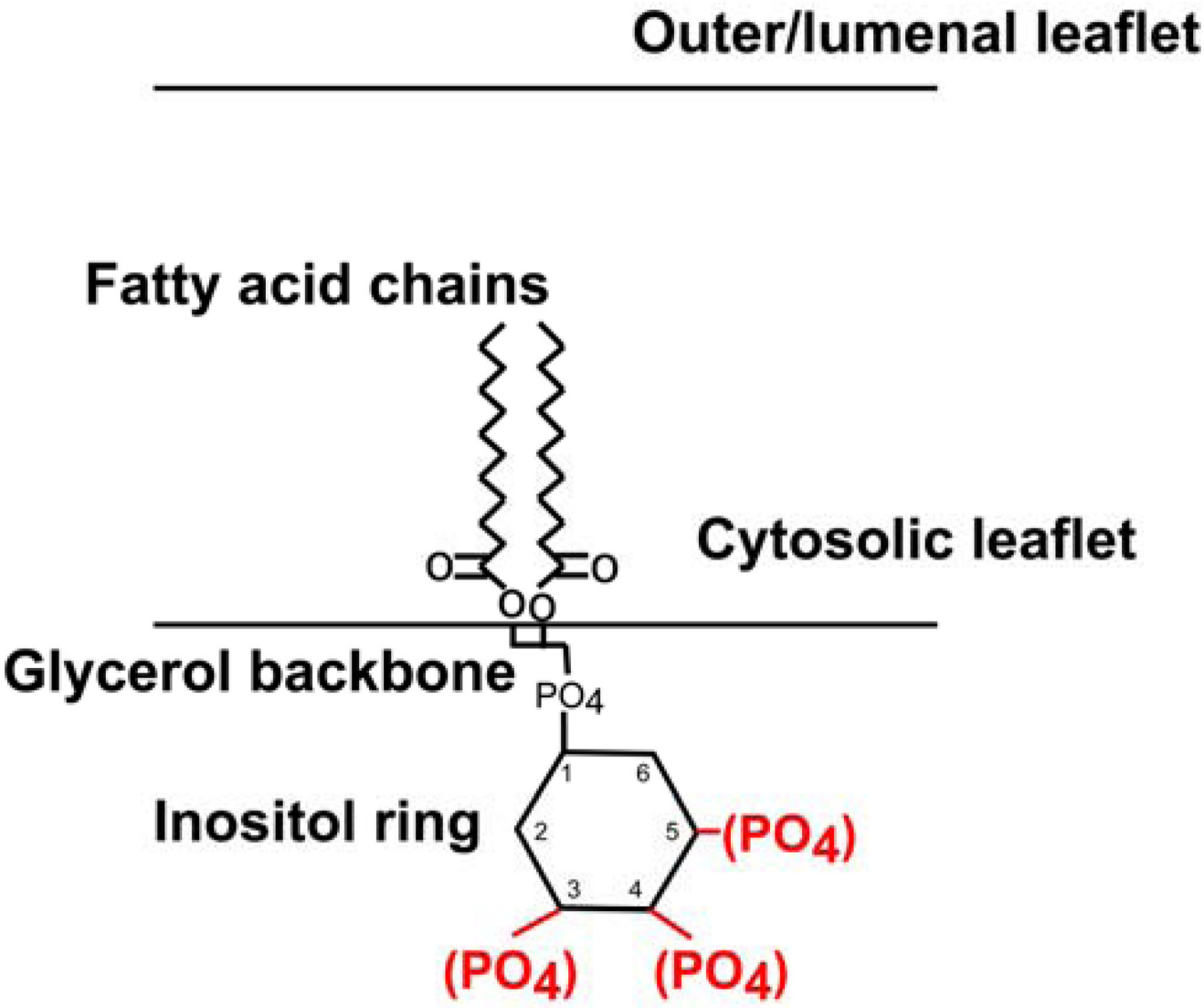

Figure 1.
Phosphoinositides are phosphorylated derivatives of PtdIns. The chemical structures of PtdIns and Phosphoinositides are shown highlighting the inositol headgroup, glycerol backbone and two fatty acyl chains. The inositol headgroup can be combinatorially phosphorylated at the D-3OH, -4OH, -5OH positions of the inositol ring as indicated in red.

