Yet1p–Yet3p interacts with Scs2p–Opi1p to regulate ER localization of the Opi1p repressor

Joshua D. Wilson, Sarah L. Thompson, and Charles Barlowe
Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755

ABSTRACT **Lipid sensing mechanisms at the endoplasmic reticulum (ER) coordinate an array of biosynthetic pathways. A major phospholipid regulatory circuit in yeast is controlled by Scs2p, an ER membrane protein that binds the transcriptional repressor protein Opi1p. Cells grown in the absence of inositol sequester Scs2p–Opi1p at the ER and derepress target genes including *INO1*. We recently reported that Yet1p and Yet3p, the yeast homologues of BAP29 and BAP31, are required for normal growth in the absence of inositol. Here we show that the Yet1p–Yet3p complex acts in derepression of *INO1* through physical association with Scs2p–Opi1p. Yet complex binding to Scs2p–Opi1p was enhanced by inositol starvation, although the interaction between Scs2p and Opi1p was not influenced by *YET1* or *YET3* deletion. Interestingly, live-cell imaging analysis indicated that Opi1p does not efficiently relocalize to the ER during inositol starvation in *yet3*Δ cells. Together our data demonstrate that a physical association between the Yet complex and Scs2p–Opi1p is required for proper localization of the Opi1p repressor to ER membranes and subsequent *INO1* derepression.**

Monitoring Editor
John York
Duke University

Received: Jul 2, 2010
Revised: Feb 14, 2011
Accepted: Feb 17, 2011

INTRODUCTION

The endoplasmic reticulum (ER) houses numerous enzymes critical for the biosynthesis of secretory proteins and lipids. In addition to these enzymatic pathways, the ER also contains proteins that monitor and regulate biosynthetic processes through the alteration of transcriptional programs. Several elegant mechanisms have been defined, explaining how cells sense and adjust transcriptional profiles to maintain protein and lipid homeostasis (Ire1p/Hac1p, Cox *et al.*, 1993; SCAP-SREBP, Wang *et al.*, 1994; Spt23p, Hoppe *et al.*, 2000; and Scs2p/Opi1p, Loewen *et al.*, 2004). In general, ER-localized sensor proteins alter their conformational or oligomeric status in response to changes in protein or lipid composition, which subsequently influences the activation state of associated transcription factors.

In the yeast *Saccharomyces cerevisiae*, the production of enzymes responsible for phospholipid biosynthesis depends on the interplay between the transcriptional activator Ino2p-Ino4p and its cognate repressor Opi1p (reviewed by Carman and Henry, 2007). When inositol is supplied in the culture medium, phospholipid biosynthesis genes containing an inositol-sensitive upstream activation sequence (UAS_{INO}) promoter element are repressed through the association of Opi1p with Ino2p (Wagner *et al.*, 2001; Jesch *et al.*, 2005). Inositol starvation apparently releases Opi1p from Ino2p, thus allowing expression of UAS_{INO}-controlled genes (Henry and Patton-Vogt, 1998; Gardenour *et al.*, 2004; Heyken *et al.*, 2005). Current evidence suggests that Opi1p is physically sequestered on the surface of ER membranes during inositol starvation through interaction with Scs2p and accumulated phosphatidic acid (Loewen *et al.*, 2003, 2004). The conversion of phosphatidic acid to phosphatidyl inositol increases with the availability of inositol, which is thought to release Opi1p from ER membranes followed by translocation into the nucleus to repress UAS_{INO} genes.

The deletion of *SCS2* results in attenuated growth in the absence of inositol, presumably by preventing ER sequestration of Opi1p (Kagiwada *et al.*, 1998; Loewen *et al.*, 2003). *INO1*, which

This article was published online ahead of print in MBoC in Press (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E10-07-0559) on March 3, 2011.

Address correspondence to: Charles Barlowe (charles.barlowe@dartmouth.edu).

Contributions of authors: J.D.W. wrote the manuscript, designed experiments, and generated data, except where noted; S.L.T. performed microscopy in Figure 4; C.B. provided advice in planning experiments and interpretation of data and edited the manuscript.

Abbreviations used: BAP31, B cell receptor associated protein of 31 kilodaltons; CCD, charge-coupled device; DSP, dithiobis[succinimidylpropionate]; ER, endoplasmic reticulum; FFAT, double phenylalanine in an acidic tract; GFP, green fluorescent protein; HA, hemagglutinin epitope; INM, inner nuclear membrane; MYC, c-Myc epitope; OD, optical density; OPI, overproducer of inositol; ORF, open reading frame; PI, phosphatidyl inositol; SCS2, suppressor of choline sensitivity; UAS, upstream activation sequence; UPR, unfolded protein response; VAMP, vesicle-associated membrane protein; VAP, VAMP-associated protein; Yet, yeast ER transmembrane protein.

encodes the rate-limiting inositol 1-phosphate synthase enzyme, is the major target of Ino2p-Ino4p activation and Opi1p repression. Highlighting the central role of *INO1* in the response to inositol starvation, overexpression of *INO1* is sufficient to rescue the inositol starvation growth defect of a *scs2*Δ mutant (Kagiwada *et al.*, 1998). An *ire1*Δ mutant, which also displays inositol starvation growth defects similar to a *scs2*Δ mutant, can similarly be rescued by overexpression *INO1* (Cox *et al.*, 1997), suggesting that an inability to properly derepress *INO1* is a major reason for inositol auxotrophy in these mutants (Cox *et al.*, 1997; Kagiwada and Zen, 2003).

Previous studies indicated that *yet1*Δ and *yet3*Δ mutants are inositol auxotrophs (Hillenmeyer *et al.*, 2008; Wilson and Barlowe, 2010). *YET1* and *YET3* encode sequence homologues of the mammalian BAP31 protein (Toikkanen *et al.*, 2006), which has reported roles in substrate-specific ER export, retention, and quality control (e.g., Lambert *et al.*, 2001; Ladasky *et al.*, 2006; Wang *et al.*, 2008). Yet1p and Yet3p form a heteromeric complex that is important for interaction with the ER translocation machinery and for inositol prototrophy (Wilson and Barlowe, 2010). In this study, we present evidence showing that the inositol-related growth defects in *yet1*Δ and *yet3*Δ mutants are caused by an inability to fully derepress *INO1* during inositol starvation. Interestingly, the Yet1p–Yet3p complex interacts with Scs2p and Opi1p in a manner that is regulated by inositol availability and requires both Yet1p and Yet3p. Together our data demonstrate that the Yet1p–Yet3p complex facilitates Scs2p-mediated sequestration of Opi1p.

RESULTS

The *yet3*Δ mutant displays an inositol starvation phenotype similar to other derepression mutants

Yeast cell survival in the absence of inositol requires the expression of inositol 1-phosphate synthase, Ino1p (Culbertson *et al.*, 1976; Donahue and Henry, 1981). The removal of genes required for the transcription of *INO1* (e.g., *ino2*Δ), results in strong inhibition of growth in the absence of inositol (Figure 1 and Culbertson and Henry, 1975). In contrast, cells harboring mutations in genes involved in the regulated derepression of *INO1*, such as *scs2*Δ or *ire1*Δ, display limited growth when starved of inositol (Figure 1 and Kagiwada *et al.*, 1998). Previous studies have indicated that cells deleted for *YET1* and/or *YET3* are comparably defective for growth in the absence of inositol (Wilson and Barlowe, 2010). Thus the findings presented in the following experiments using a *yet3*Δ mutant should be applicable to a *yet1*Δ mutant. Figure 1 compares the inositol growth defect of *yet3*Δ to other inositol starvation growth mutants. As expected, cells lacking *INO2* did not grow in the absence of inositol at either 30 or 37°C. Similar to *scs2*Δ and *ire1*Δ deletion mutants, cells with a *yet3*Δ mutation displayed growth attenuation at 30°C, whereas essentially no growth was observed at 37°C. The reason for temperature sensitivity to inositol starvation in these mutants is not entirely clear, although it has been suggested that the cellular inositol requirement may be elevated at higher temperatures (Gaspar *et al.*, 2008). Also, higher temperatures have been shown to have an inhibitory effect on *INO1* derepression in *scs2*Δ mutants (Kagiwada and Zen, 2003).

