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REVIEW

The Sac domain-containing phosphoinositide phosphatases: structure, function, and disease

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Abstract **Phosphoinositides (PIs) have long been known to have an essential role in cell physiology. Their intracellular localization and concentration must be tightly regulated for their proper function. This spatial and temporal regulation is achieved by a large number of PI kinases and phosphatases that are present throughout eukaryotic species. One family of these enzymes contains a conserved PI phosphatase domain termed Sac. Although the Sac domain is homologous among different Sac domain-containing proteins, all appear to exhibit varied substrate specificity and subcellular localization. Dysfunctions in several members of this family are implicated in a range of human diseases such as cardiac hypertrophy, bipolar disorder, Down's syndrome, Charcot-Marie-Tooth disease (CMT) and Amyotrophic Lateral Sclerosis (ALS). In plant, several Sac domain-containing proteins have been implicated in the stress response, chloroplast function and polarized secretion. In this review, we focus on recent findings in the family of Sac domain-containing PI phosphatases in yeast, mammal and plant, including the structural analysis into the mechanism of enzymatic activity, cellular functions, and their roles in disease pathophysiology.**

Keywords lipid metabolism, membrane trafficking

Introduction

Phosphoinositides (PIs), discovered in the 1950s by Marble and Lowell Hokin (Hokin M R and Hokin L E, 1953; Hokin L E and Hokin M R, 1958), are phosphorylated forms of a precursor molecule called phosphatidylinositol (PtdIns). PtdIns is composed of diacylglycerol (DAG) connected to a phosphate followed by a cytoplasmic exposed headgroup *myo*-inositol ring. The five hydroxyl groups attached to the inositol headgroup can be reversibly phosphorylated at the 3′, 4′ and 5′ positions to generate seven distinct PI isoforms, whereas positions 2′ and 6′ are obstructed presumably due to steric hindrance. In the past 30 years, the roles of PIs have been characterized extensively in both uni- and multi-cellular organisms. PtdIns comprise ~10% of total cellular lipids and PI constitute less than 1%, yet they control vital cellular processes such as cell signaling, proliferation, organization of cytoskeleton, and membrane trafficking (Odorizzi et al., 2000; De Matteis and Godi, 2004; Di Paolo and De Camilli, 2006). It has also been shown that PIs play a role in regulating ion channel activity (Wang et al., 2012), transcription, mRNA trafficking, RNA splicing (Osborne et al., 2001), chromatin structure (Viiri et al., 2012), nuclear export (Dieck et al., 2012), and bacterial infection (Pizarro-Cerdá and Cossart, 2004; Ham et al., 2011). The mechanism for controlling such divergent processes is achieved mainly by three non-mutually exclusive schemes. First, each PI has its own unique subcellular localization to allow them to selectively recruit proteins containing PI recognition modules (e.g. ANTH, ENTH, FYVE, PX, PH, PDZ, PHD, PTB, C2, GRAM, PROPPINs, Tuby, and FERM) to specific organelle membranes (Cullen et al., 2001; Lemmon, 2003; Takenawa and Itoh, 2006; Krauss and Haucke, 2007). Second, the resulting heterogeneous anionic charge (produced by the number of phosphate groups) at each organelle membranes can differentially recruit soluble polycationic proteins (Hammond et al., 2012). Third, PI in combination with specific Rab GTPase can constitute coincidence detection via recruitment of effectors with dual recognition domains (Di Paolo and De Camilli, 2006; Jean and Kiger, 2012). Coincidence detection

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increases the affinity and therefore the specificity of effector localization.

The heterogeneous distribution of PIs imparts an "organelle identity" to the cell. The maintenance of the selective concentration of specific PI species, as well as the dynamic control of PI composition in response to acute signaling inputs is achieved by a large number of PI kinases and phosphatases that are present throughout eukaryotic species. One family of these enzymes contains a conserved PI phosphatase domain termed Sac (**S**uppressor of yeast **ac**tin mutations).

The Sac phosphatase domain family in yeast and mammal

The first Sac domain-containing phosphatase, Sac1, was discovered in two independent genetic screens searching for modifiers of actin cytoskeleton defects (Novick et al., 1989) and of trans-Golgi network exocytic failure caused by inactivation of Sec14p (Bankaitis et al., 1990). Subsequently, the Sac domain was found to share considerable homology with mammalian nerve terminal protein, synaptojanin (McPherson et al., 1996) and was demonstrated to possess phosphoinositide phosphatase activity (Guo et al., 1999). With the advancement in genome sequencing, all genes containing the Sac domain have been annotated in yeast, and mammal. There are five Sac domain-containing proteins in both human and yeast. Members include the transmembrane protein Sac1, cytosolic proteins Sac2/Inpp5f, Sac3/Fig4, and synaptojanin 1 and 2. These Sac domain-containing proteins can be divided into three subfamilies: 1) Sac domain with two transmembrane motifs (Sac1); 2) Sac domain followed by along C-terminal sequences with or without recognizable structural domains (Sac2 and Sac3); 3) The N-terminal Sac domain immediately followed by a central PI 5-phosphatase domainand a C-terminal prolinerich region (synaptojanin 1 and 2) (Fig. 1A).