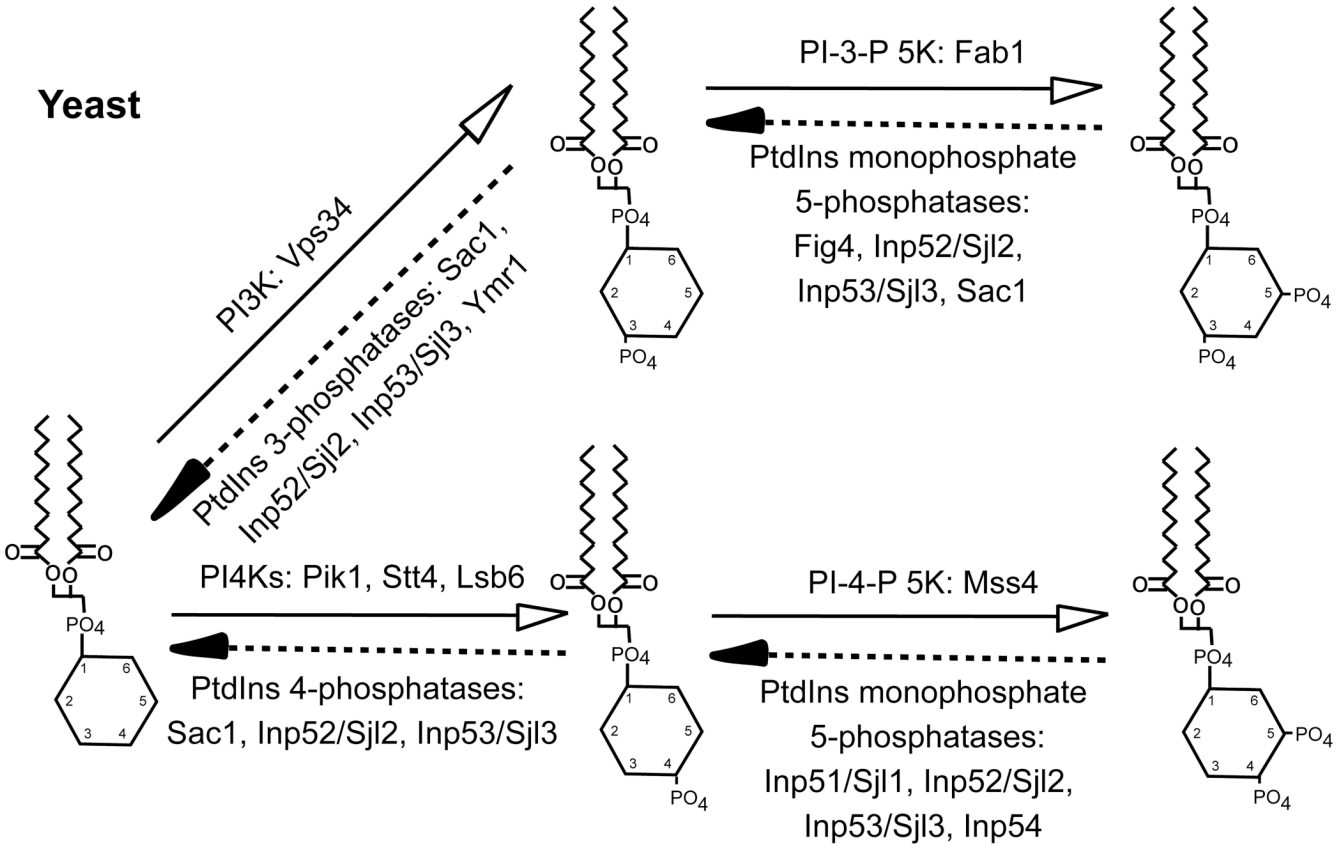

Figure 2.
Phosphoinositide metabolism in the yeast *Saccharomyces cerevisiae*. The execution points of the yeast PtdIns kinases and phosphoinositide phosphatases that regulate the synthesis and turnover of phosphoinositides, respectively, are identified.