The *yet3*Δ mutant does not maintain Ino1p expression during inositol starvation

The thermosensitive nature of the inositol starvation growth phenotype of the *yet1*Δ and *yet3*Δ mutants suggested that, like *scs2*Δ and *ire1*Δ mutants, the underlying cause of growth inhibition might be an inability to properly derepress *INO1*. To test this possibility, we examined levels of Ino1p tagged with the hemagglutinin epitope (Ino1p-HA) in wild-type and *yet3*Δ mutant cells after various periods of inositol starvation (Figure 2A). We note that prolonged inositol starvation in wild-type strains caused a notable increase in Yet3p protein levels indicative of unfolded protein response (UPR) activation (Cox *et al.*, 1997; Wilson and Barlowe, 2010). More important, the level of Ino1p-HA in *yet3*Δ mutant cells was diminished compared with wild-type cells at all time points tested, consistent with a failure to properly derepress *INO1*. Ino1p-HA levels appeared to be reduced to a greater degree after longer periods of inositol starvation, similar to observations of *INO1* mRNA levels in an *ire1*Δ mutant (Cox *et al.*, 1997). To confirm that the observed reduction in Ino1p-HA levels was the result of a failure of the *yet3*Δ mutant cells to fully derepress *INO1*, we transformed wild-type, *yet3*Δ, and *scs2*Δ mutants with an *INO1-LacZ* fusion reporter plasmid (pMR1036, Chang *et al.*, 2002) and measured reporter expression after inositol starvation. As shown in Figure 2B, both *yet3*Δ and *scs2*Δ mutant cells displayed significantly reduced levels of reporter activity compared with wild-type cells after 5 h without inositol supplement. These data demonstrate an *INO1* derepression defect in *yet3*Δ mutant cells, similar to previous studies on *ire1*Δ, *hac1*Δ, and *scs2*Δ mutants (Nikawa, 1994; Cox *et al.*, 1997; Kagiwada and Zen, 2003; Brickner and Walter, 2004).

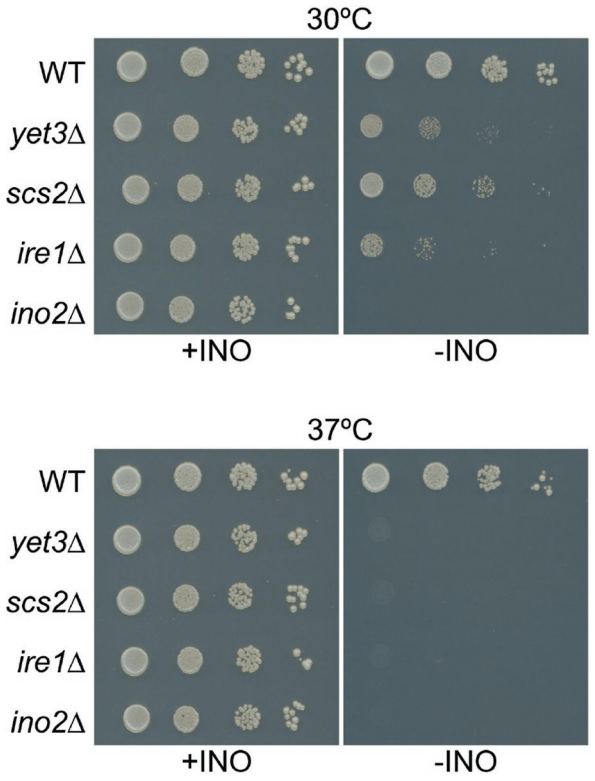


FIGURE 1: Growth comparison of *yet3*Δ with other inositol-starvation mutants. Wild-type (BY4742), *yet3*Δ (CBY2200), *scs2*Δ (CBY2819), *ire1*Δ (CBY2431), and *ino2*Δ (CBY1946) strains were prepared as described in *Materials and Methods* and grown at 30 or 37°C for 3 d in the presence or absence of inositol.

Effect of multicopy *INO1* or *SCS2* on rescue of *yet3*Δ inositol starvation growth defects

The inositol starvation–related growth defects of *INO1* derepression mutants (*ire1*Δ and *scs2*Δ) can be suppressed by overexpression of *INO1* (Cox *et al.*, 1997; Kagiwada *et al.*, 1998). Additionally, the inositol auxotrophy of a *hac1*Δ mutant was reported to be suppressed by a multicopy plasmid bearing *SCS2* (Nikawa *et al.*, 1995).

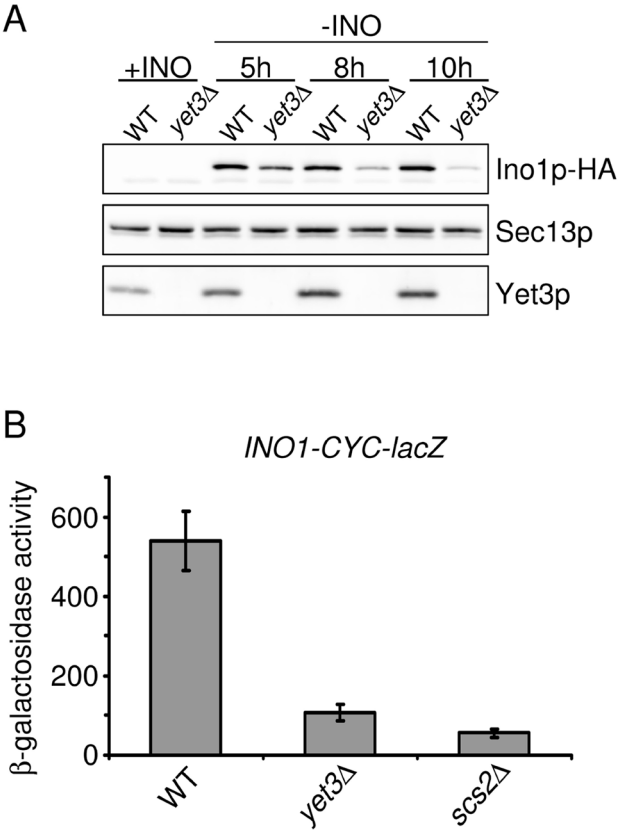


FIGURE 2: *INO1* is not properly derepressed in a *yet3*Δ strain. (A) Overnight cultures harboring HA-tagged *INO1* (wild type, CBY2938; *yet3*Δ, CBY2939) were washed and grown for the indicated times in the presence or absence of inositol. Whole cell lysates from these cultures were generated as described in *Materials and Methods* and analyzed by immunoblot. Sec13p is included as a loading control. (B) Log phase cultures of wild type (CBY3039), *yet3*Δ (CBY3040), and *scs2*Δ (CBY3041) harboring the *INO1-CYC-lacZ* reporter plasmid (pMR1036) were grown in the presence of inositol, washed, and grown for an additional 5 h in the absence of inositol before analysis of β-galactosidase activity as described in *Materials and Methods*. The mean β-galactosidase activity of three experiments is plotted (β-galactosidase activity units are A_{420} min^{-1} ml^{-1} OD_{600}-1) and error bars represent SD. Negligible β-galactosidase activities were recorded for these strains grown in parallel with 75 μM inositol.

Thus we tested whether overexpression of these genes could suppress the inositol growth defect of a *yet3*Δ mutant. Strikingly, a 2μ *INO1* plasmid suppressed the inositol growth defect of *yet3*Δ mutant cells as efficiently as this plasmid suppressed *scs2*Δ mutant cells at both 30 and 37°C (Figure 3A). This finding provides further evidence that the inositol auxotrophy in *yet3*Δ mutants is the result of an inability to properly derepress *INO1*. Notably, *yet3*Δ and *scs2*Δ mutant cells were able to activate an UPR during inositol starvation similar in magnitude to wild-type cells (unpublished data), suggesting that unlike Ire1p, Yet3p and Scs2p are not involved in UPR signaling. Interestingly, a 2μ *SCS2* plasmid had no detectable influence on the growth of *yet3*Δ cells at 30°C but clearly enhanced growth at 37°C. Multicopy *SCS2* similarly suppressed *yet1*Δ and the double *yet1*Δ *yet3*Δ mutant strains (unpublished data), suggesting that elevated levels of Scs2p partially bypass *YET1* and *YET3* function during inositol starvation. Reciprocal experiments with multicopy *YET1* and/or *YET3* showed no detectable effect on the inositol growth defect of *scs2*Δ or *ire1*Δ mutants (unpublished data). These suppression results also correlated with readouts from the *INO1-LacZ* reporter plasmid in the presence of inositol where multicopy *YET1/YET3* produced mild increases in reporter activity relative to induction by multicopy Scs2p (Supplemental Figure 1).