Sac domain structure and catalytic mechanism

The Sac domain of yeast Sac1 is the only crystal structure solved in the family to this day (Manford et al., 2010). The structure revealed that the Sac homology domain is comprised of two closely packed sub-domains: a novel N-terminal sub-domain and the catalytic phosphoinositide phosphatase sub-domain (Fig. 2A). The N-domain is comprised of a unique fold with three layers of β-sheet and three short and one long α-helix. The catalytic domain consists of a nine-stranded β-sheet flanked by five α-helixes with two and three on each side of the β-sheet. The catalytic site of Sac domain consists of a peptide motif with a sequence of CX_5R (a catalytic cysteine residue followed by 5 amino acids and a conserved arginine residue). Structural comparison of the catalytic domain of Sac1 with other CX_5R motif based proteins and lipid phosphatases revealed that the topology of the catalytic domain is conserved with other phosphatases. All the CX_5R motif based phosphatases share a common four parallel β-strands and one α-helix at the core. The loop connecting this α-helix and one of the β-strands harbors the catalytic CX_5R motif (P-loop) (Fig. 2A). The conserved structural topology of all lipid and protein tyrosine phosphatases suggests a conserved catalytic mechanism (Barford et al., 1998). First, the conserved arginine residue, together with the backbone amide groups of the P-loop will bind and position the phosphate group at the catalytic site. Second, the catalytic cysteine will attack the phosphate group to generate a cysteinyl-phosphate intermediate and the dephosphorylated product. Third, an aspartic acid residue either located in a so-called "WPD" loop (Barford et al., 1998) or within the catalytic P-loop motif will act as a general acid, donating a proton to the releasing product. Finally, the same aspartic acid residue will function as a general base to activate a water molecule, which will then hydrolyze the phosphate from the catalytic cysteine through a nucleophilic attack.

Since the lipid substrates of Sac1 are embedded in negatively charged membrane bilayer, it is not surprising that the overall surface charge of Sac1 is distributed in a way that is negatively charged on one surface and positively charged on the opposite surface. The catalytic CX_5R motif is localized on the positively charged surface, which will interface with the anionic membrane bilayer (Manford et al., 2010). Interestingly, this membrane interfacing surface is enriched with highly conserved residues as revealed by evolutionary trace method (Lichtarge et al., 1996) (Fig. 2B). This conservation suggests a general catalytic mechanism, including membrane interaction, and substrate recognition among all Sac domains.

Unlike other CX_5R-based PI phosphatases, such as PTEN (Lee et al., 1999) or MTM (Begley et al., 2003), the catalytic P-loop of Sac1 has a striking conformation in that the catalytic cysteine is far away from the conserved arginine in the CX_5R motif. These structural findings suggest that conformational change of the catalytic P-loop may be required to achieve functional arrangement of the catalytic residues. In agreement with the structural observation, Sac1 is found to be an allosteric enzyme and its activity can be stimulated by anionic phospholipids, PtdIns and phosphatidyl serine (PS) (Zhong et al., 2012). However, a detail picture of the catalytic mechanism would require co-crystal structure of Sac1 with its substrate or activator.

Sac1

Yeast Sac1

Yeast Sac1 is a 67 kDa type II transmembrane protein that localizes to both the endoplasmic reticulum (ER) and Golgi apparatus (Whitters et al., 1993). The retention of yeast Sac1

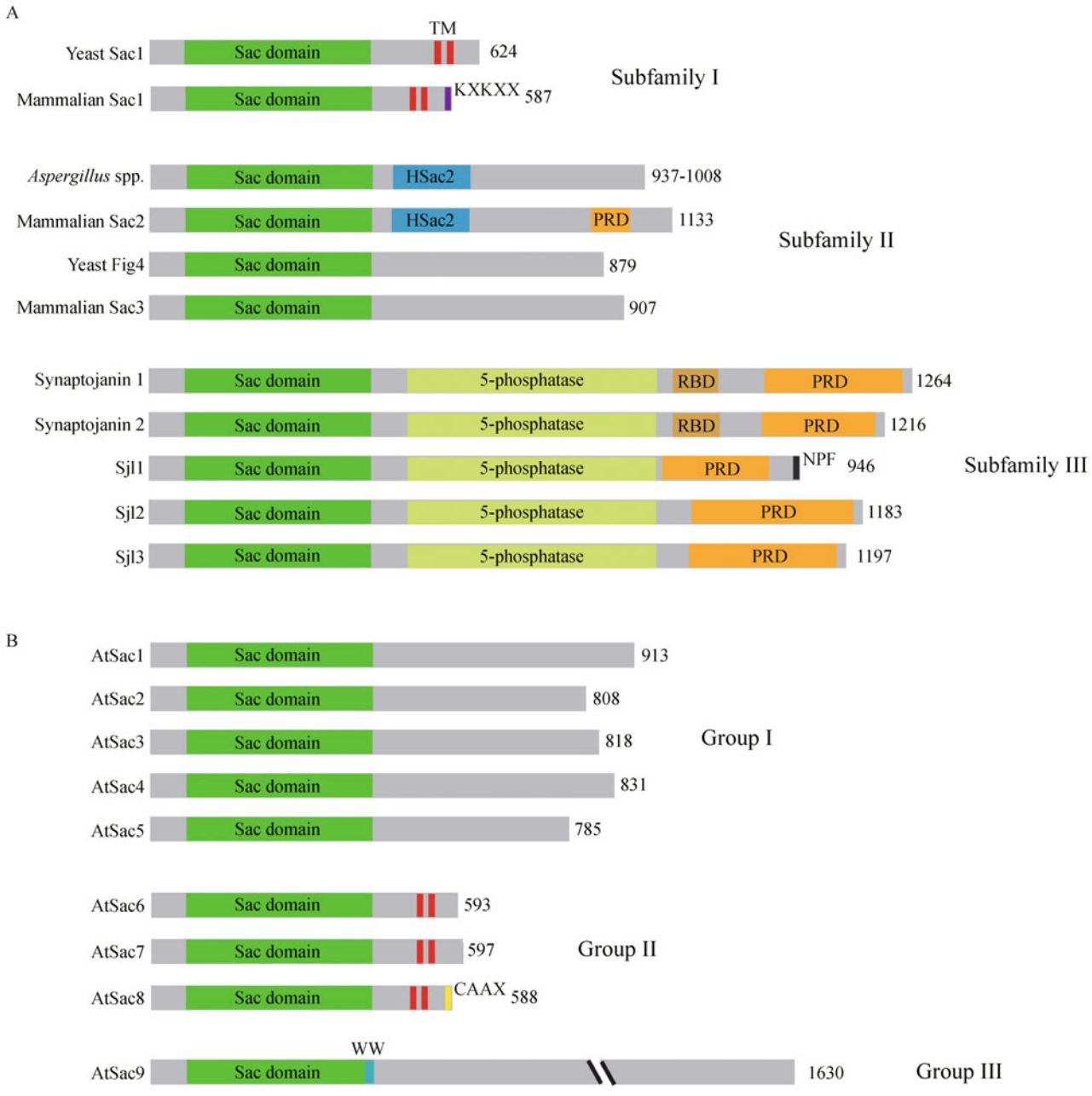

Figure 1 Sac domain-containing phosphatase family. (A) The yeast and mammalian Sac phosphatases can be divided into three subfamilies. TM: transmembrane motif; HSac2: homology of Sac2; KXKXX: COPI binding motif; RBD: RNA binding domain; PRD: proline rich domain. (B) The plant Sac domain-containing phosphatases.

