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Genetics

Efficient export of the glucose transporter Hxt1p from the endoplasmic reticulum requires Gsf2p

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ABSTRACT **Mutations in the *GSF2* gene cause glucose starvation phenotypes in *Saccharomyces cerevisiae*. We have isolated the *HXT1* gene, which encodes a low-affinity, high-capacity glucose transporter, as a multicopy suppressor of a *gsf2* mutation. We show that *gsf2* mutants accumulate Hxt1p in the endoplasmic reticulum (ER) and that Gsf2p is a 46-kDa integral membrane protein localized to the ER. *gsf2* mutants also display a galactose growth defect and abnormal localization of the galactose transporter Gal2p but are not defective in function or localization of the high-affinity glucose transporter Hxt2p. These findings suggest that Gsf2p functions in the ER to promote the secretion of certain hexose transporters.**

The *HXT* genes of the yeast *Saccharomyces cerevisiae* encode a family of hexose transporters with diverse kinetic properties and patterns of expression (1–4). Transcription of the *HXT* genes is regulated by the concentration of glucose in the environment, and this transcriptional regulation is a principal mechanism whereby cells adapt to fluctuations in glucose availability. For example, when glucose is abundant, genes encoding low-affinity glucose transporters are induced while genes encoding high-affinity glucose transporters are repressed. When glucose is scarce, this pattern of *HXT* gene transcription is reversed such that high-affinity glucose transporters are expressed (5). The *HXT1* gene encodes a major low-affinity, high-capacity glucose transporter (K_m = 100 mM) that is strongly induced in response to high levels of glucose (5–8). In this study, we have isolated *HXT1* as a multicopy suppressor of mutations in *GSF2* (*g*lucose *s*ignaling *f*actor).

The *GSF2* gene was previously identified in a screen for mutants defective in signaling the presence of high glucose levels (9). *GSF2* encodes a protein with a putative transmembrane domain and a C-terminal dilysine motif for retrieval of transmembrane proteins to the ER (see Fig. 2*A*; see also refs.9–11). In addition to relieving glucose repression of *SUC2* and *GAL10* transcription, *gsf2* mutations cause a synthetic lethal phenotype in combination with *snf1*Δ, suggesting a functional relationship between *GSF2* and *SNF1*. *SNF1* encodes a protein–serine/threonine kinase that is required to relieve transcriptional repression of many genes in response to glucose depletion (12).

Here, we have isolated multicopy suppressors of the synthetic lethal phenotype of *gsf2* and *snf1* mutations. The recovery of *HXT1* as a multicopy suppressor suggested a role for Gsf2p in glucose transporter function. We show that *GSF2* encodes a 46-kDa integral membrane protein localized to the ER and that mutations in *GSF2* lead to an accumulation of Hxt1p in the ER. These findings explain the glucose starvation and synthetic lethal phenotypes of *gsf2* mutants. *gsf2* mutations also affect the secretion of the galactose transporter Gal2p, but not the high-affinity glucose transporter Hxt2p. We suggest that Gsf2p functions in the ER to promote the secretion of certain hexose transporters.

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MATERIALS AND METHODS

Strains, Media, and Genetic Methods. *S. cerevisiae* strains were PS350 (*MATα his3-Δ200 leu2-Δ1 trp1-Δ63::TRP1 ura3–52*), PS352 (*MATα his3-Δ200 leu2-Δ1 trp1-Δ63 ura3–52 gsf2-Δ2::TRP1*; isogenic to PS350), PS4343–11B [*MATα his3-Δ200 leu2-Δ1 trp1-Δ1 ura3–52 gsf2–1 snf1-Δ10* (pSNF1-URA3, pSUC2::HIS3)], PS4342 [*MATα his3-Δ200 leu2-Δ1 trp1-Δ1 ura3–52 snf1-Δ10 gsf2-Δ2::TRP1* (pSNF1-URA3); isogenic to PS4343–11B], HY133 (*MAT***a** *his3-Δ200 leu2-Δ1 trp1Δ ura3–52 snf3Δ::HIS3 hxt1Δ::TRP1::hxt4Δ hxt2Δ::LEU2 hxt3Δ::TRP1 hxt6/7Δ gal2*; ref. 13). These strains are derivatives of S288c.

To construct strain PS1332, strain HY133 was transformed first with pMAL63-Ycp50 (14) and then with a DNA fragment containing *gsf2*Δ::*kanMX*, which was obtained by PCR using genomic DNA of strain 10523 (Research Genetics, Huntsville, AL) as template. G418-resistant colonies were selected on YP + 2% maltose containing 0.3 mg/ml geneticin (GIBCO/BRL), and then a *gsf2*Δ::*kanMX* disruptant lacking pMAL63-Ycp50 was isolated. Standard methods for yeast genetic analysis, transformation, and media preparation were used (15, 16). Media contained 2% glucose unless otherwise specified.