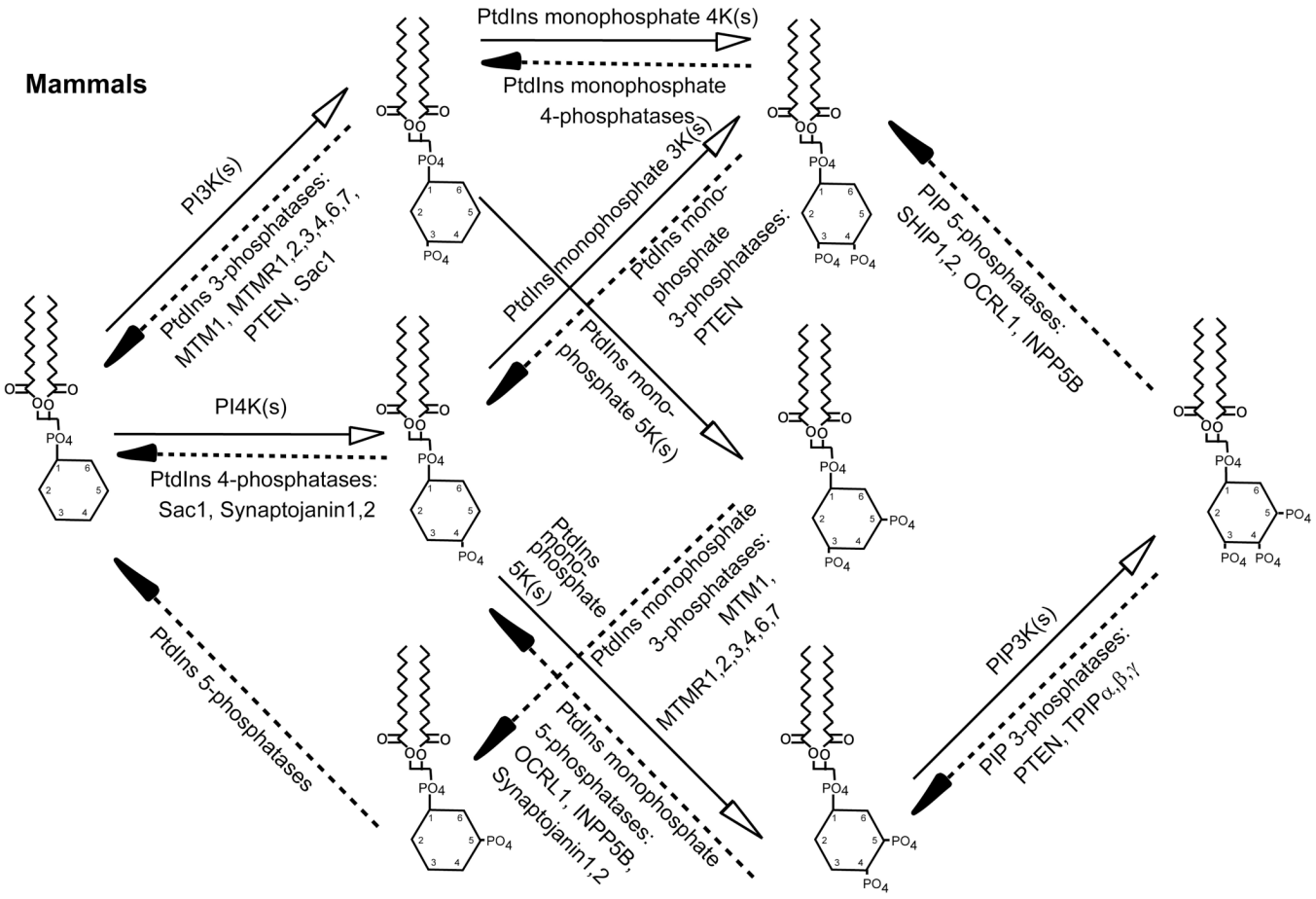

Figure 3.