in the ER is dependent on the interaction with the integral membrane protein Dpm1 during times of rapid cell growth (Faulhammer et al., 2005). Whereas the localization of Golgi is likely via interactions with other proteins such as Vps74 (the ortholog of human GOLPH3) (Wood et al., 2012). *In vitro* assays have shown that Sac1 dephosphorylates PI(3)P, PI(4)P, and PI(3,5)P_2 (Guo et al., 1999). In yeast, genetic ablation of Sac1 activity results in a nearly 10-fold increase in the steady-state levels of PI(4)P and a modest increase in the levels of PI(3)P (Rivas et al., 1999; Nemoto et al., 2000), suggesting that yeast Sac1 is the major enzyme in PI(4)P turnover *in vivo* (Table 1) (Guo et al., 1999; Strahl and Thorner, 2007). Loss of function of yeast Sac1 also affects a broad range of cellular defects such as disorganization of the actin cytoskeleton (Foti et al., 2001), secretory pathway (Schorr et al., 2001), inositol auxotrophy, cold sensitivity (Rivas et al., 1999), multiple drug sensitivity (Hughes et al., 1999), vacuolar function (Tahirovic et al., 2005), cell wall maintenance, ER protein quality control systems (Kochendörfer et al., 1999), and sphingolipid metabolism (Brice et al.,

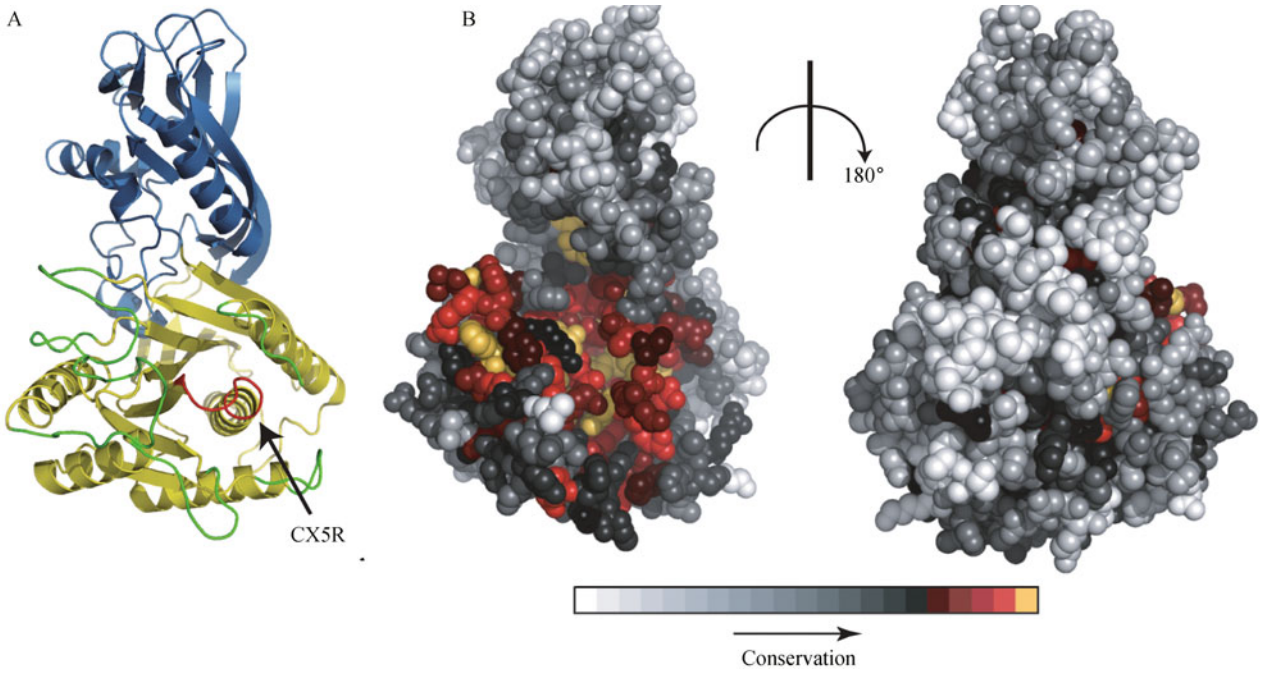

Figure 2 Sac domain structure of yeast Sac1. (A) The Sac phosphatase domain is comprised of two sub-domains: a novel N-terminal domain (blue) and the catalytic domain (yellow). The loop colored red represents the CX_5R motif. (B) Space filling model of the Sac domain with residues colored based on evolutionary conservation, which is calculated by an evolution trace method (Lichtarge et al., 1996). The least and highest conserved residues are colored from white to yellow respectively.

2009). Despite the broad impact of Sac1 on yeast cellular functions, Sac1 is not essential for cell viability.