Isolation of Multicopy Suppressors. Strain PS4343–11B, containing pSNF1-URA3 (pCE101; ref. 17) and pSUC2::HIS3 (pYSH; ref. 18), was transformed with a library of yeast genomic DNA constructed in the multicopy (2 μ) *LEU2* vector YEp13 (gift of M. A. Osley, Sloan–Kettering Institute, New York). Leu^+ colonies (43,000) were replica plated to medium containing 5-fluoroorotic acid (5-FOA) to select for those that retained viability after the loss of pSNF1-URA3. Thirty-four 5-FOA-resistant candidates were identified and were tested for growth on raffinose and for histidine prototrophy; 6 were Raf^+ His^+, and 28 were Raf^- His^-. Southern blots indicated that all 6 Raf^+ His^+ candidates carried *SNF1* plasmids, and 27 of the 28 Raf^- His^- candidates carried *GSF2* plasmids. The single remaining multicopy suppressor plasmid was designated pHCS1.

Plasmids. pADH1::HXT1 contains a PCR-generated *Bam*HI/*Xho*I fragment of *HXT1* (from start to stop codon) in the 2 μ vector pSK134 (gift of S. Kuchin, Columbia University). pHXT1 contains a 3.5-kb *Sac*I–*Nhe*I (vector site) fragment of pHCS1 in *Sac*I–*Xba*I-digested pRS315 (19). pHXT1–GFP contains a green fluorescent protein (GFP)-encoding, PCR-generated *Not*I fragment from pSF-GP1 (20) in pHXT1-N, a derivative of pHXT1 with a *Not*I site 5′ to the termination codon. p(U)HXT1 and p(U)HXT1–GFP contain a *Sac*I-*Bam*HI fragment of pHXT1 and pHXT1-GFP, respectively, in pRS316 (19). pHA-GSF2 contains a *Not*I fragment of

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Abbreviations: ER, endoplasmic reticulum; 5-FOA, 5-fluoroorotic acid; GFP, green fluorescent protein; HA, hemagglutinin; SC, synthetic complete.
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pGTEP (21), encoding a triple-hemagglutinin (HA) epitope, in pN-RM1 (9). pGFP-GSF2 contains the *Not*I GFP PCR product in pN–RM1. pGSF2 pRM1 (9) contains *GSF2* in pRS315. pAK145 and pAK146 contain *HXT2* and *HXT2-GFP*, respectively, in vector YCplac33, and pAK104 and pAK166 contain *GAL2* and *GAL2-GFP*, respectively, in pRS416 (generous gift of A. Kruckeberg, University of Amsterdam, The Netherlands).

Subcellular Fractionation. Cells were grown to midlogarithmic phase in synthetic complete (SC) − Leu medium containing 4% glucose, and subcellular fractionation was performed as described (11) except that lysis buffer contained 0.3 M mannitol, 0.1 M KCl, 50 mM Tris·HCl (pH 7.5), 1 mM EGTA (22), and Complete protease inhibitors (Boehringer Mannheim). Briefly, cleared spheroplast lysates were centrifuged at 13,000 × *g* to generate pellet (P13) and supernatant (S13) fractions. The P13 fraction was resuspended in lysis buffer, and aliquots were washed in buffer with no additions or buffer adjusted to 1 M NaCl or 1% Triton X-100. The washed fractions were centrifuged at 13,000 × *g* to generate new pellet and supernatant fractions. Proteins were precipitated with 10% trichloroacetic acid and resuspended in Laemmli sample buffer.

Immunoblot Analysis. Standard methods for SDS/PAGE and immunoblotting were used. Immunoblot analysis was performed by using anti-HA antibody (12CA5, Boehringer Mannheim) or anti-GFP antibody (7.1:13.1, Boehringer Mannheim) in combination with peroxidase-conjugated anti-mouse Ig secondary antibody (NA 931, Amersham Pharmacia) and a chemiluminescent detection system (ECL, Amersham Pharmacia).

Fluorescence Microscopy. Autofluorescence of GFP fusion proteins was visualized in unfixed cells by using a Nikon Eclipse E800 (Figs. 2, 3, and 5) or a Zeiss Axioplan II microscope (Fig. 4). Images were captured by using a digital camera (Orca-100, Inovision, Raleigh, NC or Sensicam, Cooke, Auburn Hills, MI) and imaging system (IPLab, Scanalytics, Fairfax, VA; Image Capture Software 3.1, Phase One, Northport, NY), and were converted to Adobe PHOTOSHOP files for processing.

Analysis of *gsf2-1* Allele. The *gsf2–1* allele was recovered by gap repair (23) of *Xba*I-digested pGSF2. Both strands of two independently gap-repaired plasmids containing the *gsf2–1* mutation within a 0.4-kb *Xba*I fragment were sequenced, along with the corresponding region of pGSF2.