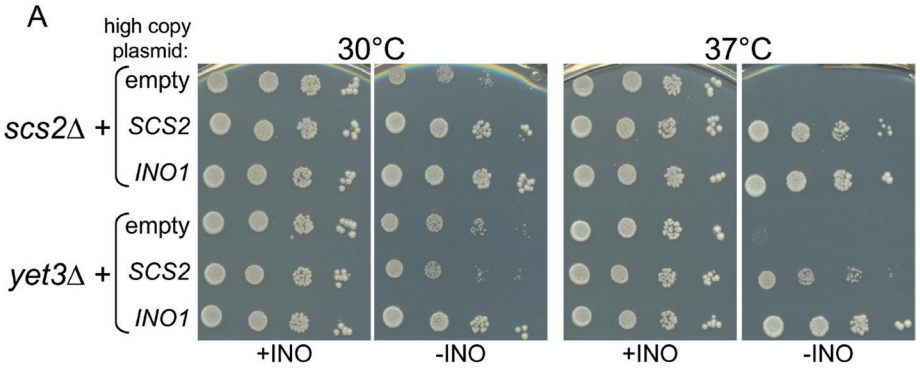
To further explore the genetic relationship between *YET3* and *SCS2*, we next examined the growth of a *yet3*Δ *scs2*Δ double mutant on medium lacking inositol. Even though the *yet3*Δ *scs2*Δ mutant displayed no apparent growth defect in the presence of inositol, essentially no growth was observed in the absence of inositol (Figure 3B). This result suggests that the Yet proteins and Scs2p can partially function (with respect to *INO1* derepression) in the absence each other, but removal of both components results in a severe defect in *INO1* derepression.

ER sequestration of Opi1p during inositol starvation is disrupted in a *yet3*Δ mutant

Opi1p sequestration on ER membranes during inositol starvation is associated with *INO1* derepression (Loewen *et al.*, 2004). Additionally, Opi1p has been reported to remain partially bound to *INO1*-associated chromatin during inositol starvation in *hac1*Δ and *scs2*Δ mutants, which have defects in *INO1* derepression (Brickner and Walter, 2004). Given our evidence that *INO1* was not properly derepressed in a *yet3*Δ mutant, we next asked whether green fluorescent protein fused to Opi1p (GFP-Opi1p) as previously described (Loewen *et al.*, 2003) was localized to the ER during inositol starvation in this mutant. As shown in Figure 4, GFP-Opi1p localized to the nuclear rim in wild-type cells when inositol was present in the medium, consistent with previous reports (Loewen *et al.*, 2003, 2004). A similar GFP-Opi1p localization pattern was observed in *yet3*Δ mutant cells grown with inositol supplement. In striking contrast, ER localization of GFP-Opi1p was not observed in *yet3*Δ mutant cells after inositol starvation as was observed in wild-type cells. Instead the majority of GFP-Opi1p appeared to remain in the nucleus of inositol-starved *yet3*Δ mutant cells (Figure 4) as also observed when Opi1p was tagged with the c-Myc epitope (Opi1p-MYC) in Supplemental Figure 2, thus correlating deficient *INO1* expression with a failure to properly sequester Opi1p in this mutant.

The Yet1p–Yet3p complex physically associates with Scs2p and Opi1p

Scs2p function in the derepression of *INO1* appears to be through binding and sequestration of the Opi1p repressor, as highlighted by aberrant Opi1p occupancy at *INO1*-associated chromatin and the lack of *INO1* derepression during inositol starvation in *scs2*Δ cells (Kagiwada and Zen, 2003; Loewen *et al.*, 2003; Brickner and Walter, 2004). Given the phenotypic similarities between the *yet1*Δ, *yet3*Δ, and *scs2*Δ mutants reported earlier in addition to the ER localization of Yet1p, Yet3p, and Scs2p (Kagiwada *et al.*, 1998; Toikkanen *et al.*, 2006; Wilson and Barlowe, 2010), we next examined whether the Yet proteins physically interact with Scs2p and Opi1p. To test for associations, we constructed a strain that expresses chromosomally tagged Scs2p-HA and Opi1p-MYC and performed immunoprecipitation experiments from digitonin solubilized semi-intact cell membranes. As shown in Figure 5A, immunoprecipitation of Scs2p-HA recovered Opi1p-MYC, as expected, but also coimmunoprecipitated Yet1p and Yet3p. Similarly, Opi1p-MYC immunoprecipitations yielded Scs2p-HA and both Yet1p and Yet3p. Interestingly, Sec61p, which we previously showed to be associated with Yet1p and Yet3p (Wilson and Barlowe, 2010), was absent from these immunoprecipitations, suggesting that the associations of Yet1p and Yet3p with the Sec complex and with Scs2p–Opi1p are mutually exclusive.



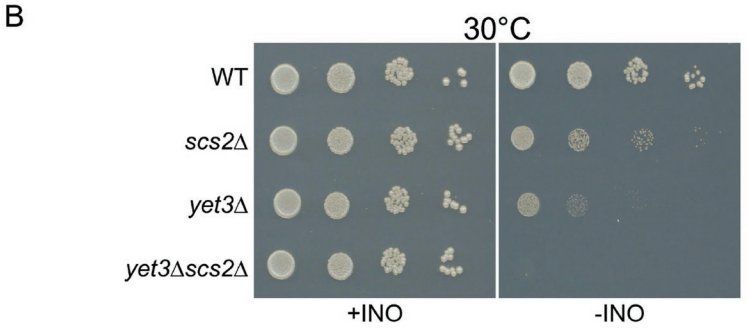


FIGURE 3: High copy *INO1* and *SCS2* rescue a *yet3*Δ mutant and a *yet3*Δ *scs2*Δ mutant displays synthetic growth defects. (A) Overnight cultures of an *scs2*Δ mutant transformed with pRS426 (CBY2908), pRS426-*SCS2* (CBY2909), or pRS426-*INO1* (CBY2910), and a *yet3*Δ mutant with pRS426 (CBY2905), pRS426-*SCS2* (CBY2906), or pRS426-*INO1* (CBY2907), were washed and plated as described in Figure 1 and grown for 4 d at 30 or 37°C. (B) Wild-type (BY4742), *yet3*Δ (CBY2200), *scs2*Δ (CBY2819), and *yet3*Δ *scs2*Δ (CBY2853) strains were prepared essentially as described in (A) and grown for 3 d at 30°C in the presence or absence of inositol.

To further characterize the association between the Yet proteins and Scs2p–Opi1p, we incubated ER microsomes (Wuestehube and Schekman, 1992) with the cross-linking reagent dithiobis[succinimidyl propionate] (DSP) and then performed denaturing to recover Scs2p-HA or Opi1p-MYC (Figure 5B). Both Yet1p and Yet3p were associated with Scs2p-HA and Opi1p-MYC in a cross-linker–dependent manner, consistent with our native immunoprecipitation results. We note that Scs2p-HA was not efficiently cross-linked to Opi1p-MYC under these conditions, presumably because primary amino groups in these proteins were not positioned to generate a cross-linked product. Given that we did not detect the Scs2p–Opi1p association using DSP, these results provide evidence that the Yet complex is proximally associated with both Scs2p and Opi1p. However, these results do not exclude the possibility that additional components are required for association of Yet1p–Yet3p with Scs2p–Opi1p.

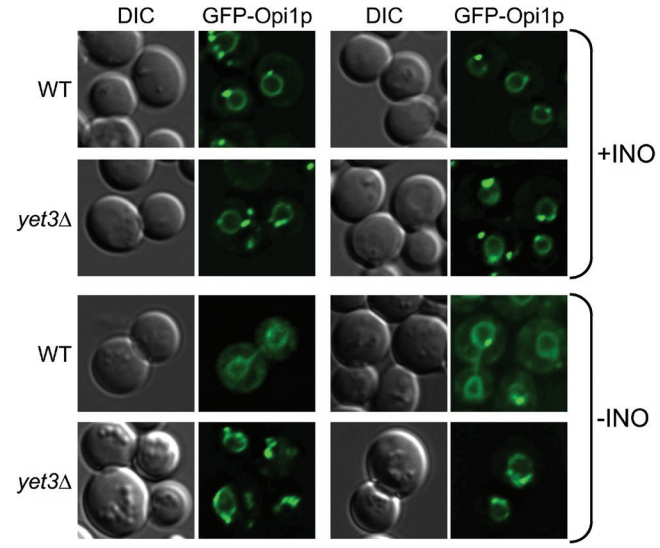


FIGURE 4: GFP-Opi1p mislocalization in a *yet3*Δ mutant during inositol starvation. Log phase cultures of wild-type (CBY3081) or *yet3*Δ mutant (CBY3084) cells expressing GFP-Opi1p were grown in the presence (75 μM) or absence of inositol for 14 h and imaged as described in *Materials and Methods*. Two representative fields are shown for each condition. Image boxes are 10 μm × 10 μm.