Interestingly, deletion of Sac1 leads to a specific increase of PI(4)P at the plasma membrane (Foti et al., 2001; Stefan et al., 2011). Since Sac1 is localized on the ER and Golgi membrane, an intriguing question was raised as to whether Sac1 is able to hydrolyze PI(4)P in a trans mode. The crystal structure of Sac1 revealed a ~70-residue flexible linker between the catalytic domain and the C-terminal transmembrane domain that allows the catalytic domain of Sac1 to stretch more than 15 nm from its anchoring membrane (Manford et al., 2010). This idea is further supported by a recent finding that Sac1 can hydrolyze PI(4)P on the plasma membrane *in trans* at ER-plasma membrane contact sites in the presence of oxysterol binding homology (Osh) proteins (Stefan et al., 2011).

Mammalian Sac1

In mammals, Sac1 is ubiquitously expressed in most mouse tissues with a particularly high level in cerebellum, hippocampus, and heart (Nemoto et al., 2000). Similar to yeast Sac1, mammalian Sac1 shuttles between ER and Golgi apparatus. In quiescent cells, mammalian Sac1 accumulates at the Golgi to downregulate anterograde trafficking by depleting PI(4)P, thus slow secretion from the Golgi apparatus (Blagoveshchenskaya et al., 2008; Blagoveshchenskaya and Mayinger, 2009; Piao and Mayinger, 2012). The Golgi localization has been shown to be dependent on the leucine zipper region of mammalian Sac1 to form oligomer and the recruitment of the coat protein COPII complex. When quiescent cells are stimulated by mitogens, Sac1 dissociates from its oligomeric state and rapidly shuttles back to the endoplasmic reticulum (ER). The retrograde trafficking of Sac1 is mediated by the coatomer COPI complex through the interaction with a canonical KXKXX motif at C-terminus of mammalian Sac1 (Rohde et al., 2003) (Fig. 1A).

Unlike the case in yeast, genetic ablation of the single murine Sac1 gene results in progeny failing to progress past E3.5 (Liu et al., 2008) (Table 1). In mammalian cells, knockdown of Sac1 expression results in disorganization of Golgi membranes, and mitotic spindle, which leads to decrease in cell viability in cells due to a blockade in progression through the G2-M cell cycle (Liu et al., 2008; Cheong et al., 2010).

Sac1 in other species

In *Drosophila*, Sac1 has been shown to regulate axon guidance in the embryonic CNS midline and Sac1 mutants die as embryos with defects in dorsal closure (Wei et al., 2003; Lee et al., 2011). Loss of *Drosophila* Sac1 leads to improper activation of several key events during development: cell shape change in the amnioserosa, increase in JNK signaling (Wei et al., 2003) and activation of Hedgehog signaling (Yavari et al., 2010). These collective data suggest an evolutionarily conserved function and point to an essential housekeeping role of Sac1 in multicellular organisms.

Sac2/INPP5f

Sac2, a 128 kDa protein encoded by the gene INPP5f, is an evolutionarily conserved protein in multi-cellular organisms from nematode to human. It is the only Sac phosphatase that lacks an ortholog in *S. cerevisiae*, but is found in other Fungi genus such as *Aspergillus* spp. and *Yarrowia lipolytica*. PFAM domain searches of Sac2 reveal that in addition to the N-terminal Sac phosphatase domain, it possesses a homology Sac2 (hSac2) domain of approximately 120 amino acids of unknown function and structure (Fig. 1A). Interestingly, this unique domain is also present in the mouse tumor protein p63-regulated gene 1-like protein (TPRGL), which is a vertebrate-specific presynaptic protein in CNS (Kremer et al., 2007). Unlike Sac1, Sac2 does not hydrolyze mono-phosphorylated lipids (Minagawa et al., 2001). Initial cloning and characterization show that this enzyme exhibits 5-phosphatase activity specific for PI(4,5)P_2 and PI(3,4,5)P_3, generating PI(4)P and PI(3,4)P_2, respectively (Minagawa et al., 2001). However, given the fact that Sac1 and Sac2 both contain identical catalytic P-loop sequence (CMDCLDRT), the molecular details regarding how Sac2 can recognize di- and tri-phosphorylated lipids (as opposed to mono-phosphorylated lipids in Sac1) remain unclear.

In human, northern blot analysis shows that Sac2 has the highest level of expression in the brain, heart, skeletal muscle, kidney and placenta (Minagawa et al., 2001). In adult mouse heart, the regulation of Sac2 expression level is controlled by histone deacetylase-2 (Hdac2) (Trivedi et al., 2007). In Hdac2 deficient mice, Sac2 expression is increased, which results in attenuated cardiac hypertrophy due to constitutive activation of glycogen synthase kinase 3 beta (Gsk3β) via the activation of Akt. However, in Hdac2 transgenic mice, Sac2 expression is transcriptionally repressed, which results in augmented cardiac hypertrophy associated with inactivated Gsk3β (Trivedi et al., 2007). The role of Sac2 in regulating cardiac hypertrophy is further demonstrated in Sac2 knockout mice. In response to stress, the Sac2 knockout mice have abnormal fetal gene reactivation and heart hypertrophy compared to wild type littermates (Zhu et al., 2009) (Table 1). These data suggest that Sac2 is an important regulator in cardiac hypertrophy response and the regulation may be through its enzymatic activity on PI(3,4,5)P_3, which in turn down-regulates PI3K/AKT signaling pathway.

Sac3/Fig4p

Yeast Sac3/Fig4p

Sac3/Fig4p, a 97 kDa protein encoded by the gene *Fig4* (Factor Induced Gene), was originally identified in a large-scale transposon tagging screen for genes induced by mating pheromone in *S. cerevisiae* (Erdman et al., 1998). Sac3/Fig4p is a phosphoinositide 5-phosphatase that specifically hydrolyzes PI(3,5)P_2 to generate PI(3)P both *in vitro* and *in vivo* (Duex et al., 2006a, 2006b) (Table 1). Unlike other Sac domain proteins, the dephosphorylation reaction is Mg^{2+} dependent (Rudge et al., 2004). Mutations in *Fig4* result in abnormal actin distribution at the shmoo tip, a failure to establish mating cell polarity leading to enlarged cells, and reduced mating efficiency (Erdman et al., 1998). Fig4p-GFP is localized to the limiting membrane of yeast vacuole, and this localization is dependent on a scaffold protein Vac14 (Rudge et al., 2004). Interestingly, Vac14 also positively regulates the Fab1 kinase that is responsible for generating PI(3,5)P_2 from PI(3)P, suggesting the formation of a multi-protein complex in regulating PI(3,5)P_2 levels (Rudge et al., 2004). In this Vac14-Fig4-Fab1 complex, Fig4 is unexpectedly required for the activation of Fab1 to synthesize PI(3,5)P_2 (Gary et al., 2002). For this reason, deletion of Fig4 elicits reduced, rather than elevated levels of PI(3,5)P_2. In agreement with this notion, Fig4 is found to be responsible for the hyperosmotic shock-induced increase as well as the turnover in PI(3,5)P_2 levels (Duex et al., 2006a).