RESULTS

Increased Dosage of the *HXT1* Glucose Transporter Gene Suppresses the Synthetic Lethal Phenotype of *gsf2 snf1* Mutants. To isolate multicopy suppressors of the synthetic lethal phenotype caused by *gsf2-1* in combination with *snf1*Δ, we sought plasmids that would allow loss of pSNF1–URA3 from a *gsf2-1 snf1*Δ strain. The strain was transformed with a multicopy library of yeast genomic DNA, and transformants that retained viability following selection for loss of pSNF1-URA3 were identified (see *Materials And Methods*). In addition to transformants carrying *SNF1* and *GSF2* plasmids, one transformant carried a putative multicopy suppressor. Sequence analysis of the plasmid revealed two ORFs, YHR095w and *HXT1*. Subcloning indicated that YHR095w was not sufficient for suppression. However, expression of the *HXT1* gene from the *ADH1* promoter on the plasmid pADH1::HXT1 suppressed the synthetic lethal phenotype of *gsf2-1 snf1*Δ and *gsf2-*Δ*2 snf1*Δ strains (Fig. 1*A* and data not shown). The *gsf2-1* mutation alters the Gln-260 codon to a stop codon (Fig. 2*A*), and *gsf2-*Δ*2* is a deletion of the entire coding region (9).

We next examined whether pADH1::HXT1 suppresses the glucose-repression defect caused by *gsf2.* Glucose repression was monitored by assaying expression of the *SUC2* promoter fused to a *HIS3* reporter carried on pSUC2::HIS3 (18). *his3*Δ strains with pSUC2::HIS3 are His$^-$ on glucose medium (Fig. 1*B*, row 2), whereas the relief of glucose repression in *gsf2 his3*Δ mutants confers a His$^+$ phenotype (9) (Fig. 1*B*, row 1). pADH1::HXT1 suppressed the glucose-repression defect (Fig. 1*B*, row 3); in contrast, a centromeric plasmid expressing *HXT1* from its own promoter did not suppress this defect, indicating that overexpression of *HXT1* is required (Fig. 1*B*, row 4).

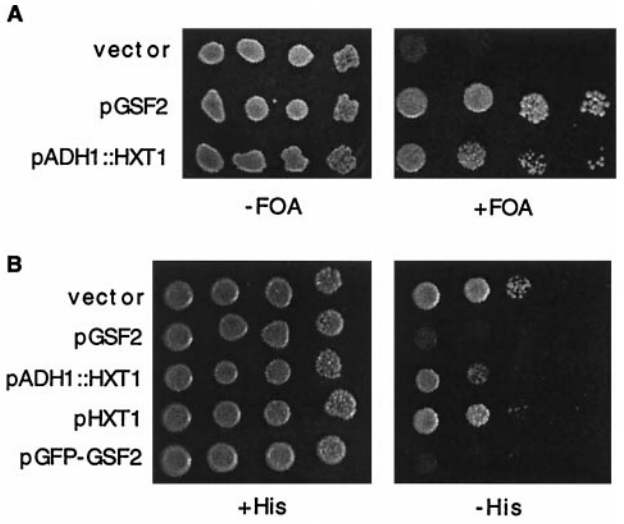

FIG. 1. Hxt1p glucose transporter overexpression suppresses *gsf2*. (*A*) A *gsf2*Δ *snf1*Δ strain (PS4342) containing pSNF1–URA3 was transformed with the vector pSK134, pGSF2, or pADH1::HXT1. Transformants were grown on medium containing uracil to allow loss of pSNF1–URA3, and 10-fold serial dilutions were spotted onto SC-Leu medium containing 5-fluoroorotic acid (5-FOA) (+FOA) or control medium lacking 5-FOA (−FOA). (*B*) A *gsf2−1* strain (PS4343–11B) containing pSNF1–URA3 and pSUC2::HIS3 was transformed with the vector pRS315 or the indicated expression plasmids, and 10-fold serial dilutions were spotted onto SD + 5% glucose medium containing histidine (+His) or 1 mM 3-aminotriazole (−His).

***gsf2* Mutation Does Not Reduce Hxt1p Biosynthesis.** The ability of *HXT1* overexpression to suppress *gsf2* mutant phenotypes suggested that *gsf2* mutants are deficient in some aspect of glucose transporter biosynthesis or function. Hxt1p and Hxt3p are closely related (86% identical) and contribute the bulk of glucose transporter activity in glucose-grown cells (3). A defect in transcription of the *HXT1* and *HXT3* genes in *gsf2* mutants is unlikely, because an *HXT1*::*lacZ* reporter is expressed at similar levels in wild-type and *gsf2* mutant strains (9), and we found that an *HXT3*::*lacZ* reporter [pBM2819 (5)] is also well expressed in both strains [154 ± 5 and 337 ± 26 units of β-galactosidase activity (24), respectively]. Moreover, expression of an Hxt1–GFP fusion from the *HXT1* promoter on a centromeric plasmid was comparable in wild-type and *gsf2*Δ strains (Fig. 3*A*). The electrophoretic mobility of Hxt1–GFP was greater in the *gsf2*Δ mutant than in wild-type, which is likely a consequence of the accumulation of Hxt1–GFP in the ER (see below).

