Research article

Expression of yeast lipid phosphatase Sac1p is regulated by phosphatidylinositol-4-phosphate
Andreas Knödler1, Gerlinde Konrad2 and Peter Mayinger*1

Address: 1Division of Nephrology and Hypertension, Oregon Health & Science University, Portland OR 97239, USA and 2Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), 69120 Heidelberg, Germany

Email: Andreas Knödler - andiknoedler@gmx.de; Gerlinde Konrad - mayinger@ohsu.edu; Peter Mayinger* - mayinger@ohsu.edu

* Corresponding author

Abstract

Background: Phosphoinositides play a central role in regulating processes at intracellular membranes. In yeast, a large number of phospholipid biosynthetic enzymes use a common mechanism for transcriptional regulation. Yet, how the expression of genes encoding lipid kinases and phosphatases is regulated remains unknown.

Results: Here we show that the expression of lipid phosphatase Sac1p in the yeast Saccharomyces cerevisiae is regulated in response to changes in phosphatidylinositol-4-phosphate (PI(4)P) concentrations. Unlike genes encoding enzymes involved in phospholipid biosynthesis, expression of the SAC1 gene is independent of inositol levels. We identified a novel 9-bp motif within the 5′-untranslated region (5′-UTR) of SAC1 that is responsible for PI(4)P-mediated regulation. Upregulation of SAC1 promoter activity correlates with elevated levels of Sac1 protein levels.

Conclusion: Regulation of Sac1p expression via the concentration of its major substrate PI(4)P ensures proper maintenance of compartment-specific pools of PI(4)P.

Background

Phosphorylated derivatives of phosphatidylinositol, collectively called phosphoinositides, play essential roles in a wide range of cellular processes situated at intracellular membranes [1]. Recent evidence indicates that phosphoinositides are not only short-lived signals that activate downstream regulatory networks, but also play constitutive roles in organelle identity and membrane dynamics [2]. A key property of individual phosphoinositides is their precisely regulated compartment-specific localization [2,3]. The control and maintenance of diverse intracellular phosphoinositide pools is achieved through the functional interplay of specific sets of lipid kinases and phosphatases. Although it has been established that deficiencies in certain lipid phosphatases can lead to severe human disease [4], it is unknown as to how the expression of these enzymes is regulated. In contrast, the transcriptional regulation of enzymes involved in the biosynthesis of major membrane phospholipids is well characterized [5]. The cellular concentrations of metabolic intermediates required for phospholipid biosynthesis, such as inositol, choline and phosphatidic acid, determine the levels of expression of their respective biosynthetic enzymes [6,7]. However, whether the expression of lipid phosphatases and kinases is controlled by similar mechanisms remains unclear.

The polyphosphoinositide phosphatase Sac1p is a major regulator of PI(4)P levels at the endoplasmic reticulum (ER) and Golgi [8-10]. The precise distribution of PI(4)P
between these two organelles is critical for coordinating cell growth with the secretory pathway [11]. Here we show that the cellular levels of yeast Sac1p are regulated at the transcriptional level. We have identified a novel 9-bp element within the SAC1 promoter region that is necessary for the regulation of promoter activity. Furthermore, we demonstrate that intracellular levels of PI(4)P correlate with Sac1p protein levels.