Inositol status regulates Yet1p and Yet3p association with Scs2p and Opi1p

We next examined the influence of inositol starvation on the interactions between Scs2p–Opi1p and the Yet proteins. For this experiment, cells were grown to log phase in the presence of inositol, washed, and then cultured for 3 h in the presence or absence of inositol. As shown in Figure 5C, immunoprecipitation of Scs2p-HA or Opi1p-MYC revealed that inositol starvation increased the amount of Yet1p and Yet3p coassociated with Scs2p-HA and Opi1p-MYC, suggesting that association of the Yet1p–Yet3p complex with Scs2p and Opi1p was stimulated by this condition. Notably, an increase in Scs2p–Opi1p association was not detected in these experiments as predicted from the model of *INO1* derepression (Loewen *et al.*, 2004). However, control experiments to assess protein association after detergent solubilization indicated that a mixture of digitonin-solubilized *SCS2-HA opi1*Δ semi-intact cells with *OPI1-MYC scs2*Δ semi-intact cells produced postsolubilization complexes in which Opi1p-MYC was efficiently coimmunoprecipitated with Scs2p-HA (Supplemental Figure 3). In contrast, a similar mixing experiment with *SCS2-HA yet3*Δ and *scs2*Δ *YET3* digitonin-solubilized semi-intact cells showed that postsolubilization association between Scs2p-HA and Yet3p was negligible (Supplemental Figure 3).

The Yet1p–Yet3p complex associates with a complex of Scs2p and Opi1p

Scs2p-HA membrane association and ER localization in *yet3*Δ mutant cells were indistinguishable from that of wild-type cells (unpublished data). In addition, the Scs2p–Opi1p interaction does not appear to depend on *YET1* (Figure 6A) or *YET3* (unpublished data), at least under conditions where membranes have been detergent solubilized. In contrast, Yet3p coassociation with Scs2p-HA or Opi1p-MYC was disrupted in *yet1*Δ mutant cells (Figure 6A). Similar results for Yet1p were obtained from Scs2p-HA immunoprecipitation experiments performed in a *yet3*Δ strain (unpublished data). These data suggest that, whereas the Yet complex does not influence the level of Scs2p–Opi1p coassociation, both members of the Yet

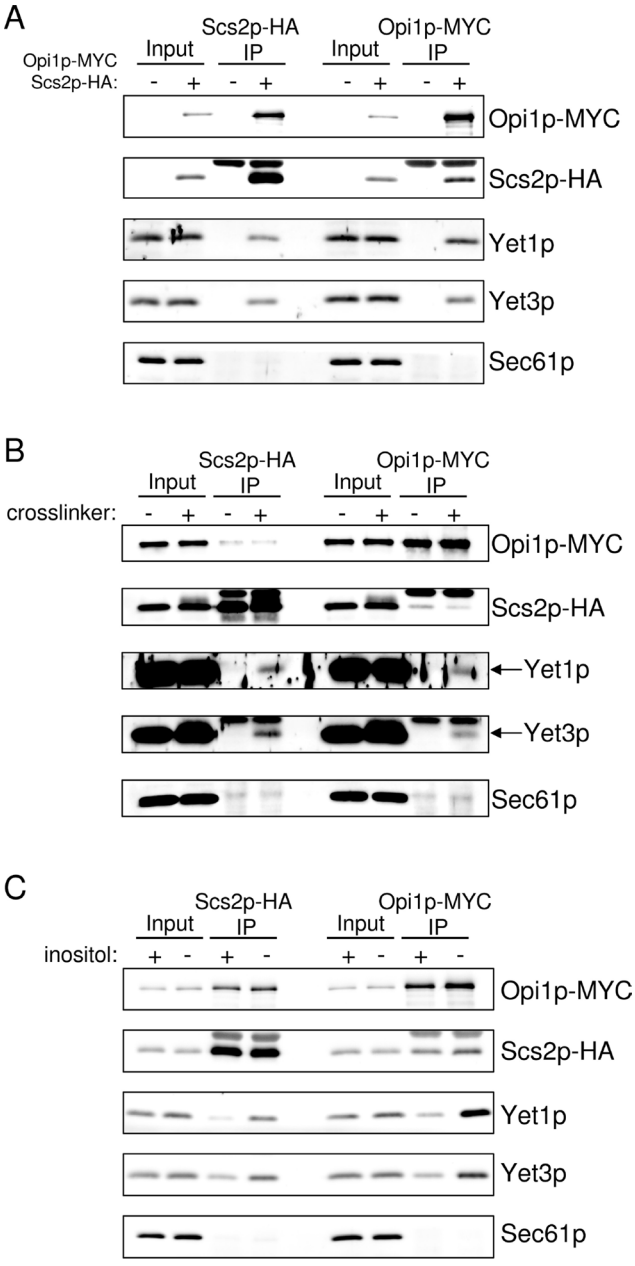


FIGURE 5: Scs2p-HA and Opi1p-MYC interact with Yet1p and Yet3p. (A) HA and MYC immunoprecipitations were carried out on digitonin-solubilized semi-intact cells after growth of the untagged strain (BY4742) and the Scs2p-HA Opi1p-MYC-tagged (CBY2903) strain in YPD. Immunoprecipitated material was analyzed by immunoblot. (B) Microsomes prepared from CBY2903 were subjected to DSP cross-linker (+) or mock treated (–), solubilized with SDS, and used for HA or MYC immunoprecipitations. (C) HA and MYC immunoprecipitations were performed as in (A) from the CBY2903 strain grown in the presence or absence of inositol for 3 h prior to harvest.

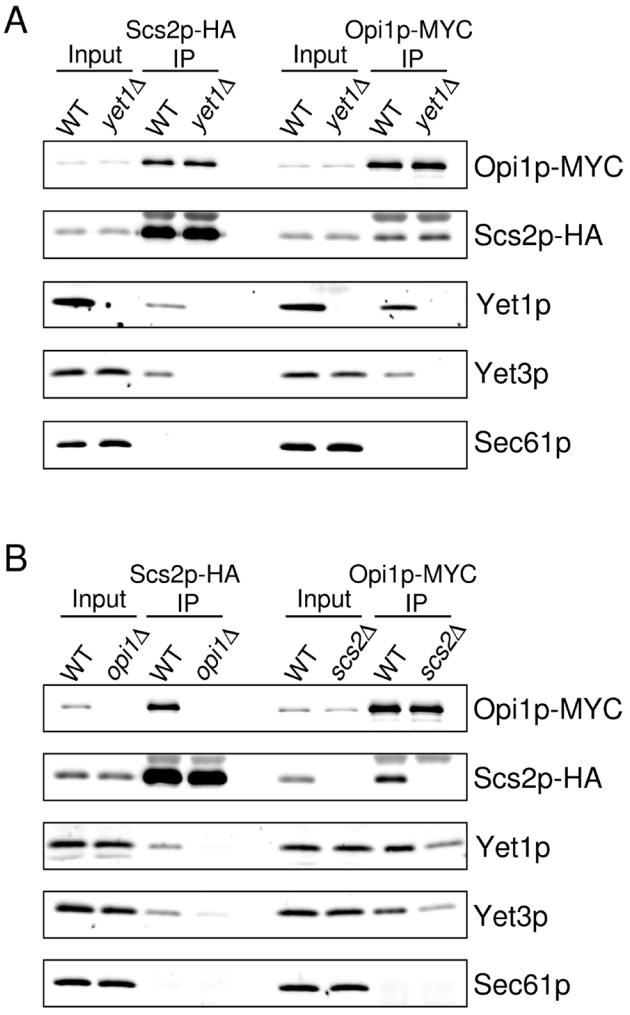


FIGURE 6: Influence of deletion mutations on Scs2p-HA and Opi1p-MYC interaction with Yet1p and Yet3p. (A) HA and MYC immunoprecipitations were performed as described in Figure 5A using wild-type (CBY2903) and *yet1*Δ (CBY2941) strains grown in YPD. (B) HA and MYC immunoprecipitations in wild-type (CBY2903), *opi1*Δ (CBY2947), and *scs2*Δ (CBY2948) strains.

complex are required for interaction with Scs2p–Opi1p. Furthermore these results correlate inositol prototrophy with an association between the Yet complex and Scs2p–Opi1p.

The protein domains required for the Scs2p–Opi1p interaction are well documented (Loewen *et al.*, 2003; Kaiser *et al.*, 2005; Loewen and Levine, 2005). Scs2p belongs to the conserved VAMP-associated protein (VAP) family in which the major sperm protein domain (N-terminal) in the cytoplasmic region of Scs2p interacts with the double phenylalanine in an acidic tract (FFAT) motif–containing domain in Opi1p (Kaiser *et al.*, 2005; Loewen and Levine, 2005). The observation that both Scs2p-HA and Opi1p-MYC coimmunoprecipitate Yet1p and Yet3p suggests that the Yet proteins interact with a complex of Scs2p and Opi1p. To test the individual importance of Scs2p and Opi1p for Yet complex association, we immunoprecipitated Scs2p-HA from *opi1*Δ cells and Opi1p-MYC from *scs2*Δ cells and then examined the levels of associated Yet1p and Yet3p (Figure 6B). Interestingly, deletion of either *OPI1* or *SCS2* decreased the level of associated Yet1p and Yet3p with the remaining Scs2p or Opi1p partner. Taken together with the cross-linking data shown in Figure 5B, these results support a model in which the Yet complex most efficiently associates with a complex of Scs2p and Opi1p. We noted that the amount of Yet complex associated with Opi1p-MYC in the *scs2*Δ mutant was higher than the amount associated with Scs2p-HA in the *opi1*Δ mutant. We reasoned that the *SCS2* homologue, *SCS22* (Loewen and Levine, 2005), might contribute to Yet complex association with Opi1p-MYC in the absence of Scs2p.