Phosphoinositide metabolism in mammalian cells. The execution points of the mammalian PtdIns kinases and phosphoinositide phosphatases that regulate the synthesis and turnover of Phosphoinositides, respectively, are identified.

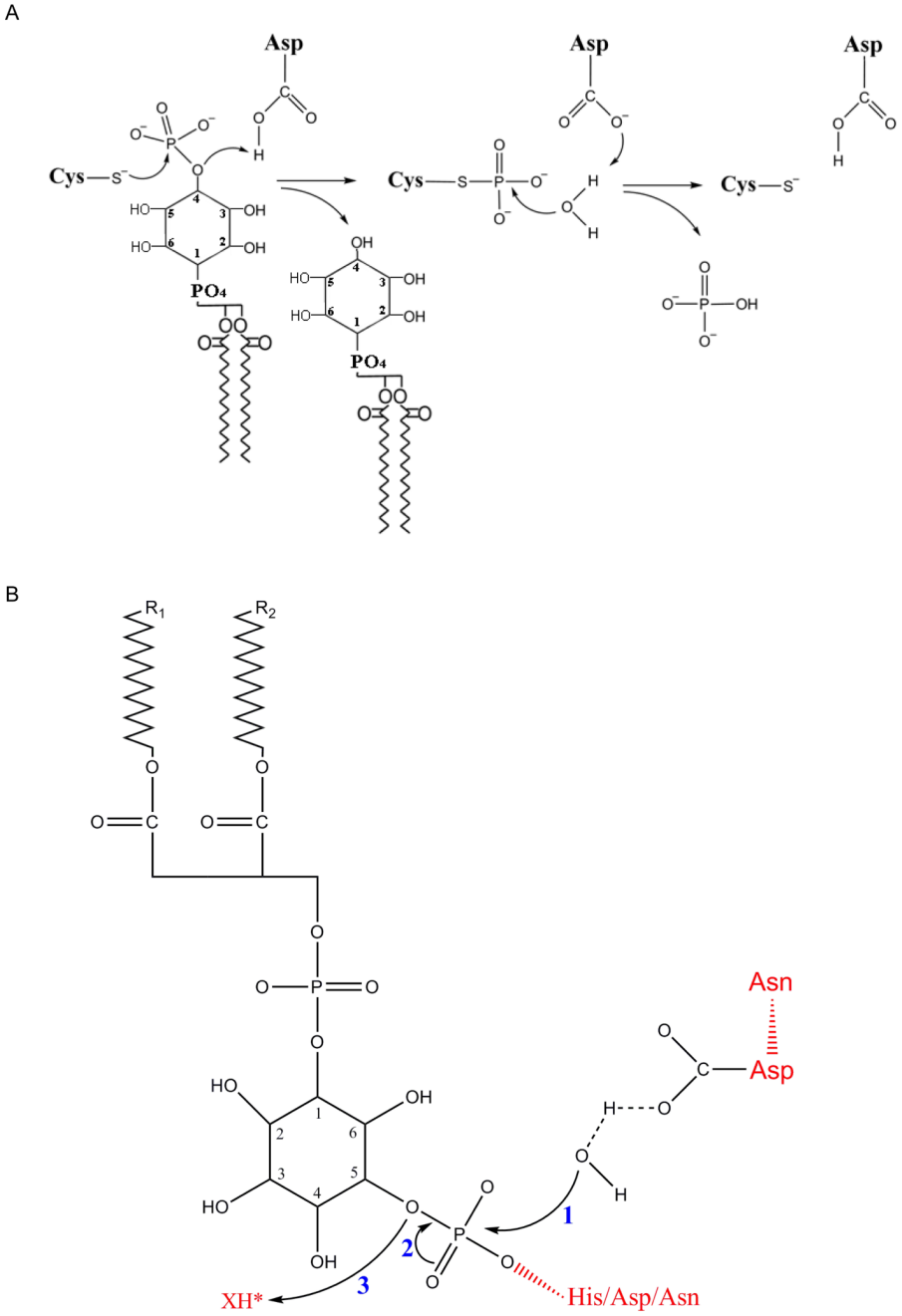

Figure 4.