**Results**

**Identification of promoter elements for regulation of SAC1 expression**

To identify the regulatory elements that are required for SAC1 gene transcription in the yeast *Saccharomyces cerevisiae*, we generated a reporter construct to examine SAC1 promoter activity. A genomic region comprising 500 bp upstream of the SAC1 open reading frame (SAC1-500/-1) was fused to the gene encoding green fluorescent protein (GFP) (Fig. 1A). The activity of the SAC1(-500/-1) 5'-UTR was then determined by monitoring intracellular GFP levels (Fig. 1B). Yeast cells in which the wild-type copy of SAC1 was deleted, showed a five-fold elevated expression from the reporter construct (Fig. 1B, C). The phosphatase-deficient sac1-8 mutant caused a similar degree of upregulated SAC1-GFP reporter activity (Fig 1D), suggesting that the SAC1 promoter is regulated by a mechanism that responds to a loss of Sac1p enzyme activity. To identify essential elements within the SAC1(-500/-1) 5'-UTR, we constructed a series of truncations within this region and assayed promoter activity (Fig 2A). Elimination of a 150-bp fragment containing a putative TATA box element (bp -50 to -46) within the 5'-UTR abolished expression (SAC1(-500/-150), Fig. 2A). Further truncations led to the discovery of a 100-bp element directly upstream of the SAC1 open reading frame that is necessary for promoter activity (Fig 2A). Significantly, the SAC1(-100/-1) minimal promoter was not only essential for gene transcription, but also sufficient for producing an elevated expression response in a sac1Δ background (Fig. 2B, C).

To further investigate SAC1 promoter elements, we constructed additional deletions within the SAC1(-500/-1) 5'-

---

**Figure 1**

**Elevated activity of the SAC1 promoter in a sac1 mutant background.** (A) Diagram depicting a reporter construct used to examine expression activity in the yeast *Saccharomyces cerevisiae*. The 5'-UTR of SAC1 ranging from bp -500 to -1 was fused to the open reading frame of GFP. (B) Expression from the GFP reporter constructs. Wild-type and sac1Δ yeast cells transformed with a CEN-based plasmid containing the SAC1(-500/-1)-GFP fusion construct were grown to early log phase at 30°C. Cell extracts were analyzed by SDS-PAGE and immunoblotting using anti-GFP and anti-glucose-6-phosphate dehydrogenase (Zwf1p) antibodies. (C, D) Quantitation of relative GFP expression levels in wild-type, sac1Δ (C) and sac1-8 (D) strain backgrounds. Data are from at least three independent experiments (+/−SE).
UTR and tested the individual deletion mutants in the GFP reporter expression assay (Fig. 3A). The deletion of base pairs from position -100 to -83 resulted in a significant loss of transcriptional activity, while removal of similar-sized fragments at either side of this region had no effect on promoter activity (Fig. 3B). Two additional deletion mutations uncovered a region consisting of the 9-bp motif ACCAGAGGT ranging from position -100 to -92, which is indispensable for expression (Fig. 3B). Further analysis using the Saccharomyces cerevisiae promoter database (SCPD) [12] showed that this motif does not overlap with any known recognition site for DNA-binding factors.

**SAC1 expression is regulated independent of inositol levels and ER stress**

Deletion of SAC1 causes specific changes in cellular phosphoinositide levels and induces characteristic cellular defects [13-16]. These in turn may indirectly affect SAC1 expression. For example, sac1Δ mutants are inositol auxotrophs, characterized by their inability to grow on inositol-depleted culture medium [17]. Changes in inositol concentrations modulate the expression of many phospholipid biosynthetic enzymes [18]. This regulation involves binding of the transcription factor complex Ino2p/Ino4p to one or several UAS_{INO} motifs in the promoter region of relevant genes [18]. Although the SAC1(-500/-1) region does not contain a canonical UAS_{INO} motif, a negative regulation of SAC1 expression by inositol would be consistent with the requirement of a func-