Mammalian Sac3/Fig4p function and disease

In mouse, Sac3/Fig4 RNA transcript is ubiquitously detected in all tissues, with highest level in the testes, spleen and heart (Chow et al., 2007), whereas Sac3/Fig4 protein level is highest in the brain (Guo et al., 2012; Sbrissa et al., 2007). The Vac14-Fig4-Fab1 ternary protein complex is also conserved in mammals and is responsible for the acute regulation of subcellular levels of PI(3,5)P_2 (Jin et al., 2008; Sbrissa et al., 2008).

Genetic mutations of Sac3/Fig4 lead to a number of diseases in human and mouse, including an autosomal recessive Charcot-Marie-Tooth disorder (CMT4J) and a subset of ALS in human (Chow et al., 2007; Zhang et al., 2008; Chow et al., 2009) as well as neurodegeneration in the "pale tremor" mouse that exhibits severe tremor, abnormal gait and diluted pigmentation (Chow et al., 2007; Lenk et al., 2011) (Table 1). *Fig4* null mice reveal a dramatic reduction in myelin in the brain, spongiform degeneration, gliosis, and junvenile lethality (Nicholson et al., 2011; Winters et al., 2011; Ferguson et al., 2012). These neurological defects have been proposed to be caused by enlarged vacuoles in neurons, which may physically interfere with normal vesicular trafficking. In support of this hypothesis, time-lapse imaging of fibroblasts from CMT4J patients displays impaired trafficking of intracellular organelles due to enlarged vacuoles (Zhang et al., 2008). Furthermore, $Fig4^{-/-}$ derived fibroblasts and neurons exhibit enlarged endosomal and lysosomal compartments, and impaired organelle trafficking (Ferguson et al., 2009).

Sac3/Fig4p disease mutations

Sac3 is a large protein with an N-terminal Sac homology domain (Fig. 1A). Several missense mutations in the gene are

Table 1 Sac domain enzymatic activities and associated phenotypes.

Protein name	*in vitro* substrate	Major *in vivo* substrate	Phenotype
Yeast Sac1	PI(3)P, PI(4)P, PI(5)P, $PI(3,5)P_2$	PI(4)P	Cold-sensitive growth, actin reorganization, inositol auxotrophy, etc.
Mammalian Sac1	PI(3)P, PI(4)P, PI(5)P, $PI(3,5)P_2$	PI(4)P	Preimplantation lethality in mouse
			N/A
Aspergillus spp.	N/A	N/A	N/A
Mammalian Sac2	$PI(4,5)P_2$, $PI(3,4,5)P_3$	$PI(3,4,5)P_3$?	Stress-induced cardiac hypertrophy
Yeast Fig4	$PI(3,5)P_2$	$PI(3,5)P_2$	Vacuole morphology defects
Mammalian Sac3	PI(3)P, PI(4)P, $PI(3,5)P_2$	$PI(3,5)P_2$	CMT4J, ALS, neurodegeneration in the pale tremor mouse and juvenile lethality
Synaptojanin 1	PI(4)P, $PI(4,5)P_2$, $PI(3,4,5)P_3$	$PI(4,5)P_2$,$PI(3,4,5)P_3$	Bipolar disorder, Down's syndrome, early postnatal lethality in mouse
Synaptojanin 2	PI(4)P, $PI(4,5)P_2$, $PI(3,4,5)P_3$	$PI(4,5)P_2$	Hearing loss associated with cochlea hair loss
Sjl1	$PI(4,5)P_2$	$PI(4,5)P_2$	Endocytic defects, increase sensitivity to temperature and neomyocin
Sjl2	PI(3)P, PI(4)P, $PI(3,5)P_2$, $PI(4,5)P_2$	$PI(4,5)P_2$	Endocytic defects, increase sensitivity to temperature and neomyocin
Sjl3	PI(3)P, PI(4)P, $PI(3,5)P_2$, $PI(4,5)P_2$	$PI(4,5)P_2$	Endocytic defects
AtSac1	$PI(3,5)P_2$	$PI(3,5)P_2$	Cell morphology defects
AtSac2	N/A	N/A	N/A
AtSac3	N/A	N/A	N/A
AtSac4	N/A	N/A	N/A
AtSac5	N/A	N/A	N/A
AtSac6	N/A	N/A	N/A
AtSac7	PI(4)P, $PI(3,5)P_2$	PI(4)P	Defective root hair and polarized growth
AtSac8	N/A	N/A	N/A
AtSac9	N/A	$PI(4,5)P_2$?	Hypersensitive to stress

found to be responsible for the CMT4J (I41T and E302K) (Chow et al., 2007; Lenk et al., 2011) and ALS disorder (D48G, D53Y, R388G, and I411V) (Chow et al., 2009). Based on homology structure modeling of the Sac phosphatase domain of human Sac3 to the crystal structure of yeast Sac1, I41T, which is a recessive mutation found in patients with CMT4J, is mapped to the Sac N-terminal sub-domain and 40 Å away from the catalytic site (Fig. 3). The I41T substitution may not affect the catalytic activity of Fig4, but the protein stability and/or protein binding ability. Consistent with this, I41T mutant shows no significant difference in its PI $(3,5)P_2$ phosphatase activity (Chow et al., 2007). In contrast to WT Fig4, I41T mutation leads to a rapid decline in protein level even in the presence of ArPIKfyve (human homolog of Vac14) (Ikonomov et al., 2010) and in yeast two-hybrid system, interaction between Fig4 I41T and Vac14 is significantly impaired (Lenk et al., 2011). Another CMT4J mutation identified at high frequency is E302K. This residue is located on a β-strand of the catalytic domain and is at the interface with the N-domain where it makes several hydrogen bonds with the surrounding residues (Fig. 3). Mutation of E302K is predicted to destabilize the protein and render the protein inactive. This is supported by the fact that Fig4 E302K construct fails to rescue the enlarged vacuole observed in *Fig4* mutant in yeast (Nicholson et al., 2011).