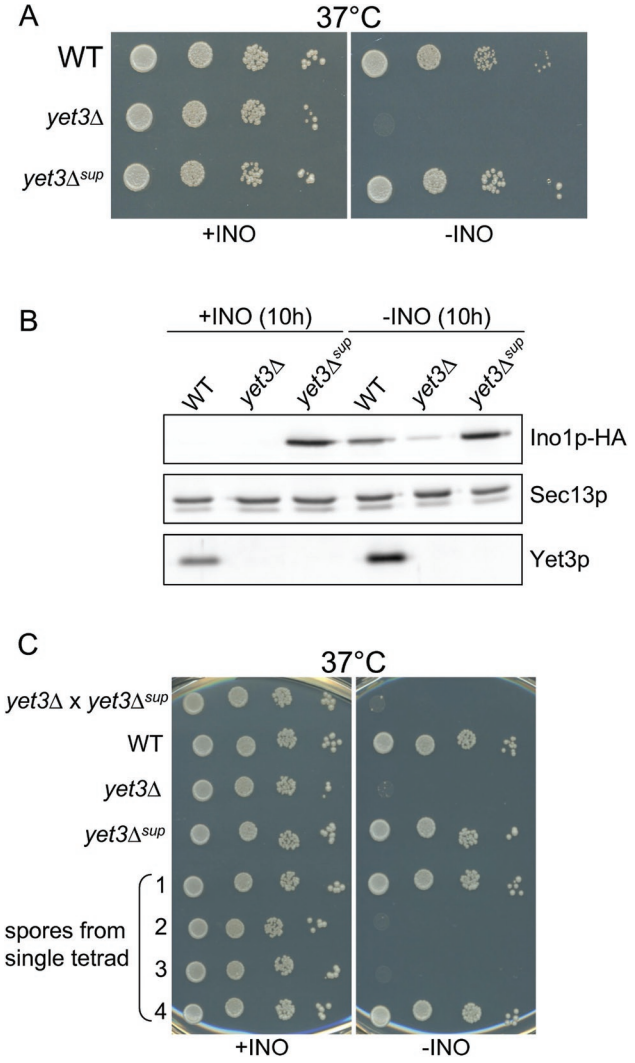


FIGURE 7: Analysis of *yet3*Δ^{sup}. (A) Wild-type (BY4742), *yet3*Δ (CBY2200), and *yet3*Δ^{sup} (CBY3096) strains were prepared as detailed in Figure 1 and grown in the presence or absence of inositol at 37°C for 3 d. (B) Whole cell lysates generated from Ino1p-HA–tagged wild-type (CBY2938), *yet3*Δ (CBY2939), and *yet3*Δ^{sup} (CBY3097) strains grown for 10 h in the presence or absence of inositol were analyzed by immunoblot. Sec13p is included as a loading control. (C) Growth assay performed as in (A) with *yet3*Δ x *yet3*Δ^{sup} (CBY3098), wild-type (BY4742), *yet3*Δ (CBY2791), and the four meiotic progeny of a single ascus of CBY3098.

However, immunoprecipitation of Opi1p-MYC in the *scs2*Δ *scs22*Δ double mutant background did not reduce the amount of Yet complex coassociated with Opi1p-MYC compared with the single *scs2*Δ mutant (Supplemental Figure 4). These observations indicate that the Yet complex can inefficiently associate with Opi1p in the absence of both Scs2p and Scs22p.

Loss of function mutation in *OPI1* suppresses inositol auxotrophy of a *yet3*Δ mutant

In experiments with *yet1*Δ *yet3*Δ mutants, we frequently observed a small number of colonies that grew up on plates lacking inositol at 37°C (unpublished data). The occurrence of such suppressor colonies was not specific to the *yet1*Δ *yet3*Δ strain or to the strain background used (BY4742), because they also appeared in a *yet3*Δ mutant and in a *yet1*Δ mutant in the FY833 background. Similar suppressor colonies were observed in *scs2*Δ and *ire1*Δ mutants at a comparable frequency (roughly 1:5000 colony-forming units after 1 wk in the absence of inositol at 37°C), suggesting that this phenomenon was not specific to cells lacking *YET1* and/or *YET3*. Importantly, comparable colony formation was not observed in an *ino1*Δ mutant, signifying a dependence on *INO1*. One such suppressed strain was isolated in a *yet3*Δ background (referred to as *yet3*Δ^{sup}) and growth was compared with the wild-type and parental *yet3*Δ strain (Figure 7A). Notably, the growth of *yet3*Δ^{sup} was more robust than that of the wild type in the absence of inositol at 37°C, whereas the parental *yet3*Δ strain showed essentially no growth under this condition. A constitutive loss of *INO1* repression that is characteristic of overproduction of inositol mutants (Opi$^-$) could explain this phenotype (Greenberg *et al.*, 1982a; 1982b). To examine the regulation of *INO1* in the *yet3*Δ^{sup} isolate, the expression of Ino1p-HA was monitored in the presence and absence of inositol. Indeed, as shown in Figure 7B, the *yet3*Δ^{sup} isolate expressed elevated levels of Ino1p-HA in both inositol-replete and -starvation conditions, demonstrating the predominant characteristic of an Opi$^-$ mutant.

To further characterize this *yet3*Δ^{sup} isolate, we crossed it to the parental *yet3*Δ mutant strain and found that the resulting diploid was sensitive to inositol starvation (Figure 7C). Tetrad dissection of this heterozygous diploid showed that the Opi$^-$ trait segregated 2:2. Together these observations provide evidence that the Opi$^-$ trait was most likely a recessive mutation. Growth of the spores from one such tetrad in the presence or absence of inositol is shown in Figure 7C. The most likely cause of the Opi$^-$ phenotype was a loss of function mutation in *OPI1*. To test this hypothesis, we sequenced the promoter and open reading frame of *OPI1* in the *yet3*Δ^{sup} strain and the parental *yet3*Δ strain and found that *yet3*Δ^{sup}, but not the parent, contained a nonsense mutation (cytosine to thymine at position +871) in the glutamine repeat region of the open reading frame. Interestingly, premature stops in this region have previously been found to result in the Opi$^-$ phenotype (White *et al.*, 1991). Thus the suppression of the inositol growth phenotype observed in the *yet3*Δ^{sup} strain indicates that, like *scs2*Δ and *ire1*Δ mutants (Cox *et al.*, 1997; Brickner and Walter, 2004), elimination of functional Opi1p is sufficient to rescue the inositol-starvation growth defect of the *yet3*Δ mutant.

DISCUSSION

In this study, we present evidence demonstrating that Yet1p and Yet3p are required for derepression of *INO1*. Cells harboring *yet1*Δ and *yet3*Δ mutations displayed comparable inositol-starvation growth phenotypes (Wilson and Barlowe, 2010) that were similar in magnitude to that of the known inositol derepression mutants *scs2*Δ and *ire1*Δ (Cox *et al.*, 1997; Kagiwada and Zen, 2003). *INO1* expression during inositol starvation was decreased in a *yet3*Δ mutant, as assessed by Ino1p-HA immunoblot and by an *INO1-LacZ* transcriptional reporter assay. Consistent with a defect in *INO1* derepression, we found that a significant amount of Opi1p remained in the nucleus during inositol starvation in a *yet3*Δ mutant. Furthermore the Yet1p–Yet3p complex interacted with Scs2p and Opi1p in manner that was stimulated by inositol starvation. Disruption of the Yet1p–Yet3p complex by *yet1*Δ mutation prevented Yet3p from interacting with Scs2p and Opi1p, connecting the inositol auxotrophy of a *yet1*Δ mutant to the loss of Yet complex association with Scs2p–Opi1p (similar results were obtained for Yet1p in a *yet3*Δ mutant, unpublished data). Together with our previous findings (Wilson and Barlowe, 2010), these results indicate that the Yet1p–Yet3p complex directly regulates Scs2p–Opi1p-mediated *INO1* derepression.

