# *SGD1* encodes an essential nuclear protein of *Saccharomyces cerevisiae* that affects expression of the *GPD1* gene for glycerol 3-phosphate dehydrogenase

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**Abstract We here report the identification of the previously uncharacterized *SGD1* gene, encoding a 102.8-kDa protein containing a leucine zipper region and a bipartite nuclear localization signal. Deletion of *SGD1* results in loss of cell viability, while an increased dosage of *SGD1* partially suppresses the osmosensitivity of *pbs2*Δ and *hog1*Δ mutants that are defective in the osmosignaling high osmolarity glycerol (HOG) mitogen-activated protein kinase pathway. The rescued mutants display a partially re-established transcriptional control of the osmostress-induced expression of *GPD1*, a target gene of the HOG pathway encoding $NAD^+$-dependent glycerol 3-phosphate dehydrogenase, and a partially recovered hyperosmolarity-induced production of glycerol. Consistent with Sgd1p affecting the transcriptional control of *GPD1*, a functional green fluorescent protein tagged Sgd1p is localized to the cell nucleus.** 

*Key words:* Gene regulation; Signal transduction; Osmoregulation; High osmolarity glycerol pathway

## 1. Introduction

In eukaryotic cells a variety of extracellular stimuli generate signals that are transmitted from cell surface receptors through multi-component signaling pathways to modify various intracellular target molecules. In many instances, evolutionarily well conserved mitogen-activated protein kinase (MAPK) modules are key elements in transmitting the signals to the downstream targets [1]. In the yeast *Saccharomyces cerevisiae* several different MAPK cascades operate, each of which is responding to different upstream signals and activating specific downstream events [2,3]. Exposed to increased osmolarity of the surrounding medium, *S. cerevisiae* activates a MAPK cascade referred to as the HOG (high osmolarity glycerol) MAPK pathway [4–6]. Activation of the HOG pathway leads to a transient phosphorylation [4,7] and transport of Hog1p into the nucleus [8,9] giving rise to transcriptional activation of several genes, including *GPD1* [10,11], encoding cytosolic glycerol 3-phosphate dehydrogenase (GPD). Increased glycerol production and intracellular accumulation is a key event in the adaptation of *S. cerevisiae* to hyperosmotic stress [12,13], and this response is dependent on a functional HOG pathway [4]. Deletion of the *HOG1* or the *PBS2* gene causes decreased glycerol production following transfer to hyperosmolarity [4], with a marked decrease of the transcriptional activation of the *GPD1* gene [10,11]. It was recently demonstrated that the short term transcriptional response to hyperosmotic stress by *GPD1* and other HOG pathway target genes is dependent on the transcriptional factors Msn1p, Msn2p, Msn4p, and the novel nuclear factor Hot1p [14]. Moreover, the osmotic induction of *GPD1* was also found to be dependent on the repressor/activator protein Rap1p [15], suggesting that the full transcriptional activation requires the cooperative interaction of a distinct set of factors.

Here we report the characterization of a novel gene, *SGD1*, which was cloned by complementation of an osmostress-sensitive, glycerol-defective mutant. The *SGD1* gene encodes an essential protein, which is localized to the nucleus and exhibits a dosage-dependent suppression of the osmosensitive phenotype of *pbs2*Δ and *hog1*Δ mutants.

## 2. Materials and methods

### 2.1. Strains, media and growth conditions

*S. cerevisiae* strains used in this study were W303-1B MATα *ade2-1 can1-100 leu2-3/112 trp1-1 ura3-1 his3-11/15* [16] and the W303 derivatives U451 MATa *ade2-1 can1-100° leu2-3/112 trp1-1 ura3*-1 and H19 MATα *ade2-1° can1-100° leu2-3/112 trp1-1 ura3-1 sup4*$^+$:pBR322:*URA3:SUP4 his3-11/15* [17]. For suppression studies isogenic *hog1*::*LEU2* [10] or *pbs2*::*LEU2* [18] or *hog1*::*TRP1 pbs2*::*LEU2* (this study) strains were used. The genotype of the diploid strain having one *SGD1* gene deleted was *ade2-1/ade2-1 can1-100/can1-100 leu2-3,112/leu2-3, 112 trp1-1/trp1-1 ura3-1/ura3-1 his3-11,15/1 his3-11,15 SGD1/sgd1*Δ::*LEU2*. For linkage studies strain M2452α MATα *ura3ade6* (kindly supplied by M. Kielland-Brandt) was used. Subcellular localization was conducted using strain ACY192 MATa *ura3-52 trp1*Δ*63 leu2*Δ*GAL*$^+$ (A.H. Corbett). The growth and maintenance of yeast strains were as previously described [18]. High osmolarity media were prepared by adding the appropriate amount of NaCl, KCl or sorbitol.