D48G and D53Y, which have been predicted to be responsible for a subset of ALS disease (Chow et al., 2009), is mapped to the exposed surface area on the N-domain. These two mutations may affect the surface property and consequently may interfere with protein–protein interactions mediated through the N-domain. The R388G mutation is located in a loop close to the catalytic P-loop (Fig. 3). This arginine residue likely contributes to positive electrostatic potentials at the catalytic site. R388G mutation may directly affect substrate binding and thus the catalytic activity of the enzyme. In fact, R388G mutant fails to rescue enlarged vacuole phenotype in *ΔFig4* yeast strain (Chow et al., 2009). The other mutation, I411V is found in a hydrophobic core on the opposite site of the P-loop (Fig. 3). The deleterious effect of this substitution by a valine residue with similar hydrophobic property as isoleucine is not clear and the I411V mutant behaves similar to wild type in yeast rescue assays (Chow et al., 2009).

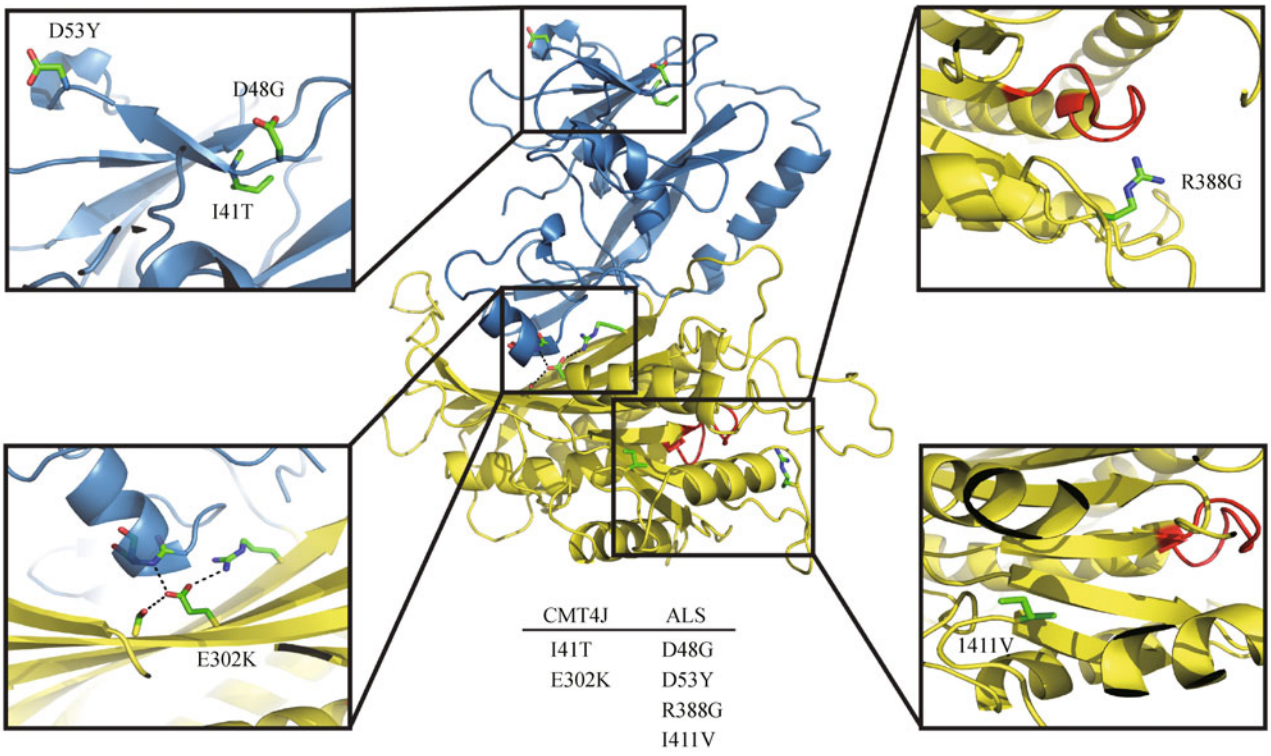

Figure 3 Disease mutations mapping on the modeled structure of the Sac domain of human Sac3/Fig4. The Sac domain of human Sac3 is modeled based on the crystal structure of yeast Sac1 with the MODELER program (Martí-Renom et al., 2000).The local structural environment of six known CMT4J and ALS associated mutations are shown in zoomed-in panels.

Synaptojanins

Enzyme activity and domain organization

Synaptojanins contain an N-terminal Sac domain, a central 5-phosphatase domain, a RNA binding domain (RBD) that is conserved in multi-cellular organisms, but not in yeast, and a C-terminal PRD (Fig. 1A). The Sac phosphatase domain is ~30% sequence identical to yeast Sac1. Biochemical characterization shows that the N-terminal Sac domain of synaptojanins predominantly dephosphorylates PI(3)P and PI(4)P, whereas the 5-phosphatase domain dephosphorylates PI(4,5)P_2 and PI(3,4,5)P_3 at the 5′ position of the inositol ring (Guo et al., 1999; Nemoto et al., 2001) (Table 1). It is speculated that the Sac domain and the 5-phosphatase domains may function in a concerted way. In the concerted model, the 5-phosphatase domain converts PI(4,5)$P_2$to PI(4)P, which is directly channeled to Sac domain for further dephosphorylation to PtIns without accumulation of the intermediate en route.