---

**Figure 2**

**Characterization of a minimal SAC1 promoter region sufficient for regulated expression.** (A) Diagram depicting deletion constructs. The constructs were fused to the open reading frame of GFP in a CEN-based vector. The plasmids were introduced into a wild-type strain background and promoter activity determined by measurement of relative GFP expression levels in cell extracts. (B) Expression of the GFP reporter. Wild-type and sac1Δ yeast cells transformed with a CEN-based plasmid containing the SAC1(-100/-1)-GFP fusion construct were grown to early log phase at 30°C. Cell extracts were analyzed by SDS-PAGE and immunoblotting using anti-GFP and anti-glucose-6-phosphate dehydrogenase (Zwf1p) antibodies. (C) Quantitation of relative GFP expression levels. Data are from at least three independent experiments (+/-SE).
tional Sac1p for growth at low-inositol conditions. To examine whether SAC1 promoter activity is regulated through inositol levels, we created a sac1Δopi1Δ double mutant. The OPI1 gene encodes a negative regulator of inositol and phospholipid biosynthesis and represses activity of INO2/INO4-mediated transcription [18]. Elimination of OPI1 causes overproduction and excretion of inositol [7,19,20]. Deletion of OPI1 in a sac1Δ background rescued the growth defect on inositol-free medium (Fig. 4A) but continued to display other sac1Δ-specific phenotypes (data not shown). However, expression of the GFP reporter from the SAC1(-500/-1) region was not attenuated but enhanced in the sac1Δopi1Δ double mutant (Fig. 4B). Yet, the elevated SAC1(-500/-1) promoter activity in a sac1Δ background did not respond to increasing concentrations of inositol in the growth medium, ruling out the possibility that OPI1 deficiency in a sac1Δ background simply stimulates SAC1 expression by increasing the cellular inositol concentrations. The sac1Δopi1Δ double mutant showed also elevated GFP expression from the minimal SAC1(-100/-1) promoter (data not shown). Because the SAC1(-500/-1) region contains no UASino motifs it remains unclear how opi1 deficiency further enhances the expression from this promoter. Combined, these results suggest that inositol is not a regulator of SAC1 expression.

Sac1p plays an important role in ER-function by promoting ATP uptake and oligosaccharide biosynthesis [11,15]. Disruption of SAC1 induces ER stress and causes constitutive activation of the unfolded protein response (UPR) [15]. To test directly whether SAC1 expression is controlled by the UPR, we induced ER stress by treating cells with the reducing agent dithiothreitol (DTT) [21]. While DTT triggered a substantial increase in the cellular levels of the ER chaperone Kar2p (Fig. 4D), expression from the SAC1(-500/-1) 5′-UTR did not change significantly (Fig. 4D). This result eliminates the possibility that SAC1 expression is under control of the UPR. sac1Δ mutants also display defects in actin cytoskeletal arrangement and are sensitive to drugs such as caffeine and Calcofluor White (CFW) [13,14]. However, treating cells with CFW, an
Figure 4

**SAC1 expression is independent of inositol levels and ER stress.** (A) Analysis of cell growth in *sac1Δ* and *opi1Δ* mutants. Cells were grown at 30°C, plated in 5-fold serial dilutions starting with a density of 10⁷ cells/ml on rich growth medium (YPD) or on inositol-free medium and incubated for 3 days. (B) *SAC1* promoter activity in *opi1Δ* mutants. Cells were transformed with a CEN-based plasmid containing the *SAC1*(-500/-1)-GFP fusion construct and grown to early log phase at 30°C. Cell extracts were analyzed by SDS-PAGE and immunoblotting. Relative GFP expression levels were quantified. Data are from at least three independent experiments (+/-SE). (C) Influence of inositol on *SAC1* promoter activity. *sac1Δ* cells transformed with a CEN-based plasmid containing the *SAC1*(-500/-1)-GFP fusion construct were grown in media containing a range of inositol concentrations. Relative GFP expression levels were quantified as above. Data are from at least three independent experiments (+/-SE). (D) Influence of ER stress on *SAC1* promoter activity. Wild-type and *sac1Δ* cells transformed with a CEN-based plasmid containing the *SAC1*(-500/-1)-GFP fusion construct were cultivated in media with or without 7 mM DTT. Cell extracts were analyzed by SDS-PAGE and immunoblotting using anti-GFP, anti-glucose-6-phosphate dehydrogenase (Zwf1p), and anti-Kar2p antibodies. Relative GFP expression levels were quantified. Data are from at least three independent experiments (+/-SE).
agent causing cell wall defects and thus activating the cell integrity pathway, had no obvious effect on SAC1 expression (data not shown).