*Escherichia coli* strains DH5α [19] and TG1 [20] were grown at 37°C, in LB (Luria broth) supplemented with ampicillin (100 μg/ml) when required.

### 2.2. DNA techniques

All recombinant DNA techniques were carried out according to standard protocols [20,21]. DNA fragments complementing an osmotically sensitive mutant (*osg3* mutant) were cloned using a YCp50 DNA library [22] that was transformed into the *osg3* mutant and colonies restoring growth on YNB plates with 1.4 M NaCl were picked for further analysis. Plasmid DNA was isolated and amplified in *E. coli*. Restriction maps of the complementing fragments were

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constructed and subcloning was performed by cloning into the centromeric vector pRS316 [23] containing *URA3* as the marker gene. A 4.8-kb *Sal*I–*Sal*I subclone, containing the functionally complementing gene (the *SGD1* gene) and 0.23 kb from the Ycp50 plasmid, was further subcloned and partially sequenced directly from the pRS316 vector, using the Sequenase kit, version 2.0 (United States Biochemicals, USA), based on the dideoxy chain termination method.

Deletion of the *SGD1* gene was performed by subcloning the *Kpn*I–*Sal*I fragment containing the entire *SGD1* gene plus 230 bp of YCp50 into a modified pBluescript (Stratagene, USA) where the *Cla*I–*Xba*I sites in the polylinker had been removed. A *Pst*I–*Cla*I fragment from the promoter-ORF region of the *SGD1* gene was removed, the *Cla*I end of the remaining insert blunt-ended prior to cutting with *Pst*I. The released fragment was replaced by the *LEU2* gene, cut with *Pst*I and *Sma*I in the YDp-L plasmid [24]. The deletion-insertion construct was cut out with *Swa*I and *Hpa*I, and transformed into the W303 diploid. The deletion was confirmed by Southern blot analysis. Total DNA was cut with *Dra*I and a 1.68-kb *Kpn*I–*Hpa*I probe of *SGD1* gene was used to detect the deletion insertion. Labeling of probe, hybridization and detection was performed according to the DIG DNA labeling and detection kits (Roche Molecular Biologicals, Germany). Sporulation of the heterozygous diploids containing the *sgd1*Δ*::LEU2* deletion was performed using standard techniques.

To assess the effect of *SGD1* overexpression the 4.8-kb *Sal*I–*Sal*I fragment was inserted into the 2 μ-based YEplac195 vector [25] having *URA3* as the marker. This plasmid was used to transform the *hog1*Δ*::LEU2* and *pbs2*Δ*::LEU2* single or double mutants. To construct a Sgd1p–green fluorescent protein (GFP) fusion, the *SGD1* gene was amplified with 5′-*Spe*I and 3′-*Sal*I ends by polymerase chain reaction (PCR) using the following primers: forward primer with *Spe*I-end: 5′-GACTAGTCTCTGCCTTGTCAGCATCATC-3′; reverse primer with *Sal*I-end: 5′-GCGTCGACACGAATCTATATTTTCCAG-3′.

The resulting PCR product was cleaved with *Spe*I and *Sal*I and cloned in frame at the 5′-*Spe*I and 3′-*Xho*I fusion site of the centromer-based vector pAC242 containing the GFP gene sequence [26]. The *SGD1–GFP* gene then was tested for suppression of the *osg3* mutation and the *SGD1* deletion to demonstrate that the fusion protein was functional.

### 2.3. Genetic analysis

To establish whether the DNA fragment complementing the *osg3* mutation contained the allelic gene, linkage analysis was performed. The complementing *Sal*I–*Sal*I fragment was cloned into the integrating vector pRS406 [23] having *URA3* as a marker. The plasmid was cut open at the *Nru*I site within the putative ORF and transformed into the *osg3* mutant. The resulting transformants were crossed with strain M2452α and the segregation of the osmosensitive phenotype was assessed in the tetrads.

### 2.4. Northern blotting

Total RNA was isolated according to standard procedures [21] from overnight cultures subjected to osmotic stress (0.7 M NaCl) by the addition of an equal volume of fresh YNB medium containing 1.4 M NaCl. Gel electrophoresis of RNA, blotting and hybridization were performed as previously described [11]. The oligonucleotides used for probing, i.e. 5′-TGTACTATTGGAGCGAAAACTTCT-3′ for *GPD1*, and 5′-AATCGATTCTCAAAATGGCGTGAGG-3′ for *ACT1*, were end-labeled with [$\gamma$-$^{32}$P]dATP, using 5 U of polynucleotide kinase (Gibco BRL, Life Technology, USA). To quantify transcript levels, the signal intensity was determined with a Molecular Dynamics (USA) Phosphoimager, each intensity being normalized to that for *ACT1* mRNA.

