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Mutations in *GSF1* and *GSF2* Alter Glucose Signaling in *Saccharomyces cerevisiae*

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ABSTRACT

One function of the *Saccharomyces cerevisiae* Snf1 protein kinase is to relieve glucose repression of *SUC*, *GAL*, and other genes in response to glucose depletion. To identify genes that regulate Snf1 kinase activity, we have selected mutants that inappropriately express a *SUC2*promoter::*HIS3* gene fusion when grown in glucose and that require Snf1 function for this phenotype. Mutations representing two new complementation groups (*gsf1* and *gsf2*) were isolated. *gsf1* mutations affect two distinct responses to glucose: the Snf1-regulated glucose repression of *SUC2* and *GAL10* transcription and the Snf1-independent induction by glucose of *HXT1* transcription. *gsf2* mutations relieve glucose repression of *SUC2* and *GAL10* transcription and, in combination with *snf1*Δ, cause an extreme slow growth phenotype. The *GSF2* gene was cloned by complementation of the *gsf2-1 snf1*Δ slow growth phenotype and encodes a previously uncharacterized 46 kD protein.

CARBON source utilization is strictly regulated in yeast. For example, certain carbon sources such as galactose and maltose induce the expression of genes required for metabolism of these sugars as much as 1000-fold. Alternatively, glucose strongly represses the expression of genes whose products are dispensable for growth on glucose, a phenomenon referred to as glucose repression. Among the genes regulated by glucose repression (*e.g.*, *GAL*, *MAL*, *GUT*, *ADH2*, and *SUC* genes) are those required for utilization of carbon sources that are metabolized less efficiently than glucose such as galactose, maltose, glycerol, ethanol, and sucrose. Consequently, cells growing in the presence of glucose plus alternate carbon sources are able to utilize the most efficiently metabolized carbon source, glucose, to the exclusion of the others. Genes required for gluconeogenesis and for respiration are also subject to glucose repression (reviewed in JOHNSTON and CARLSON 1992).

Genetic studies have identified several factors that are required to relieve glucose repression of *SUC*, *GAL*, and other genes. Certain *snf* (sucrose nonfermenting) mutations, such as *snf1*(*cat1*, *ccr1*) and *snf4*(*cat3*), define gene products required for growth on sucrose or raffinose, for derepression of *SUC2* transcription, and for other responses directly related to carbon source utilization (JOHNSTON and CARLSON 1992). Other *snf* mutations (*e.g.*, *snf2*, *snf5*, and *snf6*) are pleiotropic, and the corresponding gene products are general transcription factors (WINSTON and CARLSON 1992). A significant advance in our understanding of the mechanism of glucose repression stemmed from the finding that the *SNF1* gene encodes a protein-serine/threonine kinase whose catalytic activity is required to relieve glucose repression of *SUC2* and other glucose-repressed genes (CELENZA and CARLSON 1986; SCHULLER and ENTIAN 1987; CELENZA and CARLSON 1989). Additional genetic and biochemical studies identified the *SNF4* gene product as a positive effector of Snf1 kinase activity (SCHULLER and ENTIAN 1988; CELENZA and CARLSON 1989; CELENZA *et al.* 1989; ESTRUCH *et al.* 1992; JIANG and CARLSON 1996).

Genes required for glucose repression of *SUC2* have been identified in two principal ways: through the isolation of *ssn* (suppressor of *snf*) mutations that allow *SUC2* expression in the absence of a functional Snf1 kinase (CARLSON *et al.* 1984) and through the isolation of mutants defective in glucose repression, often by selecting *SUC2* constitutive mutants able to use raffinose or sucrose in the presence of the repressing, nonmetabolizable glucose analogue 2-deoxyglucose (ZIMMERMANN and SCHEEL 1977; TRUMBLY 1986; NEIGEBORN and CARLSON 1987). These and other studies have identified *reg1*, *glc7*, *hxk2*, *rgr1*, *grr1*, *mig1* (*ssn1*), *ssn6*, and *tup1* mutations that cause defects in glucose repression. Several lines of evidence are consistent with the view that glucose repression of *SUC2* is mediated in part by the following events: generation of a high glucose signal; inhibition of Snf1 kinase activity and stimulation or targeting of Glc7 protein phosphatase activity; and repression of *SUC2* transcription by the DNA-binding proteins Mig1 and Mig2 in conjunction with the general corepressor Ssn6-Tup1 (TU and CARLSON 1995; LUTFIYYA and JOHNSTON 1996; for review see TRUMBLY 1992; JOHNSTON and CARLSON 1992; and RONNE 1995).

The nature of the signal for glucose repression and the means by which the Snf1 kinase and Glc7 phosphatase activities may be regulated in response to this signal

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remain obscure. These elements of the glucose repression pathway are therefore of special interest for further study. To identify genes that regulate Snf1 kinase activity in response to changes in glucose availability, we have selected mutants that express a *SUC2*promoter::*HIS3* gene fusion constitutively under glucose repressing conditions. This selection was followed by a screen to exclude mutations in genes encoding components of the glucose repression pathway acting downstream of *SNF1*, including *TUP1*, several *SSN* genes, and *MIG1*. We deliberately avoided use of the analogue 2-deoxyglucose, which induces an unphysiological mode of glucose repression (discussed in SIERKSTRA *et al.* 1993), in the hope of recovering new classes of glucose repression-defective mutants.

In addition to several dominant glucose repression-defective mutations, recessive alleles representing two new complementation groups [*gsf1* and *gsf2* (glucose signaling factor)] were isolated. *gsf1* mutations affect two distinct responses to glucose: the Snf1-regulated glucose repression of *SUC2* and *GAL10* transcription and the Snf1-independent induction by glucose of *HXT1* transcription. *gsf2* mutations relieve glucose repression, and in combination with *snf1*Δ alleles, cause an extreme slow growth phenotype. The *GSF2* gene was cloned by complementation of the *gsf2-1 snf1*Δ slow growth phenotype, and encodes a previously uncharacterized 46 kD protein. Possible functions of *GSF1* and *GSF2* in glucose signaling and their relationship to *SNF1* are discussed.