**Intracellular levels of PI(4)P correlate with SAC1 promoter activity**

Disruption of SAC1 results in pleiotropic changes in cellular phosphoinositide levels. sac1Δ cells show a 2-fold elevation in PI(3)P levels and a moderate decrease in PI(4,5)P₂ [13,22]. The most dramatic phenotype is an approximate 10-fold elevation in PI(4)P levels [13,22]. In proliferating cells, Sac1p is required for confining a PI(4)P pool generated by the PI 4-kinase Stt4p to the plasma membrane. During starvation, Sac1p translocates from the ER to the Golgi and eliminates Golgi PI(4)P, which is generated by the PI 4-kinase Pik1p [13,22]. To examine whether the upregulated activity of the SAC1 promoter responds to alterations in one of these PI(4)P pools, we introduced temperature-sensitive mutant alleles of stt4 and pik1 into a sac1Δ background. Both Pik1p and Stt4p are essential for cell growth and it was shown previously that stt4ts or pik1ts strains show impaired PI(4)P biosynthesis and reduced viability at semi-permissive temperatures above 25°C [8,23]. In a sac1Δstt4ts strain cultivated at 33°C, the excess PI(4)P levels were largely reduced, whereas PI(4)P levels in the sac1Δpik1ts strain remained elevated at this temperature (Fig. 5A). These results are consistent with previous reports confirming that Sac1p controls mainly Stt4p-generated PI(4)P during normal cell growth [8,10]. As shown in Fig. 5B, the relative activity

![Figure 5](image)

**Figure 5**

**SAC1 expression responds to changes in PI(4)P levels.** (A) PI(4)P levels in sac1Δ and sac1Δ PI 4-kinase double mutants. Yeast cells were grown at 33°C and labeled with [3H]myo-inositol. Phosphoinositides were extracted, deacylated and quantified by HPLC. Data are from three independent experiments (+/-SE). (B) SAC1 promoter activity in sac1Δ and sac1Δ PI 4-kinase double mutants. Yeast cells were transformed with a CEN-based plasmid containing the SAC1(-500/-1)-GFP fusion construct and grown to early log phase at 33°C. Cell extracts were analyzed by SDS-PAGE and immunoblotting. Relative GFP expression levels were quantified. Data are from at least three independent experiments (+/-SE). (C) Correlation of increased Sac1 protein levels and PI(4)P phosphatase deficiency. Wild-type and sac1Δ yeast expressing either a myc-tagged wild-type Sac1p or phosphatase-deficient mutant myc-Sac1-22p from the SAC1(-500/-1) promoter were grown to early log phase at 30°C. Cell extracts were analyzed by SDS-PAGE and immunoblotting using anti-GFP and anti-glucose-6-phosphate dehydrogenase (Zwf1p) antibodies.
of the SAC1(-500/-1) 5'-UTR correlates significantly with the respective changes in PI(4)P in these mutant strains. This result indicates that expression from the SAC1 promoter responds to the levels of intracellular PI(4)P. To confirm that the PI(4)P-induced activity of the SAC1 promoter is reflected by increased Sac1p protein levels, we used the sac1-22 allele, encoding a phosphatase-deficient Sac1 protein [24]. In wild-type cells a myc-tagged version of Sac1-22p was expressed at the same level as Sac1p. In contrast, expression in a sac1Δ strain induced a significant increase in myc-Sac1-22p protein levels whereas protein levels of myc-tagged wild-type Sac1p remained unchanged (Fig. 5C). In summary, these results indicate that Sac1p protein levels respond to a rise in intracellular PI(4)P.