### 2.5. Immunoblotting and GFP fluorescence microscopy

To confirm expression of Sgd1p–GFP by immunoblot analysis, cells were grown to mid-logphase in YNB media and collected by centrifugation. As control, cells expressing GFP alone from the *GAL* promoter were precultured in YNB-raffinose (2% w/v) overnight, and incubated with galactose (2% w/v) for 4 h before collection. After washing with sterile water the collected cells were resuspended in PBSMT buffer (11.5 g/l $Na_2HPO_4$, 80 g/l NaCl, 2 g/l KCl, 0.5 g/l $MgCl_2$, 0.5% Triton X-100) containing protease inhibitors (0.5 mM PMSF and 3 μg/ml each of leupeptin, aprotinin, chymotrypsin and pepstatin). Glass beads were added and cells were lyzed using a bead beater at 4° with 6-s pulses 6–10 times. A total of 10–20 μg protein was resolved by polyacrylamide gel electrophoresis on a 10% gel and immunoblotted to a nitrocellulose membrane using standard methods [27]. The membrane was probed with a polyclonal anti-GFP antibody (gift of J. Kahana and P. Silver, Harvard Medical School, Boston, MA, USA) and developed using the ECL chemiluminescence kit (Amersham Pharmacia Biotech AB, Sweden).

For Sgd1p–GFP localization studies, wild-type cells expressing the Sgd1p–GFP were grown overnight in YNB medium and Sgd1p–GFP was visualized without fixation. Aliquots of 2 μl were transferred to microscope slides and covered with coverslips. Cells were viewed through a GFP optimized filter (Chroma Technology, USA) using a BX60 epifluorescence microscope (Olympus) equipped with a Quantix digital camera (Photometric, USA). The nuclear material stained with 2 μg/ml of 4′,6-diamidino-2-phenylindole (DAPI) for 10 min, and visualized under the microscope using a FITC filter.

### 2.6. Glycerol analysis, preparation of cell-free extracts and enzyme activity measurements

Cultures for glycerol analysis and enzyme assays were either grown to an $OD_{610}$ of approximately 1.0 in YNB medium containing NaCl as stated in the text, or grown to the same $OD_{610}$ in YNB medium lacking salt, followed by a shift to increased salinity by addition of an equal volume of fresh YNB medium containing an appropriate concentration of NaCl. Samples for total glycerol were withdrawn and determined as previously described [13].

For enzyme activity determination, cells were collected by centrifugation, washed twice in TRED buffer (10 mM triethanolamine, 1 mM EDTA and 1 mM dithiothreitol, pH 7.0), frozen in liquid nitrogen and stored at −20°C until used. The preparation of cell-free extracts and the assay of the specific activity of $NAD^+$-dependent GPD were as described previously [28] except that the buffers contained the following protease inhibitors: 0.5 mM PMSF and 3 μg/ml each of leupeptin, aprotinin, chymotrypsin and pepstatin.

## 3. Results

### 3.1. Isolation of the osmosensitive osg3 mutant and cloning of the SGD1 gene

The *osg3* (osmotically sensitive glycerol-defective) mutant was isolated in *S. cerevisiae* strain U451 by methods previously reported [29]. The mutant showed impaired growth on plates containing more than 0.7 M NaCl (or iso-osmolar concentrations of KCl and sorbitol) and displayed a decreased capacity to increase glycerol production at high external osmolarities (Fig. 1B). Tetrad analysis of a cross between the mutant and the wild-type cells exhibited a 2:2 segregation pattern of the osmosensitive phenotype, indicating the defect is due to a single mutation. A gene complementing the *osg3* osmosensitivity was isolated from a YCp50 DNA library [22]. Plasmid inserts that allowed growth on high osmolarity media when retransformed into the *osg3* mutant were analyzed by restriction analysis and an overlapping fragment from two complementing plasmids was chosen for subcloning. A 4.8-kb *Sal*I–*Sal*I fragment cloned into the centromeric pRS316 vector restored the osmotolerance of the mutant (Fig. 1C). Similarly, the glycerol production defect exhibited by the mutant at 1.0 M NaCl was restored in transformants containing the *Sal*I–*Sal*I fragment (data not shown). To conduct linkage analysis, the *Sal*I–*Sal*I segment was cloned into the integrating plasmid pRS406, linearized at the *Nru*I site, and transformed into the *osg3* mutant. Diploids were obtained by crossings, and the segregation of the osmotolerant phenotype was followed after sporulation. For 30 complete tetrads, osmosensitive haploid progeny was present in almost all the tetrads analyzed. From this observation we concluded that we had cloned a suppressor gene of the *osg3* mutation rather than the wild-type allele of the mutated gene. Partial DNA sequencing