MATERIALS AND METHODS

Strains, media, and genetic methods: The genotypes of strains used in this study are listed in Table 1. Standard methods for yeast genetic analysis (ROSE *et al.* 1990) and transformation (ITO *et al.* 1983) were used. Rich (YP) and defined (SD, SC) media were prepared as described (ROSE *et al.* 1990). 3-aminotriazole (3-AT; 0.25–1 mM) was occasionally included in SD-His solid medium to suppress background His^+ growth resulting from basal transcription of the *SUC2::HIS3* fusion in strains containing p*SUC2::HIS3*. 5-FOA medium contained 50 μg/ml uracil and 0.1% 5-fluoroorotic acid (BOEKE *et al.* 1984). Sensitivity to 2-deoxyglucose, nitrogen starvation, and Co^{2+} were tested as described previously (TANAKA *et al.* 1989; CONKLIN *et al.* 1993; TU and CARLSON 1994). Growth in low-glucose medium was examined by serial dilution of YP-2% glucose precultures into YP medium containing 2%, 0.5%, or 0.05% glucose followed by incubation at 30°. The gross morphology of strains grown at 30° to mid-log phase in YPD or SC medium was examined microscopically.

Plasmids: The plasmid p*SUC2::HIS3* (pYSH in TU and CARLSON 1994) contains the *HIS3* structural gene under the control of a region of the *SUC2* promoter (−900 to −384) shown previously to confer glucose-regulated transcription (SAROKIN and CARLSON 1985) and is a pRS314 derivative (*CEN6/ARSH4/TRP1*, SIKORSKI and HIETER 1989). The p*SNF1-URA3* plasmid (pCE101 in CELENZA and CARLSON 1989) contains a 2.8-kilobase (kb) *Xho*I-*Bam*HI fragment bearing the *SNF1* gene and is a YCp50 derivative (*CEN4/ARS1/URA3*, ROSE *et al.* 1987). The *GAL10::lacZ* plasmid pRY123 (WEST *et al.* 1984) contains a ~600-base pair (bp) region of the *GAL1-GAL10* promoter fused to the *Escherichia coli lacZ* gene and is a pLG670Z derivative (2μ/*URA3*, GUARENTE 1983). The *HXT1::lacZ* plasmid pBM2636 (ÖZCAN and JOHNSTON 1995) contains a 1.3-kb *Hin*dIII-*Eco*RI fragment of the *HXT1* promoter fused to the *E. coli lacZ* gene and is a YEp357R derivative (2μ/*URA3*, MYERS *et al.* 1986). The *HXT4::lacZ* plasmid pBM2800 (ÖZCAN and JOHNSTON 1995) contains the +1 to −946 region of the *HXT4* promoter fused to the *E. coli lacZ* gene and is a YEp357R derivative (2μ/*URA3*). The plasmids pAJ1 (*CYC1UAS-lacZ*) and pAJ3 (*CYC1UAS* + α2-Mcm1 operator-*lacZ*) are described by KELEHER *et al.* (1992). pRM1 (see Figure 3) contains an ~6.6-kb *Sac*I-*Bam*HI region of chromosome XIII and is a pRS315 derivative (*CEN6/ARSH4/LEU2*, SIKORSKI and HIETER 1989). pDOM1 was constructed by digesting pRM1 with *Xba*I and religating, resulting in deletion of a 0.5-kb region of the *GSF2* gene (encoding residues 148–304) and a frameshift that generates translation stop codons 3 and 33 nucleotides downstream of the *Xba*I junction. pDOR1 was constructed by digesting pRM1 with *Avr*II and religation. pOM1 contains a 1.7-kb *Hin*dIII fragment of pRM1 (containing the *GSF2* open reading frame, 155 nucleotides of 5′ noncoding sequence, and 368 nucleotides of 3′ noncoding sequence) cloned in the *Hin*dIII site of pRS315. The plasmid pΔ1-RM1 containing the *gsf2-Δ1::TRP1* allele was constructed by replacing a 0.6-kb *Bst*Z17I-*Xba*I fragment of pRM1 with a 2.4-kb *Sca*I-*Spe*I fragment of pRS304 (SIKORSKI and HIETER 1989) containing the *TRP1* marker. pN-RM1 was constructed by creating a unique *Not*I site in pRM1 immediately 3′ to the first ATG codon of *GSF2* in two steps. First, PCR products were generated that contained *Not*I sites and sequence 5′ (from the *Nhe*I site at −201 to +3) or 3′ (from +4 to the *Bsp*EI site at +314) of the first *GSF2* ATG codon. Next, these fragments were digested with *Nhe*I or *Bsp*EI, respectively, and *Not*I and ligated into *Nhe*I/*Bsp*EI-digested pRM1, resulting in the insertion of a *Not*I site in a reading frame suitable for the fusion of various moieties to the N terminus of Gsf2. The plasmid pΔ2-RM1 containing the *gsf2-Δ2::TRP1* allele was constructed by replacing a 1.3-kb *Not*I-*Spe*I fragment of pN-RM1 (containing the entire *GSF2* open reading frame) with a 1.0-kb *Not*I-*Spe*I PCR product containing the *TRP1* marker of pRS304.

Isolation of mutants: Strain PS1192 (*snf1-Δ10 ade2-101 his3-Δ200 trp1-Δ1 ura3-52*) bearing plasmids p*SUC2::HIS3* and p*SNF1-URA3* was plated on selective (SD + Ade) medium containing 5% glucose. Two hundred sixty-three spontaneously arising His^+ mutants from three independent cultures were colony purified. Isolates were patched on SD + Ade + His and replica plated to SD + Ade + 5-FOA medium containing 5% glucose to identify those mutants ($n = 120$) whose His^+ phenotype was suppressed by loss of p*SNF1-URA3*.