Catalytic mechanisms of phosphoinositide phosphatases. **(A)** The $CX_5R(T/S)$ phosphoinositide phosphatases of the PTP superfamily. These enzymes catalyze a double-displacement reaction where the leaving group alcohol is displaced by an active-site nucleophile. The resulting phospho-enzyme intermediate is resolved by transfer of the PO_3 group to an acceptor water molecule. An aspartic acid subsequently donates a proton to the leaving-group oxygen to regenerate an uncharged hydroxy group at the position from which the PO_3 group was displaced (Guan and Dixon, 1991; Fauman and Saper, 1996; Hughes *et al.*, 2000a). (**B**) The inositol polyphosphate 5'-phosphatases of the AP endonuclease superfamily. These enzymes catalyze a displacement reaction where the leaving group alcohol

is displaced by an actived water molecule as nucleophile. An invariant Asp residue, held in properly position by H-bonding with a conserved Asn, activates the nucleophilic water (**1**). A His/Asp pair cooperates with an invariant Asn to position the target phosphate bond for hydrolysis. Mg^{2+} is thought to stabilize a transition state in the reaction. There is no phosphoenzyme intermediate in this catalytic mechanism as the PO_3 group is transferred directly to the nucleophilic water molecule (**2**). Either water, or some other functional group of the enzyme (both possibilities generically designated as XH*; **3**), donates a proton to the leaving-group oxygen to regenerate an uncharged hydroxyl group at the position from which the PO_3 group was displaced.