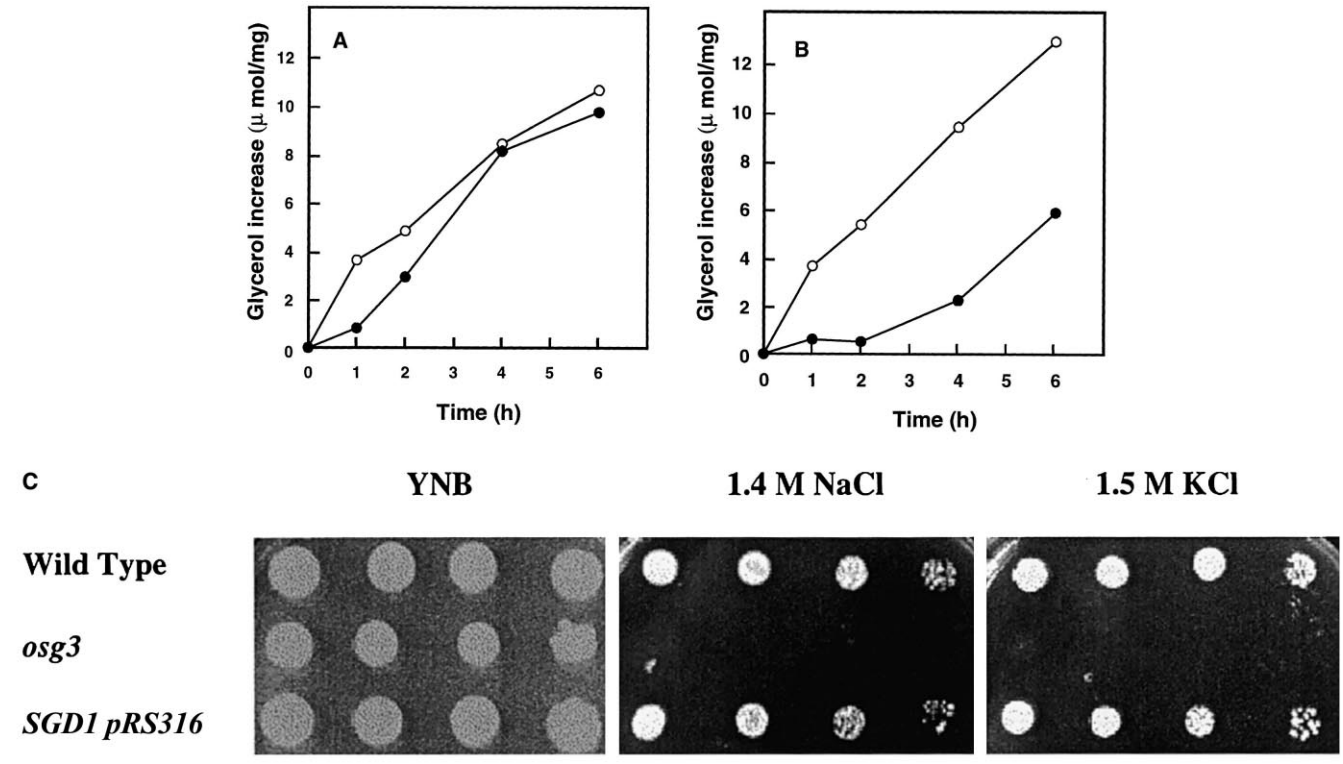


Fig. 1. Glycerol production of wild-type (○) and *osg3* mutant (●) after shifting exponentially growing cells cultured in YNB medium to fresh medium containing (A) 0.7 M NaCl or (B) 1.0 M NaCl. Samples were taken at indicated times after the shift and analyzed for total glycerol content. Values represent the mean of two independent experiments. C: Suppression of the *osg3* mutation by the *Sal*I–*Sal*I clone on high osmolarity media. Cells were grown overnight in YNB medium and spotted in 10-fold dilution steps onto YNB plates containing no added NaCl, 1.4 M NaCl or 1.5 M KCl. Growth was assessed after 7 days.

of subclones of the *Sal*I–*Sal*I fragment and database searches identified the gene, termed *SGD1*, as an open reading frame of 2700 nucleotides on chromosome 12, encoding a hypothetical protein of 899 amino acids with a calculated molecular weight of 102.8 kDa (open reading frame YLR336c). The gene was sequenced by the *S. cerevisiae* genome project (accession U19028). The deduced sequence displays 31% identity over 705 amino acids to a hypothetical 87.9-kDa *Schizosaccharomyces pombe* protein of unknown function (accession Z98601), 27% identical to an ORF in *Drosophila melanogaster* (accession AAF47640) and 24% identity over 220 amino acids to the *S. cerevisiae* Spt7p, a subunit of the nucleosomal SAGA complex [30]. Protein motif analysis of Sgd1p showed overlapping bipartite nuclear localization signals at amino acid positions 34 to 62 and a leucine zipper region at positions 505 to 536. No region of basic amino acids are found upstream of the heptad repeats of leucines, making kinship to the family of basic leucine zipper transcription factors unlikely.