Complementation analysis: The 120 His^+ mutants bearing plasmids p*SUC2::HIS3* and p*SNF1-URA3* were crossed to the *hxk2*Δ, *grr1*Δ, *reg1*Δ, *glc7-T152K*, *mig1*Δ, *rgr1*Δ and wild-type (FY250) strains listed in Table 1. Diploids were isolated by prototrophic selection on SD + His medium, and complementation was tested by replica plating to SD medium containing 5% glucose and a range of 3-AT concentrations (0, 0.25, 0.5, or 1 mM). Lack of complementation was manifested as a His^+ phenotype.

A dominant *SUC2* constitutive phenotype was evident in 13 mutants (as judged by invertase assays of glucose-grown heterozygous diploids). 5-FOA-resistant derivatives of these diploids were isolated. Invertase assays indicated that 11 strains retained the *SUC2* constitutive phenotype, while two strains reverted to wild type upon loss of p*SNF1-URA3*. However, constitutive invertase activity was low in these two strains, and we were unable to confirm that these two dominant GSF^- mutations were alleles of the plasmid-borne *SNF1* gene.

TABLE 1

***Saccharomyces cerevisiae* strains**

Strain[a]	Genotype	Source
MCY2119	*MAT***a** *snf1-Δ10 ade2-101 his3-Δ200 trp1-Δ1 ura3-52*	This laboratory
MCY2617	*MATα glc7-T152K his3-Δ200 trp1-Δ1 ura3-52 lys2-801*	This laboratory
MCY3278	*MATα reg1Δ::URA3 his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52*	This laboratory
FY250	*MATα his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52*	F. Winston
FY251	*MAT***a** *his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52*	F. Winston
A476	*MATα rgr1-Δ2::URA3 aro7 can1 gal2 trp1 ura3*	A. Sakai
PS38	*MAT***a** *gsf1-1 snf1-Δ10 ade2-101 his3-Δ200 trp1-Δ1 ura3-52 (pSNF1-URA3,* p*SUC2::HIS3)*	This study
PS43	*MAT***a** *gsf2-1 snf1-Δ10 ade2-101 his3-Δ200 trp1-Δ1 ura3-52 (pSNF1-URA3,* p*SUC2::HIS3)*	This study
PS1192	*MAT***a** *snf1-Δ10 ade2-101 his3-Δ200 trp1-Δ1 ura3-52 (pSNF1-URA3,* p*SUC2::HIS3)*	This study
PS3300-1C	*MATα gsf2-Δ2::TRP1 his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52*	This study
PS3851-3A	*MAT***a** *gsf1-1 his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52*	This study
PS3851-3B	*MATα gsf1-1 his3-Δ200 trp1-Δ63 ura3-52*	This study
PS3851-3D	*MAT***a** *his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52*	This study
PS3851-4B	*MAT***a** *gsf1-1 his3-Δ200 trp1Δ ura3-52*	This study
PS4300-21B	*MATα gsf2-1 his3-Δ200 leu2-Δ1 trp1-Δ1 ura3-52*	This study
PS4343-11B	*MATα gsf2-1 snf1-Δ10 his3-Δ200 leu2-Δ1 trp1-Δ1 ura3-52 (*p*SNF1-URA3,* p*SUC2::HIS3)*	This study
PS4350-3A	*MAT***a** *gsf2-1 his3-Δ200 trp1-Δ63 ura3-52*	This study
PS5204-1A	*MATα his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52*	This study
PS5204-1C	*MATα gsf2-Δ1::TRP1 his-Δ200 leu2-Δ1 trp1-Δ63 ura3-52*	This study
PS5959-6B	*MATα mig1Δ::URA3 his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52 lys2-801*	This study
PS7050-1D	*MATα grr1Δ::URA3 his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52*	This study
PS7851-2A	*MATα hxk2Δ::URA3 his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52*	This study

[a] All MCY, FY, and PS strains are isogenic or congenic to S288c and are *SUC2 GAL2*.

Analysis of *gsf2 snf1Δ* slow growth phenotype: In initial attempts to obtain *gsf2 snf1Δ* double mutants, *gsf2 snf1Δ* strains bearing p*SNF1-URA3* (original isolates from PS1192) were replica plated to SD + Ade + His + 5-FOA, but no 5-FOA-resistant colonies arose. The same result was obtained when heavy inocula of cells were spread on SD + Ade + His + 5-FOA plates. In each case, *snf1Δ* and *gsf1 snf1Δ* strains bearing p*SNF1-URA3* readily yielded 5-FOA-resistant growth. These findings were extended by diluting cultures of the 10 original *gsf2* mutants isolated from PS1192 and spotting cell suspensions on SD + Ade + His control plates and SD + Ade + His + 5-FOA plates. Parallel experiments with PS1192 (*snf1Δ10*) and PS38 (*gsf1-1 snf1Δ10*), also bearing p*SNF1-URA3*, were carried out. The frequency of 5-FOA resistance in *snf1Δ* ($\sim 1 \times 10^{-4}$, large colonies), *gsf1-1 snf1Δ* ($\sim 1 \times 10^{-5}$, large colonies), and *gsf2-1 snf1Δ* (1×10^{-6}, nonpropagable microcolonies) strains and the growth characteristics of the resultant colonies were estimated after 4 days of incubation at 30°.