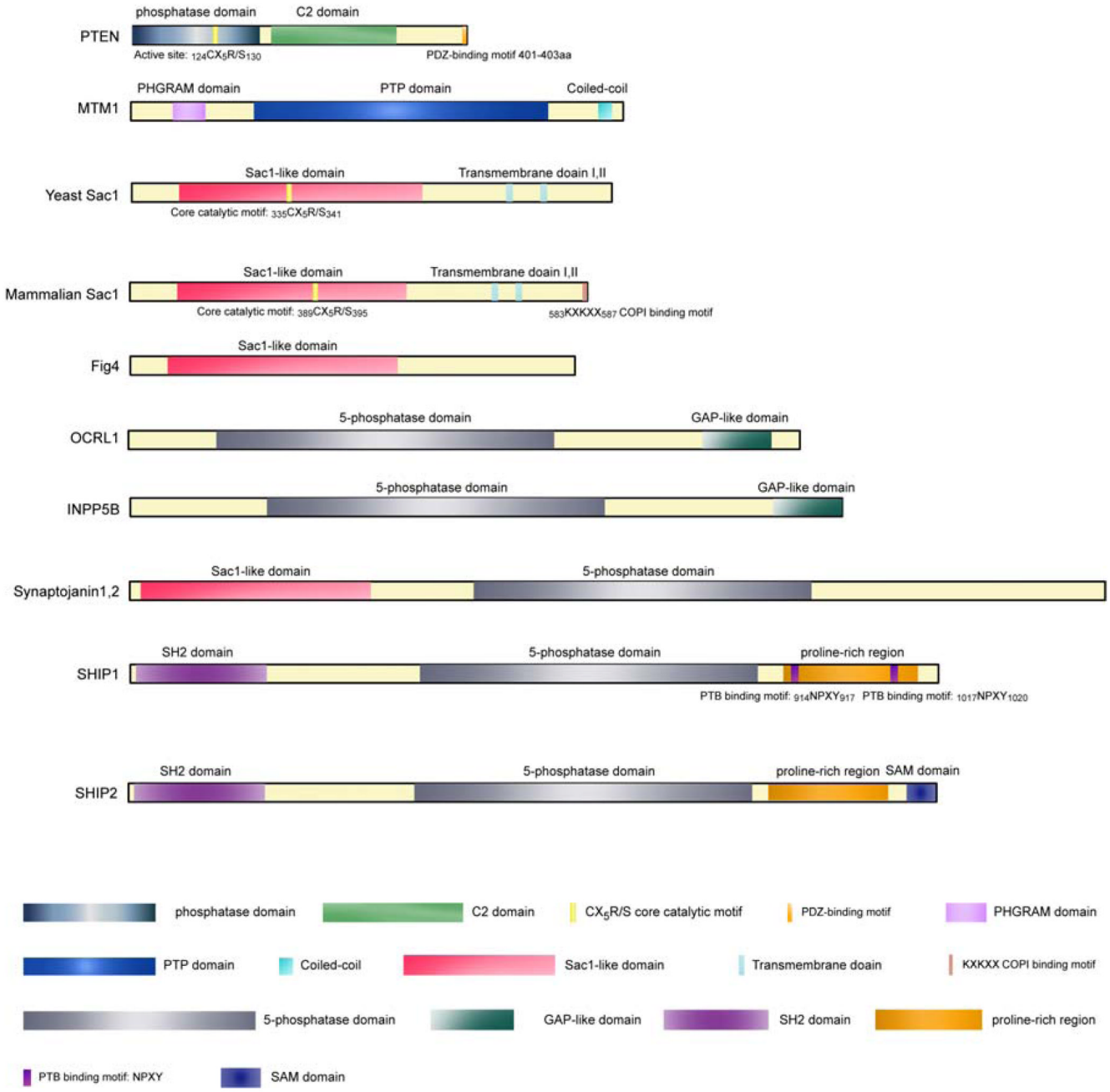

Figure 5.

Domain organization of phosphoinositide phosphatases. The domain structures of PTEN, MTM1, Sac1, Fig4, OCRL1, INPP5B, Synaptojanin 1,2, and SHIP1,2 are illustrated. Relevant functional domains and motifs are indicated.