### 3.2. Loss of the SGD1 function is lethal

A deletion of the *SGD1* gene was generated by removing 491 codons on the N-terminal side of the *SGD1* open reading frame together with 18 bases upstream of the ATG codon and replacing this part with the *LEU2* gene. A *S. cerevisiae* W303 diploid homozygous for the *leu2* mutation was transformed with the linearized *sgd1*Δ::*LEU2* plasmid and Leu$^+$ transformants isolated. Deletion of one copy of the *SGD1* gene in the diploids was confirmed by Southern blotting. The heterozygous *SGD1*/*sgd1*Δ::*LEU* diploids were sporulated and tetrads dissected. From a total of 20 tetrads, 18 contained only two viable spores, all being Leu$^-$, indicating that

Table 1
Total glycerol production and specific activity of NAD$^+$-dependent GPD in wild-type, *pbs2*Δ, and *hog1*Δ mutant cells carrying the multicopy YEplac195 vector or the same vector with the *SGD1*-containing *Sal*I–*Sal*I fragment

| | Growth conditions | WT+2 μ | WT+*SGD1*-2 μ | *pbs2*Δ+2 μ | *pbs2*Δ+*SGD1*-2 μ | *hog1*Δ+2 μ | *hog1*Δ+*SGD1*-2 μ |
|---|---|---|---|---|---|---|---|
| Glycerol | 0 M NaCl | 4.96 | 6.04 | 1.96 | 6.38 | 4.52 | 7.02 |
| (μmol/mg dry weight) | 0.7 M NaCl | 13.96 | 14.24 | 11.09 | 26.28 | 11.08 | 15.65 |
| GPD activity | 0 M NaCl | 40.9 | 70.8 | 22.5 | 35.8 | 9.3 | 28.98 |
| (mU/mg protein) | 0.7 M NaCl | 137.2 | 116 | 60.6 | 154.8 | 59.3 | 80.3 |

For glycerol analysis, cells growing exponentially in YNB medium were shifted to fresh medium containing 0 or 0.7 M NaCl followed by further incubation for 6 h, whereupon samples were removed. Values are means from two independent experiments. The GPD activities were measured in desalted crude extracts of cells grown to $OD_{610} = 1.0$ in YNB medium containing 0 or 0.7 M NaCl. The enzyme activities represent means of three independent measurements. Deviation between samples were $< 20\%$.

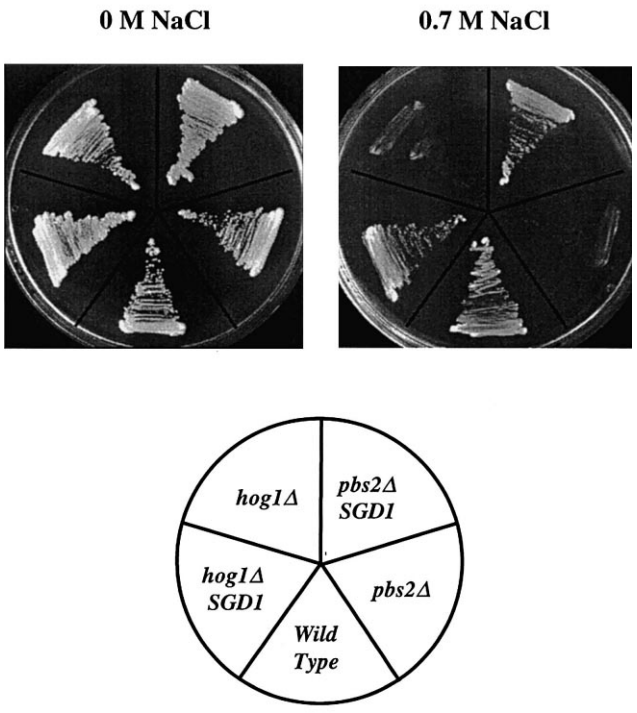


Fig. 2. Suppression of the osmosensitive growth phenotype of HOG pathway mutants by high gene dosage of the *SGD1* gene. Growth of wild-type, *pbs2*Δ, *hog1*Δ mutants containing the multicopy YEplac195 vector or the same vector with the *SGD1*-containing *Sal*I–*Sal*I fragment (*SGD1*). Growth was assessed after 5 days on YNB medium containing no added NaCl or 0.7 M NaCl.