To determine whether different carbon sources could suppress *gsf2-1 snf1Δ10* slow growth, strain PS4343-11B was patched and replica plated to SC-Trp + 5-FOA medium containing 2% glucose, 8% glucose, 2% galactose, 2% raffinose, 2% glucose + 3% glycerol, 2% glucose + 3% ethanol, or 2% glucose + 2% acetate. To determine the effects of low or high temperature, cells were replica plated to SC-Trp + 5-FOA medium containing 2% glucose and growth was monitored for several days after incubation at 18°, 23°, or 37°. Suppression of the slow growth phenotype by excess amino acids or yeast nitrogen base (YNB) was tested by spotting concentrated (10×) amino acid or YNB solutions onto lawns of strain PS4343-11B plated on SC-Trp + 5-FOA medium. In each test described above, controls with an *GSF2 snf1Δ* strain and with media lacking 5-FOA were carried out.

To obtain *gsf2-1 snf1Δ* double mutant spore clones, strain PS4300-21B was crossed to PS43 carrying p*SNF1-URA3* and the plasmid was cured from the diploid. Four colony-purified diploids were presporulated at 30° in liquid YP acetate medium, shifted to liquid sporulation medium, and sporulated at 24° or 30° for 6 days. Tetrads from a culture sporulated at 24° (cultures sporulated less well at 30°) were dissected on YP-8% glucose plates containing auxotrophic and aspartic acid supplements or on YP-2% glucose plates. These plates were incubated at 24° or 30° for 7 days. Spore germination and subsequent growth were monitored microscopically.

Cloning of the *GSF2* gene: The *GSF2* gene was cloned by transforming PS4343-11B with a *LEU2-CEN* yeast genomic DNA library (gift of P. Hieter) and replica plating Leu$^+$ transformants to SC-Leu-Trp + 5-FOA medium. 5-FOA-resistant candidates were rescreened to distinguish putative *GSF2* clones from *SNF1* clones by replica plating to SC-Leu-Trp-2% raffinose medium. PCR and *SNF1*-specific primers were used to confirm that Raf$^+$ candidates contained *SNF1* plasmids. In addition, the 5-FOA-resistant candidates were retransformed with pRS316 (*CEN6/ARSH4/URA3*, Sikorski and Hieter 1989) or p*SNF1::URA3*. Invertase activity in glucose-grown Ura$^+$ transformants was determined, and these transformants were also spotted on SD-5% glucose plates and control SD + His-5% glucose plates to determine their His phenotype.

Two independently derived putative *GSF2* clones were isolated in *E. coli* and retested for their ability to complement *gsf2-1* by retransformation of PS4343-11B and replica plating Leu$^+$ transformants to SC-Leu-Trp + 5-FOA and SD-5% glucose medium. A ~6.6-kb *Sac*I-*Bam*HI fragment from the overlapping region of these clones was subcloned into pRS315 to yield the plasmid pRM1 (see above).

Construction of *gsf2-Δ1::TRP1* and *gsf2-Δ2::TRP1* alleles: Strains containing a chromosomal *gsf2-Δ1::TRP1* allele were generated by transforming the wild-type diploid FY250 × FY251 with the 3.3-kb *Pst*I-*Spe*I fragment from pΔ1-RM1 and selecting Trp$^+$ transformants. These diploids were sporulated, Trp$^+$ mutants and Trp$^-$ haploid segregants were identified, and the Gsf phenotypes of segregants from four complete tetrads were determined by invertase assays of glucose-grown

cells. Strains containing a chromosomal *gsf2-Δ2::TRP1* allele were generated by transforming the same wild-type diploid with the 2.3-kb *Pst*I-*Avr*II fragment of pΔ2-RM1 and selecting Trp^+ transformants. Following sporulation of these diploids, Trp^+ and Trp^- segregants were identified, and invertase assays of glucose-grown segregants of four complete tetrads were carried out. The structure and heterozygosity of the *gsf2-Δ1::TRP1* and the *gsf2-Δ2::TRP1* alleles in diploid transformants was confirmed by PCR.

To confirm that the bona fide *GSF2* gene had been cloned, PS5204-1B (*gsf2-Δ1::TRP1*) was crossed to PS4300-21B (*gsf2-1*). Invertase activity of this diploid was determined in glucose-grown cells. The diploid was sporulated, and glucose-grown segregants from four complete tetrads were shown by invertase assays to exhibit the Gsf^- phenotype.

Enzyme assays: Secreted invertase activity was assayed in whole cells as described previously (GOLDSTEIN and LAMPEN 1975; modified as in CELENZA and CARLSON 1984) except that 0.5 OD_{600} units rather than 50 Klett units of cells were harvested. β-galactosidase activity was assayed in permeabilized cells (GUARENTE 1983). In experiments where invertase activity of *gsf2-1 snf1Δ* double mutants was measured, cells were transferred from slow-growing patches on YPD plates to fresh YPD liquid medium, the OD_{600} of these cell suspensions was determined, and 0.5 OD_{600} units of cells were harvested. Control wild-type (FY250), *gsf2-1* (PS4300-24C), and *snf1Δ* (MCY2119) cells were prepared similarly. To assess Ssn6-Tup1-mediated repression, *MATα* strains FY250, PS3851-3B, and PS4300-24C were transformed with pAJ1 or pAJ3, transformants were grown in SC-Ura-2% glucose to mid-log phase, and β-galactosidase activity was assayed in permeabilized cells.

RESULTS

Isolation of mutants: Our primary objective was to identify gene products that monitor extracellular glucose concentration, generate a glucose repression signal, or transduce a glucose repression signal by inhibiting Snf1 protein kinase activity. Figure 1 depicts the strategy we used to select for mutations in these signaling components upstream of Snf1 in the glucose repression pathway. In brief, we isolated *SUC2* constitutive mutants that depend on *SNF1* function for their constitutive phenotype.

The first step was to select mutations that relieve glucose repression of a *SUC2::HIS3* gene fusion containing the *HIS3* gene expressed from the *SUC2* promoter (see MATERIALS AND METHODS). The wild-type strain bearing this reporter is His^- on glucose medium owing to glucose repression of the *SUC2* promoter. Two hundred thirty-three spontaneous His^+ mutants were selected on synthetic minimal medium containing 5% glucose. These His^+ mutants could be defective in any step of the *SUC2* glucose repression pathway.