Discussion
In yeast, many enzymes required for phospholipid biosynthesis show a common pattern of transcriptional regulation [5]. Soluble and membrane-bound precursors for phospholipid biosynthesis such as inositol, choline and phosphatidic acid play a major role in this regulation [6,7]. In contrast, little is known about the transcriptional regulation of enzymes controlling the cellular levels of the phosphorylated derivatives of these phospholipids. While Sac1p function is essential when yeast cells are deprived of inositol [17], the expression of SAC1 is not regulated by inositol itself. Instead Sac1p protein levels respond to the cellular levels of PI(4)P, which is the major substrate of this lipid phosphatase. PI(4)P is concentrated in distinct intracellular pools that have diverse yet essential cellular functions such as in regulating membrane trafficking and actin cytoskeletal organization [8,10,11]. In proliferating cells, Sac1p is responsible for turning over the PI(4)P that is generated by the PI 4-kinase Stt4p [25]. We find that alterations in this Stt4p-specific PI(4)P pool are mechanistically linked to the control of SAC1 expression.

Membrane homeostasis and organelar traffic both rely on precisely regulated phosphoinositide gradients. In growing cells, Sac1p plays an important role in preventing random equilibration of PI(4)P at intracellular membranes, a phenotype commonly observed in sac1 mutants [8,9]. Linking SAC1 expression to the levels of PI(4)P ensures that sufficient levels of the lipid phosphatase are continuously available to fulfill this task. Analysis of promoter elements required for this regulation revealed the partially palindromic 9-bp motif in the 5'-UTR of SAC1 that is critical for expression. Partial palindromic sequences have also been found in other cis-acting promoter elements [26]. However, queries in the Saccharomyces cerevisiae promoter database (SCPD) indicate that the ACCACAGGT element does not overlap with any known consensus sequence for DNA binding proteins and therefore represents a novel motif. SAC1 promoters in higher eukaryotes have not yet been defined and it remains to be seen whether expression of the mammalian SAC1 homologs is regulated via a similar element.

sac1 mutants display accumulation of PI(4)P at the nuclear envelope and it is possible that nuclear phosphoinositides activate or recruit hitherto uncharacterized factors required for transcription. Recent reports indicated that phosphoinositides play important roles inside the nucleus and nuclear phosphoinositide-binding proteins have been discovered [27,28]. While our results support the idea that PI(4)P is a direct regulator of SAC1 gene expression, it is also possible that a metabolite downstream of PI(4)P is the actual signal transducer. PI(4)P can be rapidly converted to PI(4,5)P2 by the PIP kinase Mss4p [29,30]. However, sac1 mutant strains do not show elevated PI(4,5)P2 levels [22] and it is therefore unlikely that PI(4,5)P2 is directly involved in this regulation. Another potential mechanism could involve soluble inositol phosphate species. Both PI(4)P and PI(4,5)P2 can be hydrolyzed by phospholipase C giving rise to inositol-1,4-bisphosphate and inositol-1,4,5-trisphosphate respectively [31]. These soluble signal transducers can be further phosphorylated in the nucleus where they are involved in transcriptional control and mRNA export [32,33]. It remains to be determined whether these molecules play a role in regulating SAC1 expression and identifying the additional components of this signaling mechanism awaits further investigations.

Conclusion
This study characterizes a promoter element required for regulated expression of the lipid phosphatase Sac1p in yeast. This enzyme controls the distinct intracellular pools of PI(4)P required for membrane traffic and homeostasis. Distinct from phospholipid biosynthetic enzymes, whose expression is largely regulated by small soluble phospholipid precursors, the activity of the SAC1 promoter correlates with the intracellular levels of PI(4)P. We propose that the precise control of Sac1p protein levels by the membrane concentration of its major substrate ensures proper maintenance of organelle-specific phosphoinositide gradients.

Methods
Strains, reagents, and other procedures
Plasmids, strains and DNA primers are listed in Table 1 and 2. Saccharomyces cerevisiae strains were grown in standard yeast extract/pptone/dextrose (YPD) media or Hartwell’s complete media (HC). The OP11 disruption cassette was created by PCR, using the primers Opi1KOfwd, Opi1KOrev and the vector pRS413 [34] as template. The PCR product was transformed into ATY201 and STY39. Antibodies against glucose-6-phosphate dehydrogenase (Zwf1p, working dilution 1:100,000) and GFP
(working dilution 1:2,400) were purchased from Sigma-Aldrich (St. Louis, MO). [3H]myo-inositol was purchased from PerkinElmer (Wellesley, MA). SDS-PAGE and immunoblotting were performed as described [35].