*SGD1* encodes an essential gene product. Microscopic analysis of the non-viable spores showed that some of the spores had germinated and divided 2–4 times. This indicates that the *SGD1* gene is essential for growth but not for spore germination.

To further test whether there is an absolute requirement of *SGD1* for cell viability, a heterozygous *SGD1*/*sgd1*Δ diploid was transformed with the *SGD1* gene on pRS316 vector carrying the *URA3* gene as a marker. The *SGD1*/*sgd1*Δ::*LEU* diploid was sporulated and dissection yielded tetrads with four viable spores. Viable haploids having $Leu^+$ $Ura^+$ phenotype were grown in YPD overnight and tested for growth on YNB plates containing 5-fluoro-orotic acid (5-FOA) and uracil, counter-selecting against the *URA3* marker. A wild-type strain with the *SGD1* gene on pRS316 was used as the control. Loss of the *SGD1* gene contained by the *URA3* plasmid resulted in complete loss of viability for the *sgd1*Δ::*LEU2* mutant, while a considerable number of the wild-type control cells survived on the 5-FOA plates (data not shown). An additional control was performed by plating both strains on YNB plates lacking 5-FOA on which both formed colonies. From these experiments, we conclude that loss of the *SGD1* gene product results in cell death, and that *SGD1* is required for mitotic growth and has at least one essential role.

### 3.3. High dosage of SGD1 rescues HOG pathway mutants on high osmolarity media

To examine whether *SGD1* has a role in the osmoregulatory response, we transformed *hog1*Δ and *pbs2*Δ signaling defective mutants with the *SGD1* gene (the *Sal*I–*Sal*I fragment) in the YEplac195 multicopy plasmid (*SGD1*-2 μ). Increased copies of the *SGD1* gene relieved the sensitivity of *pbs2*Δ and *hog1*Δ mutants to 0.7 M NaCl stress (Fig. 2). To assess the basis for the *SGD1*-mediated suppression of defects in the HOG path-

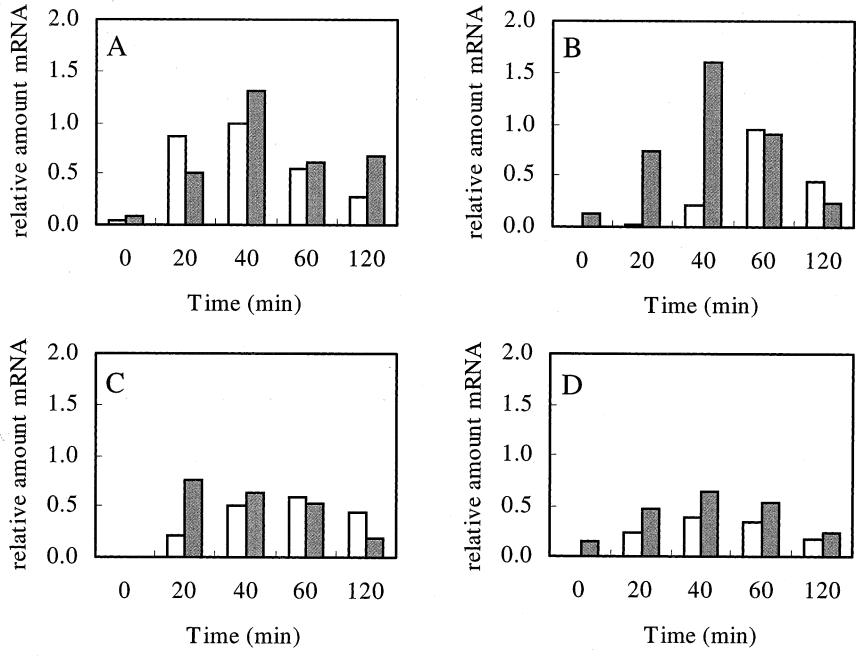


Fig. 3. Quantified Northern analysis of *GPD1* mRNA in (A) wild-type, (B) *pbs2*Δ, (C) *hog1*Δ and (D) *pbs2*Δ*hog1*Δ cells carrying the multicopy YEplac195 vector (open bars) or the same vector with the *SGD1*-containing *Sal*I–*Sal*I fragment (shaded bars). Samples were taken at indicated time points after shifting cells growing exponentially in YNB medium to fresh medium containing 0 or 0.7 M NaCl. Recorded mRNA quantities were normalized to the control *ACT1* mRNA value.