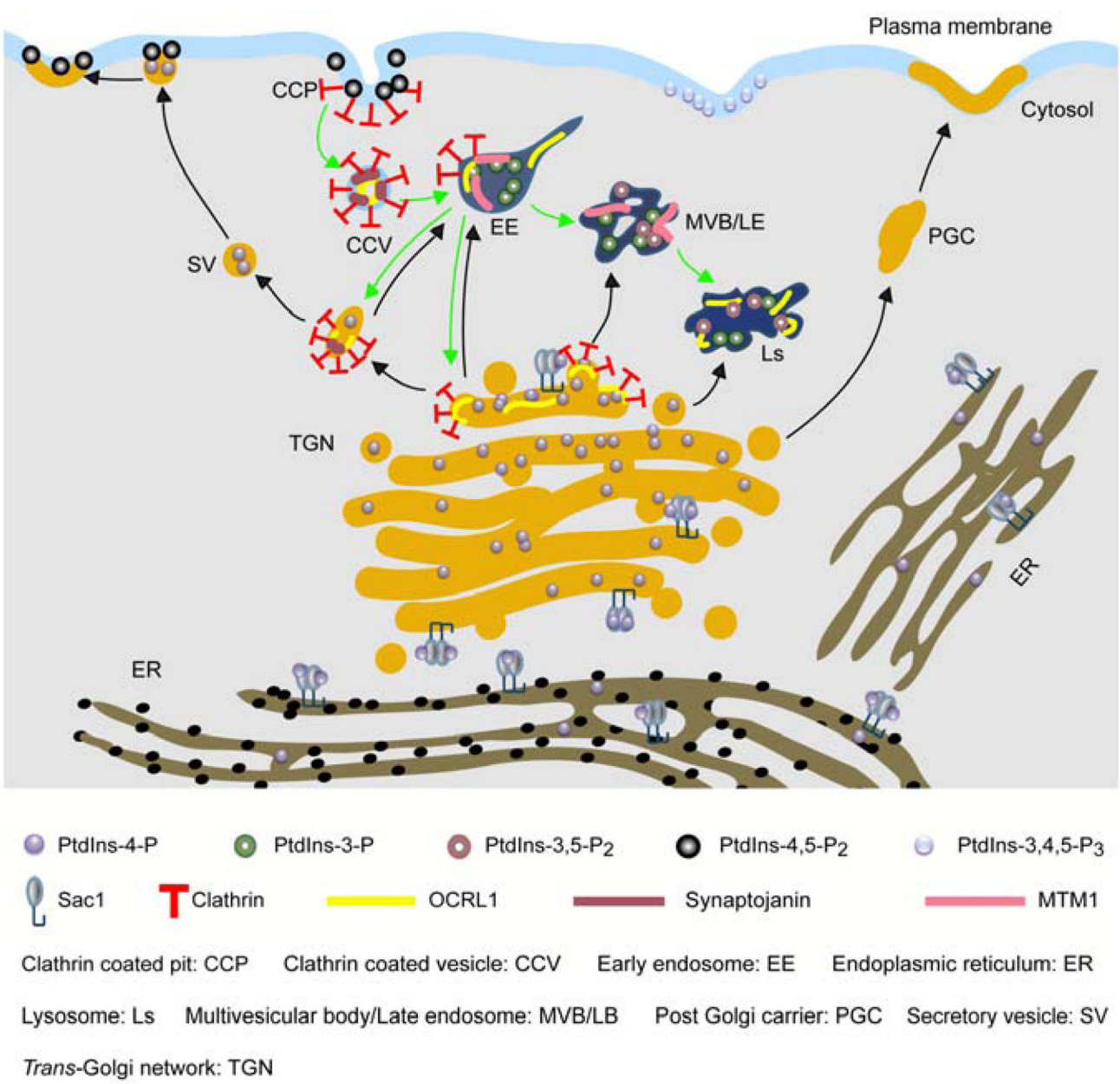

Figure 6.

Phosphoinositide phosphatases and regulation of membrane trafficking. The phosphoinositide 4-phosphatase Sac1 is localized primarily in ER and Golgi membranes. OCRL1 (yellow) is localized to the Golgi/endosomal system and is a cargo of the clathrin-coated vesicles (CCVs) responsible for bidirectional membrane trafficking between TGN and endosomes. In the neuronal presynaptic plasma membrane, a PtdIns- 4,5-P_2 pool is accessed by synaptojanin 1 during CCV uncoating. In non-neuronal cells, synaptojanin 2 (maroon) functions at an early stage of clathrin-mediated endocytosis. MTM1 (pink) regulates endocytic traffic and PTEN (red) degrades a PtdIns-3,4,5-P_3 pool involved in membrane trafficking events associated with phagocytosis. Abbreviations: CCP, clathrin-coated pit; CCV, clathrin-coated vesicle; EE, early endosome; ER, endoplasmic reticulum; Ly, lysosome; MVB/LE, multivesicular body/late endosome; PGC, post-Golgi carrier; SV, secretory vesicle; TGN, trans-Golgi network.