The next step was to exclude mutations in genes encoding components of the glucose repression pathway acting downstream of *SNF1*, including several known repressors of *SUC2* transcription such as *TUP1*, several *SSN* genes, and *MIG1*. This was achieved by replica plating the 263 original His^+ mutants to 5-FOA glucose medium lacking histidine and omitting those mutants that remained His^+ following loss of the *SNF1* plasmid.

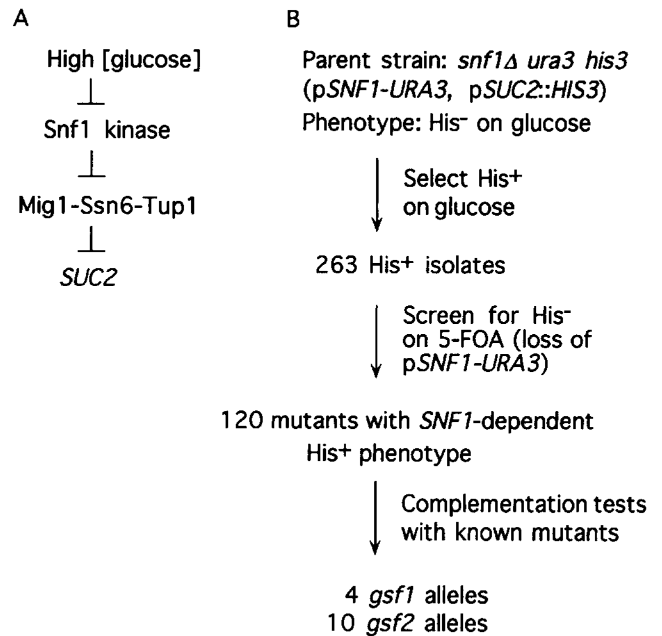

FIGURE 1.—Isolation of *gsf* mutants. (A) A simplified model of the *SUC2* glucose repression pathway. (B) Outline of the strategy for *gsf* mutant isolation. See MATERIALS AND METHODS for details.

Among the original 263 mutants, 113 remained His^+ on 5-FOA glucose medium and were not studied further. We noted, however, that every clumpy mutant isolated ($n = 30$) was in this class. This result is consistent with previous observations from this laboratory that mutations in seven *SSN* genes are characterized by a clumpy phenotype.

The remaining 120 His^+ mutants reverted to a His^- phenotype (or otherwise failed to grow) upon loss of p*SNF1-URA3* on 5-FOA glucose medium lacking histidine. These were candidates for the mutations of interest in putative upstream regulators of Snf1 function.

Complementation analysis: We tested the 120 mutants for dominance and for their ability to complement several previously isolated *SUC2* constitutive mutations. Null alleles of *HXK2, GRR1, REG1,* and *MIG1* were used in the complementation tests. Since *GLC7* and *RGR1* are essential genes, we tested sublethal, glucose repression-defective alleles of these genes (*glc7-T152K* and *rgr1-Δ2*: SAKAI *et al.* 1988; TU and CARLSON 1994). Among the 120 mutants, 18 were dominant (see below), 37 failed to complement *hxk2Δ*, 30 failed to complement *grr1Δ*, eight failed to complement *reg1Δ*, and two failed to complement *glc7-T152K*. No *mig1* or *rgr1* alleles were recovered. Eleven mutants appeared to be very weakly constitutive for expression of the chromosomal *SUC2* locus (as measured by assays of secreted invertase activity) and were not characterized further. The 14 remaining mutants were tested for their ability to complement one another. These mutants defined two complementation groups: *gsf1* (four alleles) and *gsf2* (10 alleles).

To construct strains carrying *gsf1* and *gsf2* mutations in a *SNF1* background, we backcrossed two of the original mutants, PS38 and PS43, to wild type. *gsf1-1 SNF1* and *gsf2-1 SNF1* strains carrying p*SUC2::HIS3* but not p*SNF1-URA3* were identified as Ura^- Raf^+ His^+ segregants of these crosses. The *SUC2* constitutivity indicated by the His^+ phenotype of these segregants on glucose was confirmed by assays of secreted invertase activity (see below). These segregants were then crossed to wild type, and we observed 2:2 segregation of His^+:His^- phenotypes in all tetrads in which all four segregants retained the p*SUC2::HIS3* reporter (*gsf1*, n = 8; *gsf2*, n = 12), indicating that *gsf1-1* and *gsf2-1* segregate as mutations in single nuclear genes.

Dominant *GSF*⁻ alleles: Eighteen dominant mutations were recovered in this screen. Invertase assays of glucose-grown haploid strains indicated that *SUC2* expression was constitutive in 17 of 18 mutants. While the His^+ phenotype was dominant in all 17 strains, invertase assays of glucose-repressed heterozygous diploids indicated that a dominant or semidominant *SUC2* constitutive phenotype was evident in 13 of the *GSF* strains (activity ranging from 10 to 60 units in the mutant haploids and from 4 to 19 units in the heterozygous diploids). The *SNF1* plasmid was present in the parental strain at the outset of the mutant hunt and was therefore a potential target of dominant mutations. However, none of the *GSF* mutations were linked to the *SNF1* plasmid (see MATERIALS AND METHODS).

***SUC2* expression is constitutive in *gsf* mutants:** To determine the effect of the *gsf1* and *gsf2* mutations on the regulation of chromosomal *SUC2* expression, we assayed secreted invertase activity in cultures of *gsf1* and *gsf2* mutants grown in high glucose, shifted from high glucose to low glucose, or grown in raffinose (Table 2). Invertase activity in glucose-grown cells was elevated ~100-fold in the *gsf1-1* mutant and ~60-fold in the *gsf2-1* mutant relative to wild-type levels. Thus, under repressing conditions, *gsf1* and *gsf2* mutants express constitutively both the p*SUC2::HIS3* reporter and the native *SUC2* locus.