**Generation of SAC1 promoter constructs**

Fragments of the SAC1 5’ UTR were amplified by PCR (see Table 2 for oligonucleotide sequences), ligated into pGEM-T Easy Vector (Promega, Madison, WI) and subcloned into pGK25 [24] using NotI and XhoI restriction sites. Deletion mutations within the SAC1(-500/-1) 5’-UTR were generated by mutating pGK26 using the Quick Change II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA).

**Quantification of protein levels**

Cells expressing GFP under the control of SAC1 5’-UTR constructs were grown in Hartwell’s complete media (HC) supplemented with the appropriate amino acids and harvested in early logarithmic growth phase. 5 OD cells were collected, washed in water and resuspended in 200 μl 2x Laemml buffer and 200 μl glass beads. Lysates were prepared by vortexing for one minute. Supernatants were boiled for 5 minutes and analyzed by SDS-PAGE and immunoblotting. Protein levels were measured by determination of band size and band density using NIH Image software (version 1.62). Protein amounts of GFP were normalized against protein amounts of glucose-6-phosphate dehydrogenase.

**Lipid analysis**

Since sac1 mutants are inositol auxotrophs, yeast cells were cultivated in 5.5 μM inositol prior to and during the labeling procedure. Early log phase cells were incubated with 10 μCi/ml myo-[3H]inositol for 2–3 doubling times. Labeling, extraction and deacylation of lipids was performed as described previously [36]. HPLC analysis of glycerophosphoinositols was carried out on a 250 × 4.6-mm Partisil SAX column (Whatman, Florham Park, NJ) using a Jasco HPLC system equipped with an LB 508 Radioflow detector (Berthold, Bad Wildbach, Germany). Elution and quantification of glycerophosphoinositols were performed as described [36].

**Authors’ contributions**

AK performed the experiments, contributed to the experimental design, and helped in the writing of this manuscript. GK collaborated in protein expression analyses. PM coordinated this study, provided its conceptual basis, participated in experimental design and wrote the manuscript. All authors read and approved the final manuscript.
Table 2: Oligonucleotides

| Primer | Sequence |
|--------|----------|
| Sac(-500)fwd | TTGC66GGGGC66CAC66G66CACCTA66C66CAC66T66CC |
| Sac(-242)fwd | TTGC66GGGGC66C66CA66C66AG66CTG66C66TG66C66TAT66T |
| Sac(-170)fwd | TT66GGG66CCGGCTG66C66ACTA66T66GT66C66T66T66CC |
| Sac(-125)fwd | TT66GGG66CCGG66CA66GTTAG66G66AT66G66G |
| Sac(-114)fwd | TT66GGG66CCGG66C66GA66CG66G66A66A66T66AC66CA |
| Sac(-100)fwd | TT66GGG66CCGG66CAA66C66GA66GG66A66GA66AT66AC66CA |
| Sac(-83)fwd | TT66GGG66CCGG66GA66AA66T66AGG66AA66G66AG |
| Sac(-150)rev | GGCT66CGAG66T66CT66G66A66G66G66A66AT66AT66AC66CA |
| Sac(-100)/-84fwd | G66A66A66G66GA66AA66T66AGG66AA66G66AG |
| Sac(-91)/-84fwd | GG66A66A66G66GA66AA66T66AGG66AA66G66AG |
| Sac(-83)/-70fwd | GG66A66A66G66GA66AA66T66AGG66AA66G66AG |
| Sac(-100)/-84rev | CCT66T66T66C66CT66T66T66T66T66T66T66T66CC66T |
| Sac(-83)/-70rev | GCC66A66A66G66GA66AA66T66AGG66AA66G66AG |
| Sac(-91)/-84rev | G66A66A66G66GA66AA66T66AGG66AA66G66AG |
| Sac(-100)/-92fwd | GG66A66A66G66GA66AA66T66AGG66AA66G66AG |
| Sac(-100)/-92rev | CCT66T66T66C66CT66T66T66T66T66T66T66T66CC66T |
| OpIIKfwd | CAT66AT66C66AG66CC66GA66A66CG66TC66G66CT66TT66T |
| OpIIKrev | AAC66T66A66T66T66C66GT66C66T66AT66A66AT66T66T |