Yeast synaptojanins function and regulation

S. cerevisiae encodes in its genome three synaptojanin-like genes, sjl1, sjl2, and sjl3, which are involved in endocytic membrane trafficking pathway (Singer-Krüger et al., 1998; Stolz et al., 1998). It is interesting to note that the Sac domain in sjl1 lacks the conserved CX_5R motif and consequently does not exhibit phosphatase activity (Guo et al., 1999). Although neither of these genes is essential for viability, triple mutant of Δ*sjl1-3* is inviable under normal growth conditions (Srinivasan et al., 1997) and Δ*sjl1*Δ*sjl2* double mutant displays a pronounced increase in PI(4,5)P_2 level (Stefan et al., 2002). The hydrolysis of PI(4,5)P_2 is critical for clathrin and actin-mediated endocytosis, including vesicle scission (Haucke, 2005). Consistent with this, sjl2 is shown to localize to cortical actin patches via recruitment of actin patch component Abp1. The physical interaction is critical for the regulation of vesicle formation and fission during endocytosis (Stefan et al., 2005). In addition, sjl1 and sjl2 have been implicated in eisosome receptor-mediated endocytosis based on studies using eisosome marker Pil1 (Murphy et al., 2011). Besides regulating the level of PI(4,5)P_2, sjl2 and sjl3 are also involved in mediating PI(3)P level. In Δ*ymr1*Δ*sjl3* double mutant (ymr1 is the ortholog of MTM family of PI-3-phosphatase), cells display more than two fold increase in PI(3)P level compare to either single mutant (Parrish et al., 2004). The role of sjl3 in PI(3)P level likely contributes to the AP-1/clathrin transport from TGN (trans Golgi network) to endosome (Ha et al., 2003).

Mammalian synaptojanins function and regulation

There are two members that are present in mammals:

synaptojanin 1 and 2. The founding member of this family, the mammalian synaptojanin 1 was first cloned and characterized in the search for presynaptic factors involved in synaptic vesicle recycling (McPherson et al., 1996). Synaptojanin 1 can be alternatively spliced to form a 145 kDa and 170 kDa isoforms. The 145 kDa isoform is highly expressed in adult neurons and at nerve terminals where it is involved in clathrin-dependent synaptic vesicle recycling (Cremona et al., 1999). On the other hand, the 170 kDa isoform, containing an additional C-terminal fragment involved in protein–protein interactions, is ubiquitously expressed in a variety of tissues (Ramjaun and McPherson, 1996). These two splicing isoforms have been shown to be differentially targeted to and regulate the maturation of clathrin-coated pits through the interactions with an array of proteins at sites of clathrin-mediated endocytosis. (Perera et al., 2006). It has been shown that both isoforms interact with the BAR domain protein endophilin to preferentially remove $PI(4,5)P_2$ from curved membranes as opposed to flat ones (Chang-Ileto et al., 2011). Other interacting partners include amphiphysin (Ramjaun and McPherson, 1998), intersectin (Koh et al., 2004; Irie et al., 2005), Grb2 (growth-factor-receptor-bound protein 2) (Ringstad et al., 1997), Snx9 (Yeow-Fong et al., 2005), and Myosin 1E (Krendel et al., 2007). All these interactions are critical for proper localization at clathrin-mediated endocytic sites and/or catalytic activity of the enzyme. However, only the 170 kDa isoform directly interacts with clathrin and its adaptor protein AP-2 (Haffner et al., 2000) via its unique C-terminal portion. These studies suggest a role of synaptojanin in endocytosis. The hydrolysis of $PI(4,5)P_2$ by synaptojanins may facilitate the disassembly of clathrin from endocytic vesicles (Slepnev and De Camilli, 2000).

The *in vivo* functions of synatojanin are also well studied in knock mice. Deletion of this gene in mice causes neurological defects and early postnatal lethality (Cremona et al., 1999). In neurons of synaptojanin mutant mouse, abnormal amount of clathrin-coated vesicles are observed to accumulate at the nerve termini and a delay in re-entry of recycling vesicles, which correlates with increased $PI(4,5)P_2$ levels (Cremona et al., 1999; Kim et al., 2002; Mani et al., 2007).These mutant mice are further shown to have impaired constitutive and induced endocytosis of post synaptic AMPA receptors (Gong and De Camilli, 2008). In contrast to synatojanin 1 deletion, overexpression of synaptojanin 1 in transgenic mice or neuroblastoma cell line leads to enlarged endosomes (Cossec et al., 2012). Interestingly, synatojanin 1 has been shown to be a trisomic gene located at chromosome 21 that is over-expressed in Down's syndrome patients. The increased levels of synaptojanin 1 may account for the observed enlarged endosomes in neurons and brain dysfunction in Down's syndrome patients (Arai et al., 2002; Voronov et al., 2008). Mutations of synaptojanin 1 have also been linked to bipolar disorder (Saito et al., 2001; Stopkova et al., 2004). However, the mechanism for the association of synaptojanin 1 with bipolar disorder is not clear.

Similar to synatojanin 1, synatojanin 2 also consists of several splice isoforms that are referred to as 2A and 2B, in which the 2B variant can undergo further alternative splicing to generate 2B1 and 2B2 (Khvotchev and Südhof, 1998). The 2A isoform is broadly expressed in many tissues, while the 2B isoform is predominantly expressed at nerve terminals in the brain as well as at spermatid manchette in adult testis (Nemoto and De Camilli, 1999; Nemoto et al., 2001). Unlike synaptojanin 1, the function of synatojanin 2 is less well understood. It has been shown that synatojanin 2 can be recruited to plasma membrane via the direct interaction with active Rac1 (Malecz et al., 2000). The interaction of synatojanin 2 with Rac1 has been suggested to play a role in human glioma cell invasion and migration (Chuang et al., 2004). In mouse mutant strain called Mozart, a mutation in synatojanin 2 that abolishes its enzymatic activity has been shown to be responsible for the progressive hearing loss (Manji et al., 2011), suggesting a link between phosphoinositide metabolism to hair cell survival and hearing.