To confirm linkage of the His^+ and *SUC2* constitutive phenotypes of *gsf1* and *gsf2* mutants, we assayed invertase activity of segregants from four complete tetrads derived from diploids heterozygous for *gsf1-1* or *gsf2-1*. All His^+ segregants were *SUC2* constitutive, and all His^- segregants showed glucose repression of *SUC2* (data not shown).

***snf1*Δ suppresses the *SUC2* constitutive phenotype of *gsf1* mutants:** A chief criterion of the *gsf* mutant screen was the suppression of *SUC2::HIS3* constitutive expression by *snf1*Δ. To determine whether *snf1*Δ also suppresses the constitutive expression of the chromosomal *SUC2* locus in *gsf* mutants, we first recovered *gsf1-1 snf1*Δ double mutants by the loss of p*SNF1-URA3* from PS38. Glucose-grown *gsf1-1 snf1*Δ mutants expressed ~50-fold lower invertase activity than the *gsf1-1* mutant and was nearly the same activity as a *snf1*Δ mutant (Table 2). Thus, *snf1*Δ suppresses the *SUC2* constitutive phenotype of *gsf1-1* mutants. Moreover, invertase activity in the *gsf1-1 snf1*Δ strain shifted to 0.05% glucose was equivalent to that in the *snf1*Δ strain.

Slow growth phenotype of *gsf2 snf1*Δ double mutants: We attempted to isolate *gsf2-1 snf1*Δ derivatives of PS43 that had lost p*SNF1-URA3*. However, the desired *gsf2-1 snf1*Δ double mutants could not be recovered by selecting for resistance to 5-FOA. In contrast, isogenic *snf1*Δ and *gsf1-1 snf1*Δ strains containing p*SNF1-URA3* readily yielded 5-FOA-resistant colonies (see MATERIALS AND METHODS). These findings indicated that *gsf2-1 snf1*Δ double mutants are inviable or extremely slow growing on SD 5-FOA medium. Similar results were obtained for mutants carrying each of the 10 *gsf2* alleles recovered.

None of several conditions enabled the recovery of 5-FOA-resistant derivatives of *gsf2-1 snf1*Δ strains carrying p*SNF1-URA3*, including growth at a range of temperatures from 16° to 37°, growth on raffinose or galactose, or nutritional supplements in 10-fold excess over standard concentrations (see MATERIALS AND METHODS). Two other previously isolated mutations display a slow growth or synthetic lethal phenotype in combination with *snf1*Δ: *grr1* (FLICK and JOHNSTON 1991; VALLIER *et al.* 1994) and *bcy1* (THOMPSON-JAEGER *et al.* 1991). The 8% glucose was shown to enhance the growth of *bcy1::HIS3 snf1*Δ microcolonies, which nevertheless could not subsequently be propagated (THOMPSON-JAEGER *et al.* 1991). We found that 8% glucose did not

TABLE 2

Secreted invertase activity in *gsf* mutants

Relevant genotype[b]	Secreted invertase[a] 5% glu	0.05% glu	2% raf
Wild type	1.0 ± 0.1	200 ± 20	140 ± 20
gsf1-1	93 ± 5	190 ± 20	120 ± 10
gsf2-1	64 ± 3	188 ± 3	180 ± 30
*snf1*Δ	1.2 ± 0.1	1.4 ± 0.1	NA
*gsf1-1 snf1*Δ	2.7 ± 0.3	1.8 ± 0.5	NA

[a] Micromoles glucose released/minute/100 mg (dry weight) of cells. Cultures were grown to mid-log phase in SC (wild type, *gsf1-1*, and *gsf2-1*) or YP-5% glucose medium (5% glu) (*snf1*Δ and *gsf1-1 snf1*Δ) and shifted to 0.05% glucose medium for 2–3 hr (0.05% glu) or were grown in YP-2% raffinose medium to mid-log phase (2% raf). Secreted invertase was assayed as described in MATERIALS AND METHODS. Values represent the average of three or more assays per strain. NA, not applicable, since *snf1* and *gsf1 snf1* mutants are Raf^-.

[b] The strains assayed in 5% glu and 0.05% glu were FY250, PS3851-3A, and PS4343-11B. The strains assayed in 2% raf were PS5204-1A, PS3851-4B, and PS4300-21B. Invertase activity for glucose-grown mutants of PS1192 bearing each of the four *gsf1* alleles ranged from 93 to 165, and activity for mutants bearing each of nine *gsf2* alleles ranged from 35 to 65 units, while a *gsf2-10* strain expressed 8 units.

enable the recovery of 5-FOA-resistant *gsf2-1 snf1Δ* colonies having lost p*SNF1-URA3*.

A slow growth phenotype of *gsf2-1 snf1Δ* double mutants on YPD medium was confirmed in the following way. *gsf2-1* and *gsf2-1 snf1Δ*(p*SNF1-URA3*) strains were crossed, and diploids having lost p*SNF1-URA3* were colony purified and sporulated (see MATERIALS AND METHODS). Tetrads were dissected on YPD plates containing 2% or 8% glucose and incubated at 24° or 30° for 7 days. After 24 hours, all but two spores from 40 tetrads had germinated. However, 2:2 segregation of slow:fast growth was observed in all tetrads. At 30°, slow-growing segregants gave rise to ~4–16 cells after 24 hr, while fast-growing segregants had formed colonies containing several hundred cells. After 7 days of growth, slow-growing segregants had formed barely visible microcolonies (Figure 2). Similar results were obtained for tetrads incubated at 24°. No differences in growth of tetrads on 2% *vs.* 8% glucose at either temperature were discernible. All fast-growing segregants were Suc^+ and, by inference, carried the *SNF1* allele.