Acknowledgements

We thank Lieu Than for technical help. We also thank Suparna Kanjilal and Teresa Nicolson for comments on the manuscript. This work was funded by National Institute of Health grant GM071569 (P.M.).

References

1. Di Paolo G, De Camilli P: Phosphoinositides in cell regulation and membrane dynamics. Nature 2006, 443:651-7.
2. Behnia R, Munro S: Organelle identity and the signposts for membrane traffic. Nature 2005, 438:597-604.
3. De Matteis MA, Di Camilli A, Godi A: The role of the phosphoinositides at the Golgi complex. Biochim Biophys Acta 2005, 1744:396-405.
4. Pendaries C, Tronchere H, Plantavid M, Payrastre B: Phosphoinositide signaling disorders in human diseases. FEBS Lett 2003, 546:25-31.
5. Carman GM, Henry SA: Phospholipid biosynthesis in the yeast Saccharomyces cerevisiae and interrelationship with other metabolic processes. Prog Lipid Res 1999, 38:361-99.
6. Jesch SA, Zhao X, Wells MT, Henry SA: Genome-wide analysis reveals inositol, not choline, as the major effector of Ino2p-Ino4p and unfolded protein response target gene expression in yeast. J Biol Chem 2005, 280:9106-18.
7. Loewen CJ, Gaspar ML, Jesch SA, Delon C, Kristakis NT, Henry SA, Levine TP: Phospholipid metabolism regulated by a transcription factor sensing phosphatidic acid. Science 2004, 304:1644-7.
8. Tahiriovic S, Schorr M, Mayinger P: Regulation of intracellular phosphatidylinositol-4-phosphate by the Sac1 lipid phosphatase. Traffic 2005, 6:116-30.
9. Roy A, Levine TP: Multiple pools of phosphatidylinositol 4-phosphate detected using the pleckstrin homology domain of Osh2p. J Biol Chem 2004, 279:4683-9.
10. Foti M, Audhya A, Emr SD: Sac1 lipid phosphatase and stt4 phosphatidylinositol 4-kinase regulate a pool of phosphatidylinositol-4-phosphate that functions in the control of the actin cytoskeleton and vacuole morphology. Mol Biol Cell 2001, 12:199-41.
11. Faulhammer F, Konrad G, Brankatsch B, Tahiriovic S, Knodler A, Mayinger P: Cell growth-dependent coordination of lipid signaling and glycosylation is mediated by interactions between Sac1p and Dpm1p. J Cell Biol 2005, 168:185-91.
12. Zhu J, Zhang MQ: SCFD: a promoter database of the yeast Saccharomyces cerevisiae. Bioinformatics 1999, 15:607-11.
13. Hughes WE, Pocklington MJ, Orr E, Paddon CJ: Mutations in the Saccharomyces cerevisiae gene SAC1 cause multiple drug sensitivity. Yeast 1999, 15:1111-24.
14. Cleves AE, Novick PJ, Bankaitis VA: Mutations in the SAC1 gene suppress defects in yeast Golgi and yeast actin function. J Cell Biol 1989, 109:2939-2950.
15. Kochendorfer KU, Then AR, Kearns BG, Bankaitis VA, Mayinger P: Sac1p plays a crucial role in microsomal ATP transport, which is distinct from its function in Golgi phospholipid metabolism. EMBO J 1999, 18:1506-15.
16. Tahiriovic S, Schorr M, Then A, Berger J, Schwarz H, Mayinger P: Role for lipid signaling and the cell integrity MAP kinase cascade in yeast septum biogenesis. Curr Genet 2003, 43:71-8.