Synaptojanins in other species

The phenotypes observed in both mice and human are further corroborated by studies in *Danio rerio, Drosophila melanogaster*, and *Caenorhabditis elegans*. In *D. rerio*, synaptojanin 1 mutant displays a defect in cone photoreceptor ribbon anchoring, abnormal synaptic transmission at synapses (Holzhausen et al., 2009) as well as a role in maintaining the quantity, fusion and release of synaptic vesicles at hair-cell ribbon synapses (Trapani et al., 2009). In *D. melanogaster*, synaptojanin is recruited by endophilin to promote synaptic vesicle uncoating (Verstreken et al., 2003). Over-expression of synaptojanin in *Drosophila* leads to abnormal synaptic morphology (Chang and Min, 2009). In *C. elegans*, mutations in synaptojanin (unc-26) not only disrupt synaptic vesicle recycling (Harris et al., 2000), but also enhance polyglutamine toxicity which is a phenotype observed in Huntington-interacting protein 1 mutant implicated in neuronal function in Huntington's disease (Parker et al., 2007). These data point to a role of synaptojanins in clathrin-mediated endocytic processes particularly in synaptic vesicle recycling at the nerve terminus.

Plant Sac phosphatase family

Phosphoinositides in plants regulate many cellular activities such as vesicle trafficking (Kim et al., 2001), stomatal movement (Jung et al., 2002), polar tip growth of pollen tube and root hairs (Kost et al., 1999), and responses to stress and hormonal treatments (Pical et al., 1999; De Wald et al., 2001). In *Arabidoposis thaliana*, a genome wide analysis reveals the presence of nine Sac domain-containing phosphatases with high sequence similarity (55%–69%) to Sac domain of yeast

Sac1, which are subsequently named AtSac1 to AtSac9 (Zhong and Ye, 2003). These nine genes can be sub-divided into three groups based on sequence similarity: 1) Group I contains C-terminal sequences with a range of 252–338 amino acids. 2) Group II contains a C-terminal transmembrane motif. 3) Group III consists of only one member, AtSac9, with a long stretch of C-terminal sequences of ~1100 amino acids (Zhong and Ye, 2003) (Fig. 1B). Although other Sac homologs are found in other plant species, the following section will mainly focus on the Sac domain phosphatases in the context of *Arabidoposis*.

The group I enzymes consist of AtSac1–AtSac5, which are considered as Fig4 homologs based on sequence identity. Their gene expression is widely distributed in most plant organs, with relatively lower level in mature leaves. Among this group, AtSac1 has been well characterized. AtSac1 is a PI-phosphatase specific for $PI(3,5)P_2$ and mutations in AtSac1 cause defects in cell morphogenesis, such as a decrease in cell wall synthesis, a reduction in cell elongation, defect in actin cytoskeleton and a global change in plant architecture (Zhong et al., 2005).

The group II, which consists of AtSac6–AtSac8, is most closely related to yeast Sac1, which contains two transmembrane motifs at its C-terminal end. AtSac7 and AtSac8 are widely expressed in most plant organs, whereas AtSac6 is only present in flowers (Zhong and Ye, 2003). Among the three enzymes, only AtSac7 has been characterized so far. AtSac7 has been shown to be enriched at the TGN-like compartment at the tips of growing root hairs (Thole et al., 2008). Mutation of this gene is responsible for the root hair defective 4-1 (rhd4-1) phenotypes that display aberrant root hair and polarized tip growth. This phenotype is associated with an accumulation of PI(4)P in the tip root hairs, consistent with the *in vitro* assay showing its preferred PI phosphatase activity toward PI(4)P (Thole and Nielsen, 2008; Thole et al., 2008).

AtSac9 is the only enzyme belongs in Group III due to its distinct domain features that include a long C-terminal domain and the presence of a WW domain within the Sac domain (Fig. 1B). Microarray analysis shows highest expression in the roots and lower expression in the leaves and flowers (Zhong and Ye, 2003). AtSac9 mutant displays elevated $PI(4,5)P_2$ and water soluble $Ins(1,4,5)P_3$ levels in the roots compared to wild type plants (Williams et al., 2005). The AtSac9 mutants are hypersensitive to external stress and constitutively overexpress stress-induced genes and over-accumulate reactive-oxygen species (Williams et al., 2005).

Concluding remarks

It has only been a little more than 20 years since the discovery of the first Sac domain-containing protein, the yeast Sac1. With the advance of whole-genome sequencing, all the Sac homology domain-containing PI phosphatases have been annotated in quite a few species, including yeast, mammal, and plant. Extensive functional studies on this family of PI phosphatases have been documented and many genetic diseases have been linked to mutations in genes encoding PI phosphatases in this family. Meanwhile, crystal structure for the Sac phosphatase domain provides an excellent model for molecular understanding of genetic mutations occurred on Sac domain-containing phosphatases. Despite the current progress in this field, there are still several key questions remain to be addressed. Given that the catalytic pocket residues are highly conserved across species (Fig. 2B), the mechanistic detail for how substrate recognition and specificity is achieved would require detail structural information of the Sac domain with its cognate substrate. Moreover, how the activity of individual Sac domain containing proteins is precisely controlled by sophisticated signaling networks under physiological or disease state remains to be explored in the future. With the availability of disease models that have deletions or mutations of the Sac domain family members from a variety of organisms, future studies to screen for small-molecules that interfere with phosphoinositide metabolism may lead to potential therapeutic agents for the treatment of human diseases.

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Compliance with ethics guidelines

FoSheng Hsu and Yuxin Mao declare that they have no conflict of interest. This article does not contain any studies with human and animal subjects performed by any of the authors.

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