Several *gsf2-1 snf1Δ* double mutant segregants from this cross were patched to YPD medium in attempts to propagate them for detailed analysis. However, these strains readily acquired spontaneous suppressors of their slow growth phenotype, making propagation of pure *gsf2-1 snf1Δ* cultures difficult. Therefore, invertase activity was measured in *gsf2-1 snf1Δ* double mutants taken directly from slow-growing patches of cells on YPD plates prior to the appearance of fast-growing revertants. These *gsf2-1 snf1Δ* strains expressed 5 units of invertase activity; for comparison, *snf1Δ*, *gsf2-1*, and wild-type strains prepared in the same manner expressed 2, 100, and 50 units of secreted invertase activity, respectively (see MATERIALS AND METHODS). In addition, suspensions of *gsf2-1 snf1Δ* cells were spotted on YP-sucrose, YP-galactose, and YPD plates along with wild-type, *gsf2-1*, and *snf1Δ* control cells. Growth of *gsf2-1 snf1Δ* strains was very slow on YPD and was undetectable on YP-sucrose and YP-galactose medium. Thus, although *gsf2-1 snf1Δ* strains grow very slowly on YPD, they appear to have a Snf^- phenotype with respect to *SUC2* and *GAL* gene regulation.

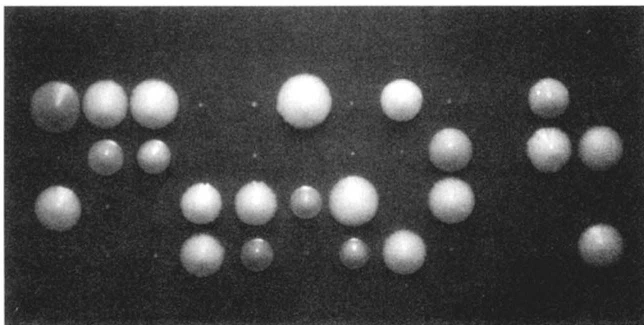

FIGURE 2.—Tetrad analysis of *gsf2-1/gsf2-1 SNF1/snf1Δ* diploid. PS4300-21B and PS43 were crossed, and the diploid was subjected to selection for loss of p*SNF1-URA3* on 5-FOA medium. Ura^- diploids were sporulated, and tetrads were dissected on YPD medium and incubated at 30° for 7 days. The growth of spore clones from 11 tetrads, demonstrating 2:2 segregation of slow:fast growth, is shown.

The *gsf* mutations do not affect transcriptional repression mediated by Ssn6-Tup1: In addition to mediating glucose repression, the Ssn6(Cyc8)-Tup1 corepressor is required for transcriptional repression of a large number of diversely regulated genes (KELEHER *et al.* 1992 and references therein). The *gsf* mutant hunt was designed to eliminate mutations in genes encoding general repressors such as *TUP1* and *SSN6*. Nevertheless, we decided to test directly whether the glucose repression defects observed in the *gsf* mutants could be attributed to a loss of Ssn6-Tup1 repressor activity. This was particularly important to test in the *gsf2-1* mutant, since the extreme slow growth phenotype of *gsf2-1 snf1Δ* mutants precluded a clear determination of the relationship of *GSF2* and *SNF1* function with respect to *SUC2* regulation. We therefore examined the ability of Ssn6-Tup1 to repress **a**-specific promoters in the *gsf* mutants.

In α cells, repression of **a**-specific genes is mediated by the α2-Mcm1 operator (JOHNSON and HERSKOWITZ 1985) and requires the α2 and Mcm1 DNA-binding proteins and the Ssn6-Tup1 corepressor (KELEHER *et al.* 1992). Repression established by Ssn6-Tup1 at the α2-Mcm1 operator was examined by comparing the expression of a pair of *CYC1UAS-lacZ* reporters, one containing an α2-Mcm1 operator (pAJ3) and one lacking the operator (pAJ1). The expression level of the operator-containing reporter in strains of genotype α*gsf1-1* (0.4 ± 0.04 units) and α *gsf2-1* (0.5 ± 0.06 units) was >100-fold lower than that of the reporter lacking the operator (α *gsf1-1*, 56 ± 2.3 units; α *gsf2-1*, 60 ± 6.6 units). The magnitude of operator-mediated repression in the mutants was similar to that in a wild-type strain (KELEHER *et al.* 1992). Repression by Ssn6-Tup1 thus appears to be intact in the *gsf* mutants. The glucose repression defects observed in these mutants are therefore unlikely to be caused by a loss of Ssn6-Tup1 function.

Effects of *gsf* mutations on expression of other glucose-regulated genes: *gsf1-1* and *gsf2-1* mutations abolish or greatly decrease glucose repression of *SUC2* expression (Table 2). To determine whether the *gsf* mutations affect glucose repression of other glucose-repressed genes, the expression of a *GAL10::lacZ* fusion was measured in *gsf* mutants (Table 3). *GAL10::lacZ* expression was induced to wild-type levels in the *gsf1-1* and *gsf2-1* mutants grown in galactose. During growth in glucose plus galactose, however, *GAL10::lacZ* expression was elevated ~1,000-fold in the *gsf1-1* and *gsf2-1* mutants relative to wild type, indicating that the *gsf* mutants are defective in glucose repression of *GAL10* expression.

The expression of a number of glucose transporters is regulated by extracellular glucose concentration (BIS-

SON *et al.* 1993 and references therein; KO *et al.* 1993; ÖZCAN and JOHNSTON 1995; LIANG and GABER 1996). For example, transcription of the *HXT1* gene is induced >200-fold by high glucose concentrations. We reasoned that defects in signaling high glucose concentration could affect glucose-induced processes as well as glucose-repressed ones. Therefore, we tested whether the *gsf* mutations affect glucose induction of *HXT1* transcription. In glucose-grown cells, expression of an *HXT1::lacZ* reporter was reduced approximately fourfold in the *gsf1-1* strain relative to wild type (Table 3). The magnitude of the *HXT1* induction defect in *gsf1-1* mutants was similar to that seen in *reg1*Δ, *hxk2*Δ, and *rgt1*Δ strains with the identical *HXT1::lacZ* reporter (ÖZCAN and JOHNSTON 1995). The existence of a second *GRR1*-mediated arm of the *HXT1* induction pathway probably accounts for the relatively mild effect of *gsf1-1*, *reg1*Δ, *hxk2*Δ, and *rgt1*Δ mutations on *HXT1* expression (ÖZCAN and JOHNSTON 1995). In contrast, no effect of the *gsf2-1* mutation on the levels of *HXT1::lacZ* expression was observed (Table 3).