Gsf2p Is an Integral Membrane Protein Localized to the ER. Because Gsf2p contains a putative transmembrane domain (Fig. 2*A*), we determined whether Gsf2p is present in a membrane fraction. A *gsf2*Δ strain was transformed with the centromeric plasmid pHA-GSF2, which bears the native *GSF2* promoter and encodes a functional Gsf2p tagged at the N terminus with a triple HA epitope. Crude spheroplast lysates were prepared and fractionated into 13,000 × *g* pellet (P13) and supernatant (S13) fractions. Immunoblot analysis detected a 46-kDa species corresponding to HA–Gsf2p only in the P13 fraction (data not shown). Washing the P13 fraction with 1 M NaCl was not sufficient to render HA–Gsf2p soluble; however, washing the P13 fraction with 1% Triton X-100

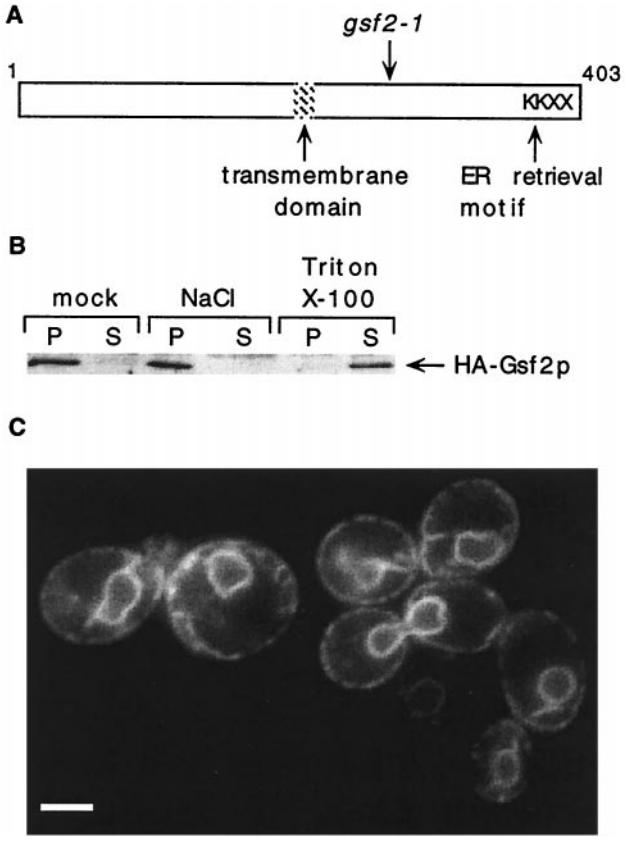

FIG. 2. Localization of Gsf2p to the ER. (*A*) Schematic diagram of Gsf2p indicating the position of a putative transmembrane domain (residues 177–198, LVAQWLFFVMHIFKVGIITLFL), the *gsf2-1* mutation (Q260 CAG codon → TAG stop codon), and a C-terminal dilysine (KKXX) ER-retrieval motif (10, 11). (*B*) Immunoblot analysis of HA–Gsf2p. A spheroplast lysate was prepared from a *gsf2*Δ strain (PS352) carrying pHA–GSF2 and was centrifuged at 13,000 × *g*. The P13 fraction was resuspended in buffer. Aliquots were washed with buffer (mock) or with buffer adjusted to 1 M NaCl or 1% Triton X-100 and centrifuged at 13,000 × *g* to generate pellet (P) and supernatant (S) fractions. Proteins were resolved by SDS/10% PAGE, and HA–Gsf2p was detected by immunoblotting with anti-HA antibody. (*C*) Fluorescence microscopy of GFP–Gsf2p. The *gsf2*Δ strain PS352 was transformed with pGFP–GSF2, and cultures were grown to mid-logarithmic phase in SC-Leu + 4% glucose. A 0.5-ml aliquot was centrifuged briefly at low speed, and 1 μl of a concentrated cell suspension was visualized by using fluorescence microscopy. Exposure time was 2 sec. (Bar = 2.5 μm.)

readily solubilized HA–Gsf2p (Fig. 2*B*). These results indicate that Gsf2p is an integral membrane protein.

Gsf2p contains a C-terminal dilysine motif for retrieval of transmembrane proteins to the ER (Fig. 2*A*; refs. 10 and 11). To determine the subcellular localization of Gsf2p, we constructed the centromeric plasmid pGFP–GSF2, which bears the *GSF2* promoter and encodes an N-terminal fusion of GFP to Gsf2p. This plasmid complements a *gsf2*Δ mutant (Fig. 1*B*, row 5). *gsf2*Δ cells containing pGFP–GSF2 were grown to mid-logarithmic phase in glucose medium and examined by fluorescence microscopy. A perinuclear pattern of fluorescence with discontinuous peripheral cisterna (25) was observed (Fig. 2*C*), consistent with an ER localization of Gsf2p (26–29). Similar fluorescence patterns were observed in cells grown to stationary phase in glucose and in cells grown to mid-logarithmic phase in 5% glycerol/2% ethanol (data not shown).