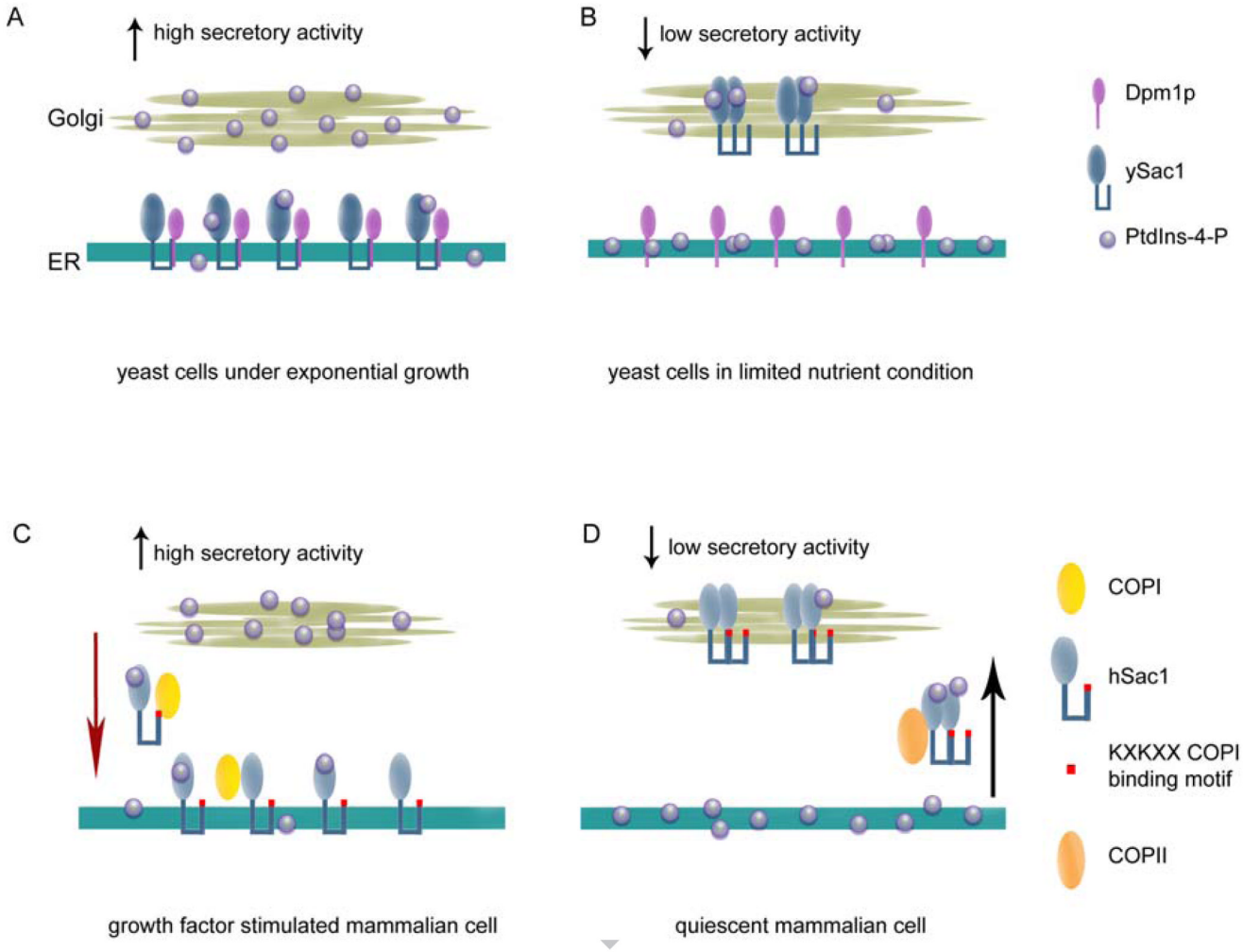

Figure 7.

Dynamic regulation of Sac1 localization in yeast and mammals. When yeast cells are in exponential growth, the ER dolicholphosphate mannose synthase Dpm1p interacts with yeast Sac1 (ySac1) via their respective trans-membrane domains. This interaction restricts Sac1 to the ER, results in elevated Golgi PtdIns-4-P, and promotes robust secretory activity **(A)**. Upon nutrient limitation, the ySac1 interaction with Dpm1p is broken and ySac1 escapes to Golgi membranes. Increased Golgi ySac1 reduces PtdIns-4-P levels and, subsequently, secretory activity **(B)**. In mammalian cells, growth factor signaling promotes retrograde transport of Sac1 from Golgi membranes to the ER via an ARF- and COPI-dependent pathway. Consequent elevation of Golgi PtdIns-4-P promotes optimal secretion **(C)**. In quiescent cells, Sac1 oligomerizes and translocates to the Golgi via a COPII-mediated pathway. Increased Golgi Sac1 reduces PtdIn-4-P pools and downregulates secretory activity **(D)**.