17. Whisters EA, Cleves AE, McGee TP, Skinner HB, Bankaitis VA: SAC1p is an integral membrane protein that influences the cellular requirement for phospholipid transfer protein function and inositol in yeast. J Cell Biol 1993, 122:79-94.
18. Bachhawat N, Ouyang Q, Henry SA: Functional characterization of an inositol-sensitive upstream activation sequence in yeast. A cis-regulatory element responsible for inositol-choline mediated regulation of phospholipid biosynthesis. J Biol Chem 1995, 270:25087-95.
19. Greenberg ML, Reiner B, Henry SA: Regulatory mutations of inositol biosynthesis in yeast: isolation of inositol-excreting mutants. Genetics 1982, 100:19-33.
20. Ashburner BP, Lopes JM: Regulation of yeast phosphoinositide biosynthetic gene expression in response to inositol involves two superimposed mechanisms. Proc Natl Acad Sci USA 1995, 92:7922-6.
21. Kohno O, Norminton K, Sambrook J, Gething MJ, Mori K: The promoter region of the yeast KAR2 (BIP) gene contains a regulatory domain that responds to the presence of unfolded proteins in the endoplasmic reticulum. Mol Cell Biol 1993, 13:877-890.
22. Guo S, Stolz LE, Lemrow SM, York JD: SAC1-like domains of yeast SAC1, INP52, and INP53 and of human synaptojanin encode polyphosphoinositide phosphatases. J Biol Chem 1999, 274:12900-5.
23. Audhya A, Foti M, Emr SD: Distinct roles for the yeast phosphatidylinositol 4-kinases, Stt4p and Pkl1p, in secretion, cell growth, and organelle membrane dynamics. Mol Biol Cell 2000, 11:2673-89.
26. Mori K, Ogawa N, Kawahara T, Yanagi H, Yura T: Palindrome with spacer of one nucleotide is characteristic of the cis-acting unfolded protein response element in Saccharomyces cerevisiae. J Biol Chem 1998, 273:9912-20.
27. Bunce MW, Bergendahl K, Anderson RA: Nuclear PI(4,5)P(2): a new place for an old signal. Biochim Biophys Acta 2006, 1761:560-9.
28. Deleris P, Gayral S, Breton-Douillon M: Nuclear PtdIns(3,4,5)P3 signaling: an ongoing story. J Cell Biochem 2006, 98:469-85.
29. Homma K, Terui S, Minemura M, Qadota H, Anraku Y, Kanaho Y, Ohya Y: Phosphatidylinositol-4-phosphate 5-kinase localized on the plasma membrane is essential for yeast cell morphogenesis. J Biol Chem 1998, 273:15779-86.
30. Desrivieres S, Cooke FT, Parker PJ, Hall MN: MSS4, a phosphatidylinositol-4-phosphate 5-kinase required for organization of the actin cytoskeleton in Saccharomyces cerevisiae. J Biol Chem 1998, 273:15787-93.
31. Flick JS, Thorner J: Genetic and biochemical characterization of a phosphatidylinositol-specific phospholipase C in Saccharomyces cerevisiae. Mol Cell Biol 1993, 13:5861-76.
32. York JD, Odom AR, Murphy R, Ives EB, Wente SR: A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. Science 1999, 285:96-100.
33. Sikorski RS, Hieter P: A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 1989, 122:19-27.
34. Harlow E, Lane D: Antibodies: A laboratory manual. Cold Spring Harbor Laboratory; 1988.
35. Schorr M, Then A, Tahirovic S, Hug N, Mayinger P: The phosphoinositide phosphatase Sac1p controls trafficking of the yeast Chs3p chitin synthase. Curr Biol 2001, 11:1421-6.