To determine whether the expression of Gsf2p is modulated by glucose availability, a *gsf2*Δ strain transformed with pHA–GSF2 was grown in medium containing either 5% glucose, 3% galactose/3% glycerol/2% ethanol, or 5% glycerol/2% ethanol. Immunoblot analysis revealed no significant differences in the levels of HA–Gsf2p and no differential posttranslational modification (data not shown).

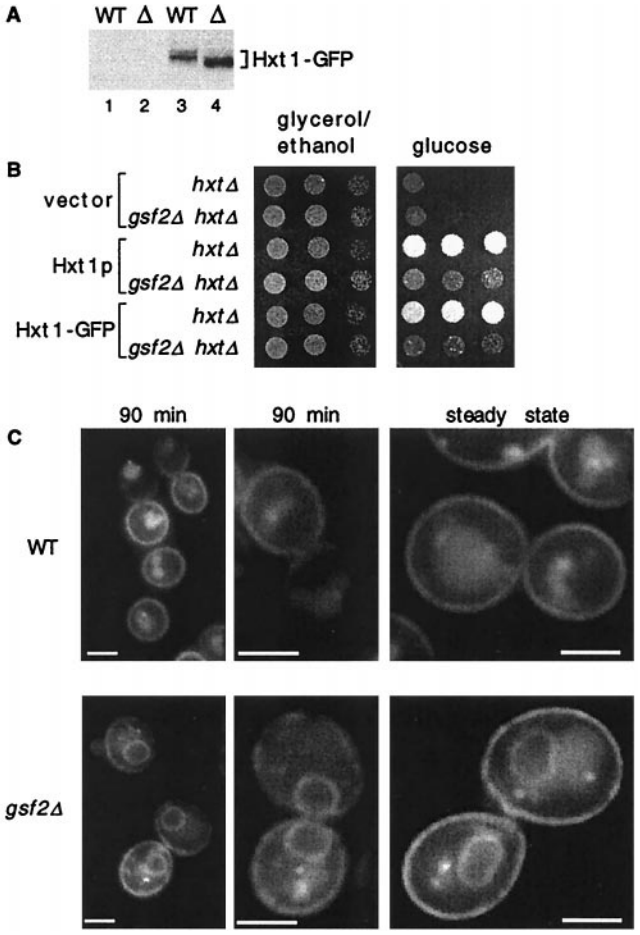

FIG. 3. Function and localization of Hxt1p. (*A*) Isogenic wild-type (WT, PS350) and *gsf2*Δ (Δ, PS352) strains containing p(U)HXT1 (lanes 1 and 2) or p(U)HXT1–GFP (lanes 3 and 4) were grown in SC − Ura + 5%glycerol/2%ethanol, glucose was added to 4% to induce HXT1–GFP expression, and aliquots were harvested 90 min after glucose addition. Whole-cell extracts were prepared by glass bead lysis of cells resuspended in Laemmli sample buffer plus protease inhibitors, proteins were resolved by SDS/10% PAGE, and Hxt1–GFP was detected by using immunoblot analysis with anti-GFP antibody. No prominent proteolytic fragments were detected. (*B*) Isogenic *hxt*Δ and *gsf2*Δ *hxt*Δ strains lacking all major hexose transporter genes (HY133 and PS1332) were transformed with the vector pRS316, p(U)HXT1, or p(U)HXT1–GFP. Transformants were grown in SC − Ura + 5%glycerol/2%ethanol, and 10-fold serial dilutions were spotted onto SC − Ura + 5%glycerol/2%ethanol and SC − Ura + 5%glucose media. The glucose plate was incubated anaerobically to inhibit respiration. Pregrowth of transformants in glucose for several generations did not improve the growth of *gsf2*Δ mutants relative to wild-type on glucose (data not shown). (*C*) Wild-type (PS350) and *gsf2*Δ (PS352) strains carrying pHXT1–GFP were grown in SC-Leu + 5%glycerol/2%ethanol, glucose was added to 4%, and aliquots were harvested 90 min after glucose addition (*Left* and *Center*), or cells were grown for many generations in SC-Leu + 4%glucose (*Right*). Cells were prepared for fluorescence microscopy as described in Fig. 2. Exposure times for all images were 2 sec. (Bar = 2.5 μm.)