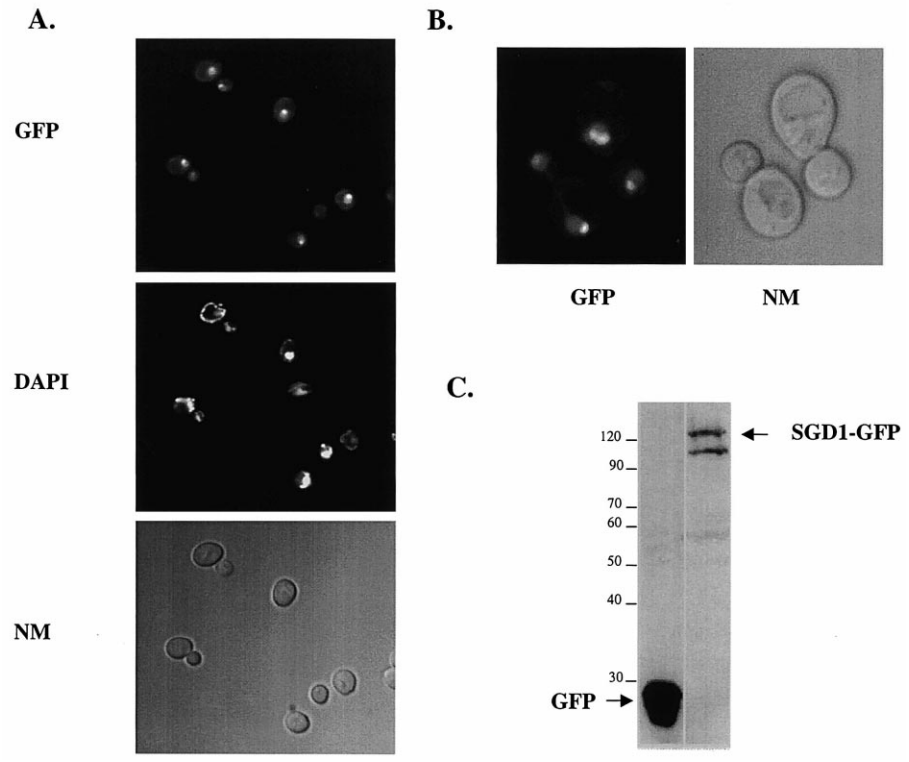


Fig. 4. Subcellular localization of Sgd1p–GFP in living yeast cells and confirmation of Sgd1p–GFP expression by Western blot. *S. cerevisiae* strain ACY192, expressing Sgd1p–GFP from the centromer-based vector pAC242, was grown in YNB liquid medium to early exponential phase and cell samples were taken and processed for microscopy as described in Section 2. The same field of cells was photographed to detect (A) Sgd1p–GFP-associated fluorescence, (GFP), DNA fluorescence after staining with DAPI or whole cells by Nomarski optics (NM). In living cells DAPI gives bright staining of mitochondrial DNA, whereas chromosomal DNA gives a diffuse spot. B: Enlarged cells to demonstrate the distribution of Sgd1p–GFP fluorescence in the nucleus. C: Immunoblot analysis of extracts from overnight cells cultured in selective media and resolved on a 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis blotted onto a nitrocellulose membrane. The GFP was detected by polyclonal anti-GFP antibodies. The left lane shows an extract of cells harboring a control plasmid containing GFP expressed from the *GAL1* promoter, while the right lane shows an extract of cells expressing the Sgd1p–GFP fusion protein from the pAC242 vector. The upper band has a position corresponding to the expected Sgd1p–GFP size (130 kDa); the lower band probably represents a degradation product.

way, we measured glycerol production and the activity of GPD (Table 1) for cells exposed to 0 or 0.7 M NaCl, when carrying an empty vector or *SGD1*-2 μ. The presence of multiple copies of *SGD1* did not cause any apparent change in glycerol production of wild-type cells, while in *hog1*Δ and *pbs2*Δ mutants the defective glycerol production was restored to levels similar to or higher than (*pbs2*Δ) for wild-type cells (Table 1). In a *hog1*Δ*pbs2*Δ double mutant, the effects of *SGD1*-2 μ resembled those obtained in the *hog1*Δ mutant. Hence, the introduction of multiple copies of *SGD1* enhances basal as well as the stress-induced levels of glycerol production in HOG pathway mutants. Measurements of the activity of GPD (Table 1), which has a key role in glycerol biosynthesis [13], gave results that paralleled what was observed for glycerol production. To further analyze the suppression effect caused by a high copy number of *SGD1*, we quantified the levels of *GPD1* transcript in wild-type cells and HOG pathway mutants, after a shift of cells to increased (0.7 M) NaCl (Fig. 3). The observed effects demonstrated that dosage effects of *SGD1* are exerted at the transcriptional level in the HOG pathway mutants. While increased dosage has small or insignificant effects on the osmostress-mediated transient induction of *GPD1* in wild-type cells, the decreased and delayed induction in the *pbs2*Δ mutant is restored to not only wild-type levels but, importantly, also to wild-type kinetics. Hence, in this mutant the introduction of *SGD1*-2 μ causes restoration of the HOG pathway defect. In *hog1*Δ and *hog1*Δ*pbs2*Δ mutants there is a similar recovery of the response, but not to the extent observed for the *pbs2*Δ mutant. The *Sal*I–*Eco*RI region, containing the portion of the upstream *VRP1* gene that is included in the *Sal*I–*Sal*I fragment, did not cause suppression when introduced on its own in a multicopy plasmid (pRS424) into *pbs2*Δ or *hog1*Δ mutants. Hence, we conclude that it is the authentic *SGD1* gene that is required for the suppression.