We note that, whereas 2μ overexpression of *SCS2* partially rescued *yet3*Δ (Figure 3A), similar overexpression of *YET1* and/or *YET3* had no detectable suppressive effect on an *scs2*Δ mutant (unpublished data). These results are consistent with a model in which the Yet complex confers inositol prototrophy through Scs2p. In other words, excess Yet complex provides no benefit in the absence of *SCS2*. It seems paradoxical then that an *scs2*Δ mutant displays a more modest growth phenotype than a *yet3*Δ mutant at 30°C and that the *yet3*Δ *scs2*Δ double mutant exhibits a clear synthetic defect in the absence of inositol. However, yeast contains a homologue of *SCS2*, known as *SCS22*, that has been reported to play a minor role in phospholipid metabolism because an *scs2*Δ *scs22*Δ double mutant showed a synthetic growth defect in the absence of inositol (Loewen and Levine, 2005). Thus one explanation for this paradox is that although Scs22p does not notably influence the association of the Yet complex with Opi1p in the absence of Scs2p (Supplemental Figure 4), it may possess a basal level of derepression activity that requires the Yet complex but is not significantly enhanced by its overexpression.

The observation that Yet1p–Yet3p interactions with Scs2p and Opi1p were increased by inositol starvation suggests that this association facilitates Scs2p-mediated *INO1* derepression. It does not seem to be simply a matter of modulating the association between Scs2p and Opi1p because the *yet1*Δ mutation did not detectably affect this interaction, at least under the detergent solubilization conditions we tested. However, the observation that Opi1p was not correctly localized to the ER in a *yet3*Δ mutant during inositol starvation suggests a role for the Yet1p–Yet3p complex in this process. Our favored model is that the Yet proteins bind to and sequester Scs2p–Opi1p complexes at ER membranes during inositol starvation, thus preventing diffusion and transport to the inner nuclear membrane (INM) and subsequent Opi1p-mediated repression of UAS_{INO} genes. Previous studies have shown that, in the absence of inositol, Opi1p binds to Scs2p and an increased pool of ER-localized phosphatidic acid to remain outside the nucleus (Loewen *et al.*, 2004). While this study was under review, two genome-wide screens of the yeast deletion collection identified >200 genes that influence growth rates in the absence of inositol, including *yet1*Δ and *yet3*Δ (Villa-Garcia *et al.*, 2010; Young *et al.*, 2010). Interestingly, several of these gene deletions cause reductions in intracellular pH, which decreases binding of Opi1p to phosphatidic acid and prevents efficient sequestration of Opi1p (Young *et al.*, 2010). The Yet1p–Yet3p complex does not appear to influence intracellular pH but may act to further stabilize the association between Opi1p-Scs2p and the pool of ER-localized phosphatidic acid to ensure stringent derepression during inositol starvation.

Alternatively, the Yet complex could play a more active role in the movement of Scs2p–Opi1p. The mechanism by which Opi1p exits the nucleus upon inositol starvation has not been described. ER localization of Opi1p does not depend on the major nuclear export receptors (Loewen *et al.*, 2004), and it has been suggested that Opi1p binds Scs2p on the INM (Brickner and Walter, 2004). One possibility is that Opi1p exits the nucleus in complex with Scs2p. If this is the case, Yet1p–Yet3p may somehow facilitate the INM to ER relocalization of Scs2p–Opi1p during inositol starvation. These models are consistent with observations in mammalian systems showing that the Yet protein homologue BAP31 influences localization of specific substrate molecules (e.g., Annaert *et al.*, 1997; Paquet *et al.*, 2004; Szczesna-Skorupa and Kemper, 2006). A final possibility is that the Yet1p–Yet3p complex facilitates Scs2p-mediated nuclear membrane recruitment of *INO1*, a requisite part of its activation (Brickner and Walter, 2004; Brickner *et al.*, 2007). Remarkably, artificial recruitment of the *INO1* locus to the nuclear membrane bypassed the *SCS2* requirement in the inositol-starvation response (Brickner and Walter, 2004). In contrast, this strategy was not sufficient to overcome the inositol requirement of a *hac1*Δ strain.

The Yet complex is required for Scs2p–Opi1p function although the Yet1p and Yet3p proteins appear to possess distinct functional properties. A similar relationship was reported for the mammalian homologues BAP29 and BAP31, which perform overlapping but nonidentical functions (Ladasky *et al.*, 2006; Abe *et al.*, 2009). We observed that inositol starvation has a more significant effect on Yet1p association with Scs2p and Opi1p (clearly apparent for Opi1p-MYC coimmunoprecipitation; Figure 5C) when compared with Yet3p association with Scs2p and Opi1p. Moreover, 2μ overexpression of *YET1* in the presence of inositol elevated basal *INO1* promoter activity, whereas 2μ overexpression of *YET3* reduced this promoter activity (Supplemental Figure 1). Taken together, these results suggest that increasing the relative amount of Yet1p in association with Yet3p positively influences the ability of the Yet complex to act in *INO1* derepression.

We previously reported genetic and physical interactions between the Yet1p–Yet3p complex and the Sec translocation complex (Wilson and Barlowe, 2010). More specifically, *yet1*Δ and *yet3*Δ mutants displayed synthetic growth defects in the absence of inositol when combined with *sec63–1*, *sec61–2*, or *sec71*Δ mutations. To test whether these genetic relationships are shared with other mutations in the *INO1* derepression pathway, we examined the growth of *scs2*Δ *sec63–1* and *scs2*Δ *sec71*Δ double mutant strains (Supplemental Figure 5). Interestingly, both *scs2*Δ *sec63–1* and *scs2*Δ *sec71*Δ mutant strains showed synthetic growth defects in the absence of inositol. These findings indicate some type of a connection between Scs2p-mediated *INO1* derepression and the ER translocation apparatus. We also considered the possibility that a general reduction in phosphatidyl inositol (PI) levels was not well tolerated by translocation-defective cells because PI levels are known to be diminished when cells are starved for inositol (Chang *et al.*, 2002) and are presumably reduced further in *yet*Δ and *scs2*Δ mutants. However, this possibility seems unlikely because the *ire1*Δ mutation, which also attenuates *INO1* derepression (Cox *et al.*, 1997), did not display synthetic growth defects in the absence of inositol when combined with *sec71*Δ (Supplemental Figure 5). These results support a specific genetic connection between *YET1*, *YET3*, and *SCS2* with components of the ER translocation apparatus.

In this report, we demonstrate a direct role for the yeast BAP31 homologues in regulation of Scs2p–Opi1p-mediated derepression of *INO1*. Scs2p belongs to a conserved family of ER-localized VAPs, which interact with a variety of intracellular proteins often through binding to a FFAT motif as found in Opi1p (Lev *et al.*, 2008). Many FFAT motif–containing proteins, including oxysterol binding proteins and ceramide transport proteins, functionally interact with VAPs to regulate lipid synthesis and transport in animal cells (Wyles *et al.*, 2002; Kawano *et al.*, 2006). Interestingly, a mutation in one of the human VAP genes produces late-onset familial motor neuron disease (Nishimura *et al.*, 2004). On the basis of sequence conservation and preserved ER localization for the BAP31 and VAP families, we speculate that Yet1p–Yet3p regulation of Scs2p–Opi1p function in yeast represents a conserved regulatory module that controls lipid synthesis and/or transport in other eukaryotic species. Further mechanistic dissection of this pathway in yeast should contribute to our general understanding of BAP31- and VAP-controlled processes with potential connections to human disease.

Strain	Genotype	Source
BY4741	*Mata his3Δ met1Δ0 leu2Δ0 ura3Δ0*	Research Genetics
BY4742	*Matα his3Δ leu2Δ0 lys2Δ0 ura3Δ0*	Research Genetics
FY834	*Matα his3Δ200 ura3–52 leu2Δ1 lys2Δ202 trp1Δ63*	Winston *et al.*, 1995
CBY1946	BY4742 with *ino2::kanMX6*	Research Genetics
CBY2200	BY4742 with *yet3::kanMX6*	Research Genetics
CBY2253	BY4741 with *sec71::kanMX6*	Research Genetics
CBY2431	BY4742 with *ire1::kanMX6*	Research Genetics
CBY2708	BY4741 with *sec63::sec63–1natMX4*	Wilson and Barlowe, 2010
CBY2791	BY4741 with *yet3::natMX4*	Wilson and Barlowe, 2010
CBY2819	BY4742 with *scs2::kanMX6*	Research Genetics
CBY2838	BY4742 with *INO1::HIS3MX6–3HA*	This study
CBY2839	CBY2200 with *INO1::HIS3MX6–3HA*	This study
CBY2853	BY4742 with *yet3::natMX4 scs2::kanMX6*	This study
CBY2901	BY4741 with *OPI1::13MYC-kanMX6 MET1*	This study
CBY2902	BY4742 with *OPI1::13MYC-kanMX6 yet3::natMX4 LYS2*	This study
CBY2903	BY4741 with *OPI1::13MYC-kanMX6 SCS2::HIS3MX6–3HA MET1*	This study
CBY2904	CBY2903 except *yet3::natMX4*	This study
CBY2905	CBY2200 with pRS426	This study
CBY2906	CBY2200 with *SCS2*/pRS426	This study
CBY2907	CBY2200 with *INO1*/pRS426	This study
CBY2908	CBY2819 with pRS426	This study
CBY2909	CBY2819 with *SCS2*/pRS426	This study
CBY2910	CBY2819 with *INO1*/pRS426	This study
CBY2941	CBY2903 with *yet1::kanMX6 lys2Δ0*	This study
CBY2947	BY4741 with *SCS2::HIS3MX6–3HA opi1::kanMX6 MET1*	This study
CBY2948	BY4742 *OPI1::13MYC-kanMX6 scs2::kanMX6 LYS2*	This study
CBY3015	BY4742 with *scs2::kanMX6 sec63::sec63–1natMX4*	This study
CBY3020	BY4742 with *scs2::kanMX6 sec71::kanMX6*	This study
CBY3039	BY4742 with pMR1036	This study
CBY3040	CBY2200 with pMR1036	This study
CBY3041	CBY2819 with pMR1036	This study
CBY3058	BY4742 with *ire1::natMX4 sec71::kanMX6*	This study
CBY3081	FY834 *ura3–52::PHO5-GFP-OPI1-URA3*	This study
CBY3084	CBY3081 with *yet3::HIS3MX6*	This study
CBY3096	CBY2200 except *yet3Δ*^{sup}	This study
CBY3097	CBY3096 with *INO1::HIS3MX6–3HA*	This study
CBY3098	*Mata/α* CBY2791 × CBY3096	This study
CBY3226	CBY2948 except *scs22::HIS3MX6*	This study
CBY3233	CBY3039 with *YET1*/pRS423 and *YET3*/pRS426	This study
CBY3234	CBY3039 with *YET1*/pRS423	This study
CBY3235	CBY3039 with *YET3*/pRS426	This study
CBY3236	CBY3039 with *SCS2*/pRS426	This study