***gsf2*Δ Mutations Cause an Accumulation of Hxt1–GFP in the ER and a Glucose Growth Defect.** Because Gsf2p is localized to the ER and *HXT1* overexpression suppresses *gsf2* mutations, we examined the possibility that Gsf2p influences an ER-localized aspect of Hxt1p maturation or secretion. The centromeric plasmid pHXT1–GFP bears the *HXT1* promoter

and encodes an Hxt1–GFP fusion protein which restores glucose transporter function as effectively as native Hxt1p to a strain deleted for all major glucose transporter genes (*hxt*Δ, Fig. 3B). pHXT1–GFP was introduced into wild-type and *gsf2*Δ cells, cultures were grown in glycerol/ethanol medium, and Hxt1–GFP synthesis was induced by the addition of glucose. The localization of Hxt1–GFP was monitored by using fluorescence microscopy (Fig. 3*C*). No fluorescence was detected before glucose addition (data not shown). At 90 min after glucose addition, fluorescence was observed in the periphery of wild-type cells, consistent with the expected plasma membrane localization of Hxt1p (Fig. 3*C*). In *gsf2*Δ cells, patchy fluorescence was observed in the cell periphery, and a striking pattern of fluorescence was observed in the ER of nearly all cells (Fig. 3*C*). In contrast, ER-localized fluorescence was never observed in wild-type cells. Similar fluorescence patterns were observed at 30, 60, and 120 min after glucose addition; however, at 30 min, overall fluorescence intensity was lower and the patterns were less distinct (data not shown). *gsf2*Δ cells grown for several generations in glucose medium also displayed ER-localized and peripheral fluorescence, whereas wild-type cells did not show any ER-localized fluorescence (Fig. 3*C*).

Consistent with the observation that *gsf2*Δ does not completely block secretion of Hxt1–GFP, disruption of *GSF2* in the *hxt*Δ strain decreased, but did not abolish, the ability of Hxt1p and Hxt1–GFP to support growth on glucose (Fig. 3*B*). In addition, these growth phenotypes indicate that at least a portion of the plasma membrane-localized Hxt1p and Hxt1–GFP in *gsf2*Δ mutants is functional.

***gsf2* Mutations Affect the Localization of the Galactose Transporter Gal2p and Cause a Galactose Growth Defect.** The *S. cerevisiae* galactose transporter, Gal2p, is closely related to Hxt1p [69% identity, 83% similarity; (6)] and is capable of transporting glucose in addition to galactose (13). We therefore examined whether mutations in *GSF2* affect galactose utilization. A *gsf2*Δ strain bearing pGSF2 or the parent vector pRS315 was tested for growth on galactose at 24°, 30°, and 37°. A growth defect of *gsf2*Δ cells was observed at all temperatures and was most pronounced at 24° (Fig. 4*A*). A multicopy plasmid containing *GAL2* partially suppressed this growth defect (data not shown).

We next examined the subcellular localization of a Gal2–GFP fusion protein in wild-type and *gsf2*Δ strains. The centromeric plasmid pAK166 bears the *GAL2* promoter and encodes Gal2–GFP, which restores galactose transporter function as effectively as native Gal2p to a *gal2* strain (Fig. 4*B*). Immunoblot analysis showed that full-length Gal2–GFP is expressed in the *gsf2*Δ mutant, although levels were lower than in the wild-type 90 min after galactose induction (Fig. 4*C*). Wild-type and *gsf2*Δ strains bearing pAK166 were grown in glycerol/ethanol medium, Gal2–GFP synthesis was induced by the addition of galactose, and the localization of Gal2–GFP was monitored by using fluorescence microscopy. At 90 min after galactose addition, fluorescence was observed in the periphery of wild-type cells, consistent with the expected plasma membrane localization of Gal2p (Fig. 4*D*). In the *gsf2*Δ mutant, however, a punctate cytoplasmic pattern of fluorescence was observed (Fig. 4*D*). After growth for many generations in galactose, both wild-type and mutant cells displayed peripheral fluorescence and occasional cytoplasmic concentrations of fluorescence; however, an abnormal pattern remained evident in the *gsf2*Δ mutant (Fig. 4*D*). Consistent with these findings, the *gsf2*Δ mutation decreased, but did not abolish, the ability of both Gal2p and Gal2–GFP to support growth on galactose (Fig. 4*B*).

Specificity of Gsf2p Function. To determine whether *gsf2*Δ affects other hexose transporters, we next examined Hxt2p function and localization in a *gsf2*Δ mutant. Hxt2p is a high-affinity glucose transporter that belongs to the subfamily of