### 3.4. Sgd1p–GFP localizes to the nucleus

Since the assumed function of Sgd1p is likely to be exerted inside the nucleus, or by control of nuclear entry, a fusion of the GFP to the C-terminal end of the Sgd1p was constructed, to allow for in vivo localization of the protein. The *SGD1–GFP* gene expressed from its own promoter in a centromeric plasmid fully suppressed the lethality of the *SGD1* deletion as well as the salt sensitivity of the *osg3* mutant (data not shown), demonstrating that the fusion protein is functional. Furthermore Western blot analysis demonstrated that the transformed cells express a GFP-linked protein of the expected size for Sgd1p–GFP (Fig. 4C). The subcellular distribution, as revealed by Sgd1p–GFP fluorescence, demonstrated that the protein is concentrated to the cell nucleus and colocalizes with DAPI-stained DNA (Fig. 4A). Distribution to the nucleus was also confirmed using indirect immunofluorescence with antibodies directed against GFP (data not shown). No

obvious influence on the nuclear localization of Sgd1p was observed by the presence of a *pbs2*Δ or *hog1*Δ mutation or by exposure of the cells to salt stress (0.7 M NaCl, data not shown). The distribution of the Sgd1p–GFP fluorescence within the nuclear region suggests that the protein is enriched in the nucleolus (Fig. 4B).

## 4. Discussion

We here report the cloning of *SGD1*, encoding a nuclear protein that is essential for cell viability and exhibits a dosage-dependent suppression of HOG pathway defects. The *SGD1* gene was isolated as an extragenic suppressor of the osmosensitive and glycerol-defective phenotype of a *S. cerevisiae* mutant. This pointed towards a possible function for *SGD1* in the osmoregulatory glycerol response, which involves a controlled induction of *GPD1* expression to raise the intracellular glycerol concentration following exposure to hyperosmotic stress [29]. This response represents, at least partly, a HOG pathway-dependent reaction [10], critical for cellular adaptation to high osmolarity conditions. Several observations suggest that an increased copy number of *SGD1* suppresses the osmosensitivity of the HOG pathway mutants by affecting the expression of the *GPD1* gene. High dosage of *SGD1* partially restored the defective transcriptional response of *GPD1* in HOG pathway mutants subjected to osmotic stress (Fig. 2). In agreement with this finding multicopy expression of *SGD1* also promoted increased activity of glycerol-3-phosphate dehydrogenase and increased glycerol production (Table 1) in mutants deleted for *PBS2* or *HOG1*. Somewhat surprizingly, the restoration of *GPD1* expression, GPD activity and glycerol production were stronger in mutants lacking the MAP kinase activator Pbs2p, than in cells lacking the downstream MAP kinase, Hog1p. However, the fact that suppression of a *hog1*Δ single mutant was similar to that observed for a *hog1*Δ*pbs2*Δ double mutant, suggests that the presence of Hog1p might be required for an effective suppression of the osmosensitivity of a signaling defective mutant. These arguments place the function of *SGD1* downstream of the HOG MAPK cascade, consistent with the nuclear residence of the protein, as demonstrated by the localization pattern of a functional Sgd1p–GFP fusion (Fig. 4). The mechanism whereby Sgd1p may affect *GPD1* transcription is open to speculation. Since there is no direct experimental evidence that Sgd1p is a substrate for the HOG pathway we cannot disregard the possibility that Sgd1p might be operating in a different regulatory context. This supposition finds some support in the fact that Sgd1p exhibits sequence homology in its N-terminal to Spt7p, a subunit of the nucleosomal SAGA histone acetylation complex [30]. It was recently demonstrated that osmotic induction of *GPD1* requires binding of Rap1p to its promoter and specific inactivation of all Rap1p binding sites completely abolished the osmotic induction of *GPD1* [15]. Rap1p is a DNA binding repressor activator protein with an array of functions that all appear to be linked to a chromatin opening property of the protein [31]. Hence, changes of the chromatin environment might be a prerequisite for the osmostress-induced activation of *GPD1* expression. Increased dosage of *SGD1* might interfere in this context, mediating activated transcription of the gene. Participation in such a general function would be in agreement with the vital role of Sgd1p for yeast growth.

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