TABLE 1: Yeast strains used in this study.

MATERIALS AND METHODS

Yeast strains and media

Yeast strains used in this study are listed in Table 1. All C-terminal epitope tagging was achieved using described methods (Longtine *et al.*, 1998), and all yeast transformations were performed using the lithium acetate technique (Ito *et al.*, 1983). Deletion mutants containing the *natMX4* cassette were created with p4339 (Tong *et al.*, 2001). Yeast cells were grown at 30°C in 1% yeast extract, 1% peptone, and 2% dextrose (YPD) medium unless otherwise noted. For plasmid selection, yeast cells were grown in 0.67% yeast nitrogen base without amino acids, 2% dextrose, and requisite supplements (YMD). For inositol-starvation growth assays, strains were grown overnight in YMD. After washing with sterile water, strains were plated on YMD with or without 75 μM inositol and grown at indicated temperatures. For experiments shown in Figures 2, 4, 5C, and 7B, starter cultures were grown in 0.67% yeast nitrogen base (without inositol) and complete supplement (MP Biomedicals, Solon, OH), 2% dextrose and 75 μM inositol (CSMD). After washes with sterile water, cells were resuspended and grown for the indicated time periods in either CSMD without inositol or CSMD with 75 μM inositol. All defined growth media lacked choline.

Plasmid construction

Genomic DNA from BY4742 was used as the template for the construction of plasmids. For *SCS2*/pRS426, *SCS2* including 479 nucleotides upstream of the open reading frame (ORF) and 298 nucleotides downstream of the ORF were cloned into *Bam*HI and *Eco*RI sites in the polylinker of pRS426 (Christianson *et al.*, 1992). For *INO1*/pRS426, *INO1* including 504 nucleotides upstream of the ORF and 337 nucleotides downstream of the ORF were cloned into *Bam*HI and *Eco*RI sites in the polylinker of pRS426; pMR1036 (Chang *et al.*, 2002) and pTL211 (Loewen *et al.*, 2003) have been described.

Antibodies

Antiserum directed against Yet1p (Toikkanen *et al.*, 2006), Yet3p (Wilson and Barlowe, 2010), Sec13p (Salama *et al.*, 1993), Kar2p

(Brodsky *et al*., 1993), and Sec61p (Stirling *et al*., 1992) have been described. The sheep anti–mouse and donkey anti–rabbit secondary horseradish peroxidase–linked antibodies were from GE Healthcare (Piscataway, NJ), and monoclonal antibodies against MYC (9E10) and HA (HA.11) were from Covance (Princeton, NJ).

Yeast cell lysates, immunoblotting, immunoprecipitation, and chemical cross-linking

Lysates (Figures 2A and 7B) were derived from ~0.5–1.0 OD_{600} equivalents of yeast cells and prepared using the bead-beat method in JR lysis buffer (20 mM HEPES pH 7.4, 0.1 M sorbitol, 50 mM potassium acetate, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride). Lysates were then centrifuged for three minutes at 1000 × *g* at 4°C to create a low-speed supernatant. This low-speed supernatant was mixed 2:1 with 5× reducing sample buffer, heated for 6 min at 75°C, and resolved by SDS–PAGE. After transfer to nitrocellulose membranes and antibody incubations, immunoblots were developed with SuperSignal West Pico chemiluminescent substrate (Pierce Chemical, Rockford, IL) and visualized by charge-coupled device (CCD) camera (UVP BioImaging, Upland, CA). Native immunoprecipitation experiments were performed with digitonin-solubilized semi-intact cell membranes as detailed previously (Wilson and Barlowe, 2010). For cross-linking experiments, 0.5 mM DSP (Pierce Chemical), or an equivalent volume of dimethyl sulfoxide (vehicle) was incubated for 15 min at 20°C with ~0.6 A_{280} units of microsomal membranes (Wuestehube and Schekman, 1992) prepared from log phase cells grown for 3 h in CSMD without inositol prior to harvest. After quenching with glycine, SDS was added to 1% and samples were heated to 75°C for 3 min. Samples were clarified by centrifugation and 100 µl of the supernatant fluid was diluted 10-fold with 15 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, and 2 mM sodium azide and used in immunoprecipitations.

β-Galactosidase assay

Cells transformed with pMR1036 were grown to log phase in CSMD-leucine with 75 µM inositol, washed in sterile water, resuspended in CSMD-leucine without 75 µM inositol, and grown for 5 h. β-Galactosidase assays were performed according to the manufacturer's instructions on β-galactosidase assays in yeast (ThermoFischer Scientific, Rockford, IL).

Microscopy

Stationary cultures grown in CSMD with 75 µM inositol were washed twice with CSMD without inositol and then diluted into either CSMD with 75 µM inositol or CSMD without inositol and grown for 14 h to an OD_{600} of 0.25–0.5. At this point, images were acquired with a Hamamatsu Orca R2 cooled CCD camera mounted on an Eclipse Ti Nikon microscope. Optical sections (0.2 µm) in the z axis were collected in GFP and differential interference contrast (DIC) channels with a 100× 1.4 NA objective. Iterative restoration and contrast enhancement were performed using Volocity software (Perkin Elmer, Waltham, MA).

ACKNOWLEDGMENTS

We thank C. Bentivoglio for technical assistance, S. Henry for pMR1036, T. Levine for pTL211, and M. Makarow for Yet1p antiserum. We also thank D. Compton and W. Wickner for the use of their microscopes. We are grateful to J. Merritt, J. Scarcelli, A. Lorente, C. Hodge, V. Starai, C. Stroupe, W. Wickner, L. Myers, J. Brickner, and C. Cole for helpful advice. This work was supported by NIH grant GM52549.

REFERENCES

Abe F, Van Prooyen N, Ladasky JJ, Edidin M (2009). Interaction of Bap31 and MHC class I molecules and their traffic out of the endoplasmic reticulum. J Immunol 182, 4776–4783.

Annaert WG, Becker B, Kistner U, Reth M, Jahn R (1997). Export of cellubrevin from the endoplasmic reticulum is controlled by BAP31. J Cell Biol 139, 1397–1410.

Brickner DG, Cajigas I, Fondufe-Mittendorf Y, Ahmed S, Lee PC, Widom J, Brickner JH (2007). H2A.Z-mediated localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. PLoS Biol 5, e81.

Brickner JH, Walter P (2004). Gene recruitment of the activated INO1 locus to the nuclear membrane. PLoS Biol 2, e342.

Brodsky JL, Hamamoto S, Feldheim D, Schekman R (1993). Reconstitution of protein translocation from solubilized yeast membranes reveals topologically distinct roles for BiP and cytosolic Hsc70. J Cell Biol 120, 95–102.

Carman GM, Henry SA (2007). Phosphatidic acid plays a central role in the transcriptional regulation of glycerophospholipid synthesis in *Saccharomyces cerevisiae*. J Biol Chem 282, 37293–37297.

Chang HJ, Jones EW, Henry SA (2002). Role of the unfolded protein response pathway in regulation of INO1 and in the sec14 bypass mechanism in *Saccharomyces cerevisiae*. Genetics 162, 29–43.