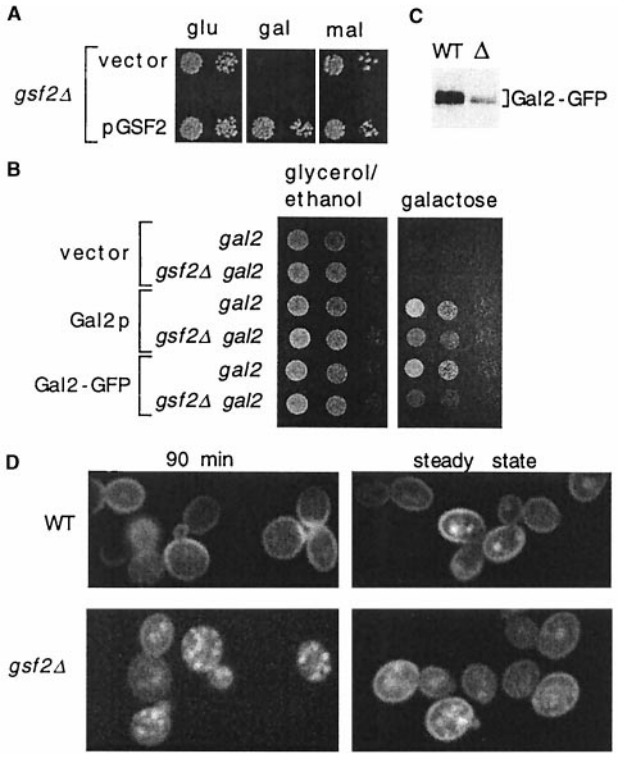

FIG. 4. Function and localization of Gal2p. (*A*) The *gsf2*Δ strain PS352 was transformed with pMAL63-YCp50 (14) to provide Mal activator function and pGSF2 or the vector pRS315. Serial dilutions (10-fold) were spotted onto SC-Leu-Ura medium containing 1 μg/ml antimycin A to inhibit respiration and 2% glucose, 2% galactose, or 2% maltose as carbon source. Plates were incubated at 24°. (*B*) Isogenic *gal2 hxt*Δ and *gsf2*Δ *gal2 hxt*Δ strains (HY133 and PS1332) were transformed with the vector pRS316 or plasmids expressing Gal2p (pAK104) or Gal2–GFP (pAK166). Transformants were grown in SC − Ura + 5%glycerol/2%ethanol, and 10-fold serial dilutions were spotted onto SC − Ura + 5%glycerol/2%ethanol and SC − Ura + 2%galactose media. The galactose plate was incubated anaerobically. Pregrowth of transformants in galactose for several generations did not improve the growth of *gsf2*Δ mutants relative to wild-type on galactose (data not shown). (*C*) Wild-type and *gsf2*Δ strains (PS350, PS352) containing pAK166 were grown in SC − Ura + 5%glycerol/2%ethanol, galactose was added to 4% to induce Gal2–GFP expression, and aliquots were harvested 90 min later. Immunoblot analysis was carried out as described in Fig. 3. No prominent proteolytic fragments were detected. (*D*) Cultures were prepared as in *C* (*Left*) or were grown for many generations in SC − Ura + 4%galactose (*Right*), and cells were prepared for fluorescence microscopy as described in Fig. 2. Exposure times were 0.6 sec.

hexose transporters that includes Hxt1p and Gal2p (4). Hxt2p is 62% identical, 78% similar to Hxt1p (1). The *gsf2*Δ mutation did not noticeably impair the ability of an *hxt*Δ strain expressing Hxt2p or Hxt2-GFP to grow on glucose (Fig. 5*A*). Moreover, no striking difference in the localization of Hxt2–GFP in wild-type and *gsf2*Δ strains was observed (Fig. 5*B*).

We also examined the ability of *gsf2*Δ mutants to utilize the disaccharide maltose. The strains used in this study express two maltose transporters, Mal31p and Agt1p (30), which are more distantly related to Hxt1p [each ≈23% identical, 43% similar to Hxt1p; (1, 30)]. No growth defect of *gsf2*Δ cells on maltose was observed at 24°C or 30°C (Fig. 4*A*; data not shown), and both wild-type and *gsf2*Δ cells grew poorly on maltose at 37°C (data not shown). Deletion of *GSF2* in a strain expressing the maltose transporter Mal61p also did not impair growth on maltose (data not shown).

We next examined whether Gsf2p has a role in the trafficking of amino acid permeases similar to that of Shr3p. Shr3p is an ER-resident integral membrane protein that is required for