Christianson TW, Sikorski RS, Dante M, Shero JH, Hieter P (1992). Multifunctional yeast high-copy-number shuttle vectors. Gene 110, 119–122.

Cox JS, Chapman RE, Walter P (1997). The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane. Mol Biol Cell 8, 1805–1814.

Cox JS, Shamu CE, Walter P (1993). Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. Cell 73, 1197–1206.

Culbertson MR, Donahue TF, Henry SA (1976). Control of inositol biosynthesis in *Saccharomyces cerevisiae*; inositol-phosphate synthetase mutants. J Bacteriol 126, 243–250.

Culbertson MR, Henry SA (1975). Inositol-requiring mutants of *Saccharomyces cerevisiae*. Genetics 80, 23–40.

Donahue TF, Henry SA (1981). myo-Inositol-1-phosphate synthase. Characteristics of the enzyme and identification of its structural gene in yeast. J Biol Chem 256, 7077–7085.

Gardenour KR, Levy J, Lopes JM (2004). Identification of novel dominant INO2c mutants with an Opi^- phenotype. Mol Microbiol 52, 1271–1280.

Gaspar ML, Jesch SA, Viswanatha R, Antosh AL, Brown WJ, Kohlwein SD, Henry SA (2008). A block in endoplasmic reticulum-to-Golgi trafficking inhibits phospholipid synthesis and induces neutral lipid accumulation. J Biol Chem 283, 25735–25751.

Greenberg ML, Goldwasser P, Henry SA (1982a). Characterization of a yeast regulatory mutant constitutive for synthesis of inositol-1-phosphate synthase. Mol Gen Genet 186, 157–163.

Greenberg ML, Reiner B, Henry SA (1982b). Regulatory mutations of inositol biosynthesis in yeast: isolation of inositol-excreting mutants. Genetics 100, 19–33.

Henry SA, Patton-Vogt JL (1998). Genetic regulation of phospholipid metabolism: yeast as a model eukaryote. Prog Nucleic Acid Res Mol Biol 61, 133–179.

Heyken WT, Repenning A, Kumme J, Schuller HJ (2005). Constitutive expression of yeast phospholipid biosynthetic genes by variants of Ino2 activator defective for interaction with Opi1 repressor. Mol Microbiol 56, 696–707.

Hillenmeyer ME *et al*. (2008). The chemical genomic portrait of yeast: uncovering a phenotype for all genes. Science 320, 362–365.

Hoppe T, Matuschewski K, Rape M, Schlenker S, Ulrich HD, Jentsch S (2000). Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. Cell 102, 577–586.

Ito H, Fukuda Y, Murata K, Kimura A (1983). Transformation of intact yeast cells treated with alkali cations. J Bacteriol 153, 163–168.

Jesch SA, Zhao X, Wells MT, Henry SA (2005). 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 280, 9106–9118.

Kagiwada S, Hosaka K, Murata M, Nikawa J, Takatsuki A (1998). The *Saccharomyces cerevisiae* SCS2 gene product, a homolog of a synaptobrevin-associated protein, is an integral membrane protein of the endoplasmic reticulum and is required for inositol metabolism. J Bacteriol 180, 1700–1708.

Kagiwada S, Zen R (2003). Role of the yeast VAP homolog, Scs2p, in INO1 expression and phospholipid metabolism. J Biochem 133, 515–522.

Kaiser SE, Brickner JH, Reilein AR, Fenn TD, Walter P, Brunger AT (2005). Structural basis of FFAT motif-mediated ER targeting. Structure 13, 1035–1045.

Kawano M, Kumagai K, Nishijima M, Hanada K (2006). Efficient trafficking of ceramide from the endoplasmic reticulum to the Golgi apparatus requires a VAMP-associated protein-interacting FFAT motif of CERT. J Biol Chem 281, 30279–30288.

Ladasky JJ, Boyle S, Seth M, Li H, Pentcheva T, Abe F, Steinberg SJ, Edidin M (2006). Bap31 enhances the endoplasmic reticulum export and quality control of human class I MHC molecules. J Immunol 177, 6172–6181.

Lambert G, Becker B, Schreiber R, Boucherot A, Reth M, Kunzelmann K (2001). Control of cystic fibrosis transmembrane conductance regulator expression by BAP31. J Biol Chem 276, 20340–20345.

Lev S, Ben Halevy D, Peretti D, Dahan N (2008). The VAP protein family: from cellular functions to motor neuron disease. Trends Cell Biol 18, 282–290.

Loewen CJ, Gaspar ML, Jesch SA, Delon C, Ktistakis NT, Henry SA, Levine TP (2004). Phospholipid metabolism regulated by a transcription factor sensing phosphatidic acid. Science 304, 1644–1647.

Loewen CJ, Levine TP (2005). A highly conserved binding site in vesicle-associated membrane protein-associated protein (VAP) for the FFAT motif of lipid-binding proteins. J Biol Chem 280, 14097–14104.

Loewen CJ, Roy A, Levine TP (2003). A conserved ER targeting motif in three families of lipid binding proteins and in Opi1p binds VAP. EMBO J 22, 2025–2035.

Longtine MS, McKenzie A 3rd, Demarini DJ, Shah NG, Wach A, Brachat A, Philippsen P, Pringle JR (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast 14, 953–961.

Nikawa J (1994). A cDNA encoding the human transforming growth factor beta receptor suppresses the growth defect of a yeast mutant. Gene 149, 367–372.

Nikawa J, Murakami A, Esumi E, Hosaka K (1995). Cloning and sequence of the SCS2 gene, which can suppress the defect of INO1 expression in an inositol auxotrophic mutant of *Saccharomyces cerevisiae*. J Biochem 118, 39–45.

Nishimura AL *et al*. (2004). A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. Am J Hum Genet 75, 822–831.

Paquet ME, Cohen-Doyle M, Shore GC, Williams DB (2004). Bap29/31 influences the intracellular traffic of MHC class I molecules. J Immunol 172, 7548–7555.

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

Stirling CJ, Rothblatt J, Hosobuchi M, Deshaies R, Schekman R (1992). Protein translocation mutants defective in the insertion of integral membrane proteins into the endoplasmic reticulum. Mol Biol Cell 3, 129–142.

Szczesna-Skorupa E, Kemper B (2006). BAP31 is involved in the retention of cytochrome P450 2C2 in the endoplasmic reticulum. J Biol Chem 281, 4142–4148.

Toikkanen JH, Fatal N, Hildén P, Makarow M, Kuismanen E (2006). *YET1*, *YET2* and *YET3* of *Saccharomyces cerevisiae* encode BAP31 homologues with partially overlapping functions. J Biol Sci 6, 446–456.

Tong AH *et al*. (2001). Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294, 2364–2368.

Villa-Garcia MJ, Choi MS, Hinz FI, Gaspar ML, Jesch SA, Henry SA (2010). Genome-wide screen for inositol auxotrophy in *Saccharomyces cerevisiae* implicates lipid metabolism in stress response signaling. Mol Genet Genomics 285, 125–149.

Wagner C, Dietz M, Wittmann J, Albrecht A, Schuller HJ (2001). The negative regulator Opi1 of phospholipid biosynthesis in yeast contacts the pleiotropic repressor Sin3 and the transcriptional activator Ino2. Mol Microbiol 41, 155–166.

Wang B, Heath-Engel H, Zhang D, Nguyen N, Thomas DY, Hanrahan JW, Shore GC (2008). BAP31 interacts with Sec61 translocons and promotes retrotranslocation of CFTRDeltaF508 via the derlin-1 complex. Cell 133, 1080–1092.

Wang X, Sato R, Brown MS, Hua X, Goldstein JL (1994). SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. Cell 77, 53–62.

White MJ, Hirsch JP, Henry SA (1991). The OPI1 gene of *Saccharomyces cerevisiae*, a negative regulator of phospholipid biosynthesis, encodes a protein containing polyglutamine tracts and a leucine zipper. J Biol Chem 266, 863–872.

Wilson JD, Barlowe C (2010). Yet1p and Yet3p, the yeast homologues of BAP29 and BAP31, interact with the ER translocation apparatus and are required for inositol prototrophy. J Biol Chem 285, 18252–18261.

Winston F, Dollard C, Ricupero-Hovasse SL (1995). Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. Yeast 11, 53–55.

Wuestehube LJ, Schekman RW (1992). Reconstitution of transport from endoplasmic reticulum to Golgi complex using endoplasmic reticulum-enriched membrane fraction from yeast. Methods Enzymol 219, 124–136.

Wyles JP, McMaster CR, Ridgway ND (2002). Vesicle-associated membrane protein-associated protein-A (VAP-A) interacts with the oxysterol-binding protein to modify export from the endoplasmic reticulum. J Biol Chem 277, 29908–29918.

Young BP *et al*. (2010). Phosphatidic acid is a pH biosensor that links membrane biogenesis to metabolism. Science 329, 1085–1088.