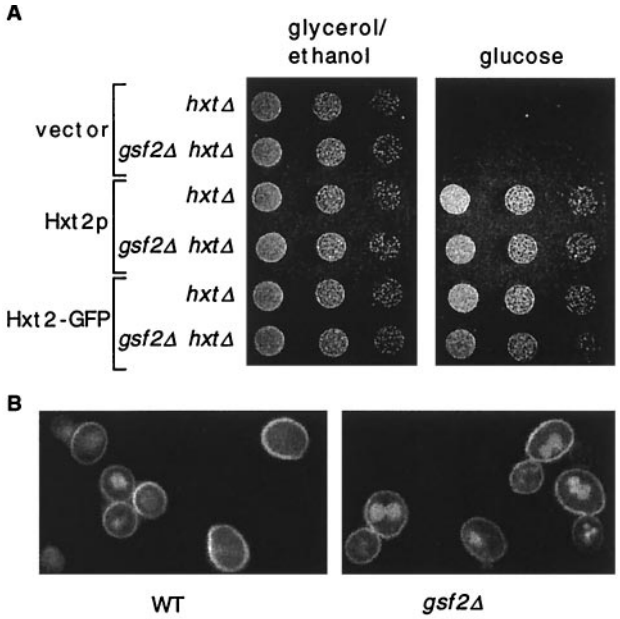

FIG. 5. Function and localization of Hxt2p. (*A*) Strains HY133 (*hxt*Δ) and PS1332 (*gsf2*Δ *hxt*Δ) containing the vector pRS316 or plasmids expressing Hxt2p (pAK145) or Hxt2–GFP (pAK146) were grown in SC − Ura + 5%glycerol/2%ethanol, and 10-fold serial dilutions were spotted onto SC − Ura + 5%glycerol/2%ethanol and SC − Ura + 2%glucose media. The glucose plate was incubated anaerobically. (*B*) Wild-type (PS350) and *gsf2*Δ (PS352) strains containing pAK146 were grown in SC − Ura + 4%glucose and shifted to SC − Ura + 0.1%glucose to induce Hxt2–GFP expression. Cells were harvested 90 min after the shift to low glucose and were prepared for fluorescence microscopy as described in Fig. 2. Exposure times were 4 sec.

the export of amino acid permeases from the ER (28, 31, 32). Because *shr3*Δ mutants are defective in amino acid transport, *shr3*Δ is synthetic lethal in combination with amino acid auxotrophic mutations, including *leu2*, *his3*, and *trp1* (28). In contrast, *gsf2*Δ *his3*, *gsf2*Δ *leu2 his3*, and *gsf2*Δ *leu2 his3 trp1* mutants are viable (this study; ref. 9). *gsf2*Δ mutants therefore do not have an amino acid transport defect similar to that conferred by *shr3*Δ.

DISCUSSION

The *GSF2* gene was previously identified in a screen for mutants defective in signaling the presence of high glucose levels (9). In this study, we isolated *HXT1* as a multicopy suppressor of a *gsf2* mutation, indicating a role for Gsf2p in glucose transporter function. We have shown that Gsf2p is an integral membrane protein localized to the ER and that Hxt1–GFP accumulates in the ER of *gsf2*Δ mutants. The localization of the galactose transporter Gal2p is also aberrant in *gsf2*Δ mutants.

Several lines of evidence suggest that the function of Gsf2p is specific for certain transporters. *gsf2*Δ mutants show normal localization of Hxt2p and exhibit growth phenotypes indicating normal maltose transporter and amino acid permease function. The mutants also do not display defects in derepression of secreted invertase activity or other phenotypes associated with global alterations in secretion (9). Thus, Gsf2p appears to play a role in the secretion of a select group of proteins.

Several possible functions for Gsf2p can be envisioned. One plausible model is that Gsf2p assists the maturation of Hxt1p and Gal2p into conformations competent for trafficking through the secretory pathway. This model is compatible with the different effects of *gsf2*Δ on Hxt1p and Gal2p and with the partial localization of Hxt1p and Gal2p to the plasma membrane. Alternatively, Gsf2p could affect the entry of certain cargo into transport vesicles. In this model, Hxt1p molecules accumulate in the ER of *gsf2*Δ mutants because of a blockage or delay in vesicle entry; it is less clear how this model could account for the different localization pattern observed for Gal2p.

The function of Gsf2p may be related to the roles of other ER integral membrane proteins, such as Shr3p, Erv14p, or p24 proteins. Mutants defective in the ER-resident protein Shr3p accumulate amino acid permeases in the ER (28). Coat protein II (COPII)-coated vesicles transport proteins from the ER to the Golgi apparatus (33), and Shr3p is required for the formation of amino acid permease–COPII complexes in the ER prior to vesicle budding (32). However, Shr3p is not included in these complexes and appears to function before the recognition of permeases by COPII components (31, 32). Erv14p is required for the export of the membrane glycoprotein Axl2p from the ER (34). Erv14p appears to cycle between the ER and Golgi, and it has been proposed that interactions of Erv14p with Axl2p mediate the entry of Axl2p into COPII-coated vesicles (34). Finally, p24 proteins, including Emp24p/Bst2p and Erv25p, may regulate vesicle assembly or function as cargo adaptors for selected secretory proteins (35–37).

The observation that Hxt1p accumulates in the ER of *gsf2* mutants can account for the glucose repression defect of these mutants. A decrease in the number of glucose transporters at the plasma membrane could reduce glucose transport to a level insufficient to trigger glucose repression (8). Overexpressing Hxt1p in *gsf2* mutants presumably increases the number of glucose transporters at the plasma membrane and suppresses the glucose-repression defect.

Our findings also explain the synthetic lethal interaction of *gsf2* and *snf1* mutations (9). The defect in glucose transporter function in *gsf2* mutants essentially subjects cells to glucose limitation, as indicated by the relief of glucose repression of *SUC2* and *GAL10* transcription (9). Because the Snf1 protein kinase is required for growth in glucose-limiting conditions (12), *gsf2* and *snf1* exhibit a synthetic-lethal phenotype.

Glucose transporter structure and function are widely conserved among eukaryotes (1, 3, 4). In addition, mammalian sequences with homology to Gsf2p are present in several databases. Given the importance of glucose transport and its regulation in human physiology and pathology, it will be interesting to further explore Gsf2p-related functions in yeast and other organisms.

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