Additional phenotypes of *gsf* mutants: Several additional phenotypes of *gsf1-1* and *gsf2-1* strains were tested. First, *gsf1-1* and *gsf2-1* strains are sensitive to the nonmetabolizable glucose analogue 2-deoxyglucose, as is wild type, while several previously identified *SUC2* constitutive mutants (*e.g.*, *grr1*, *reg1*, *glc7*, *hxk2*, and *mig1*) are 2-deoxyglucose resistant. In contrast to the growth defect of *grr1* mutants in YP-glucose medium (VALLIER *et al.* 1994), both *gsf1-1* and *gsf2-1* mutants are proficient for growth in YP medium containing 2%, 0.5%, or 0.05% glucose. We also tested whether these mutants display any of three additional *grr1* mutant phenotypes: sensitivity to nitrogen starvation (FLICK and JOHNSTON 1991), resistance to Co^{2+} (CONKLIN *et al.* 1993), and elongated morphology (BAILEY and WOODWARD 1984). Both *gsf1-1* and *gsf2-1* mutants appeared wild type with respect to these phenotypes. Finally, *gsf1-1* and *gsf2-1* mutants were neither temperature sensitive nor cold sensitive for growth on glucose or raffinose medium, and homozygous *gsf1-1* and *gsf2-1* diploids were proficient in sporulation.

TABLE 3

Effects of *gsf* mutations on *GAL10::lacZ* and *HXT1::lacZ* expression

	β-galactosidase activity[a]			
	GAL10::lacZ[b]		*HXT1::lacZ*[c]	
Genotype[d]	glu + gal	gal	glu	gal
Wild type	0.5 ± 0.1	2800 ± 100	260 ± 6	2.3 ± 0.1
gsf1-1	470 ± 50	1760 ± 20	73 ± 5	1.3 ± 0.1
gsf2-1	530 ± 40	1880 ± 40	360 ± 10	3.0 ± 0.1

[a] β-galactosidase activity is expressed in Miller units. All values are the average of three to four assays of two to four independent transformants.

[b] Cultures were grown to mid-log phase in SC-Ura plus 4% glucose/3% galactose/3% glycerol (glu + gal) or SC-Ura plus 3% galactose/3% glycerol (gal). The *GAL10::lacZ* fusion plasmid was pRY123.

[c] Cultures were grown to mid-log phase in SC-Ura plus 4% glucose (glu) or SC-Ura plus 3% galactose/3% glycerol (gal). The *HXT1::lacZ* fusion plasmid was pBM2636.

[d] The strains used were PS3851-3D, PS3851-3A, and PS4350-3A.

Cloning of the *GSF2* gene: The *GSF2* gene was cloned by complementation of the *gsf2-1 snf1*Δ slow growth phenotype (see MATERIALS AND METHODS). A *gsf2-1 snf1*Δ strain carrying p*SNF1-URA3* and p*SUC2::HIS3* was transformed with a *LEU2-CEN* yeast genomic DNA library and Leu^+ transformants were selected for resistance to 5-FOA. Only those transformants containing *GSF2* or *SNF1* library plasmids were expected to remain viable following loss of the p*SNF1-URA3* plasmid. Thirteen candidates were identified and rescreened to distinguish putative *GSF2* clones (Raf^-) from *SNF1* clones (Raf^+). The two Raf^+ candidates were confirmed to contain *SNF1* by PCR. The 11 Raf^- *GSF2* candidates were retransformed with p*SNF1-URA3*, and invertase assays of glucose-grown cells indicated that expression of *SUC2* was repressed in all *GSF2* candidates.

The library plasmids were isolated from two independently derived *GSF2* candidates and, on retransformation of PS4343-11B, complemented both the *SUC2* constitutive and the *snf1* synthetic slow growth phenotypes caused by the *gsf2-1* mutation. DNA sequencing of the ends of the inserted yeast sequences in these clones revealed an ~8-kb overlapping region from the left arm of chromosome XIII containing two complete open reading frames (Figure 3). Subcloning of this region indicated that plasmids bearing only YML048w complement both the *snf1* synthetic lethal and the *SUC2* constitutive phenotypes of an *gsf2-1* mutant; moreover, deletion of YML048w abolished the ability of subclones to complement *gsf2-1* (Figure 3). Subsequent disruption of YML048w confirmed that this gene corresponds to *GSF2* (see below).

Characterization of *gsf2* null mutants: To confirm that the bona fide *GSF2* gene had been cloned, an *gsf2-*Δ*1::TRP1* allele was constructed in which codons 148–304 of YML048w were deleted and replaced with the *TRP1* marker. A diploid strain isogenic to FY250 and heterozygous for this allele was constructed by one-step gene disruption, and *SUC2* constitutive and Trp^+ phenotypes cosegregated in tetrads derived from sporulation of this diploid (see MATERIALS AND METHODS). The average invertase activity of six glucose-grown *gsf2-*Δ*1::TRP1* segregants from three tetrads (11 ± 1.0 units) was 12-fold higher than the average activity of six wild-type segregants (0.9 ± 0.1 units). The original point mutants expressed higher activity but these mutants were isolated in a different S288c-related genetic background. An *gsf2-*Δ*1::TRP1* segregant was then

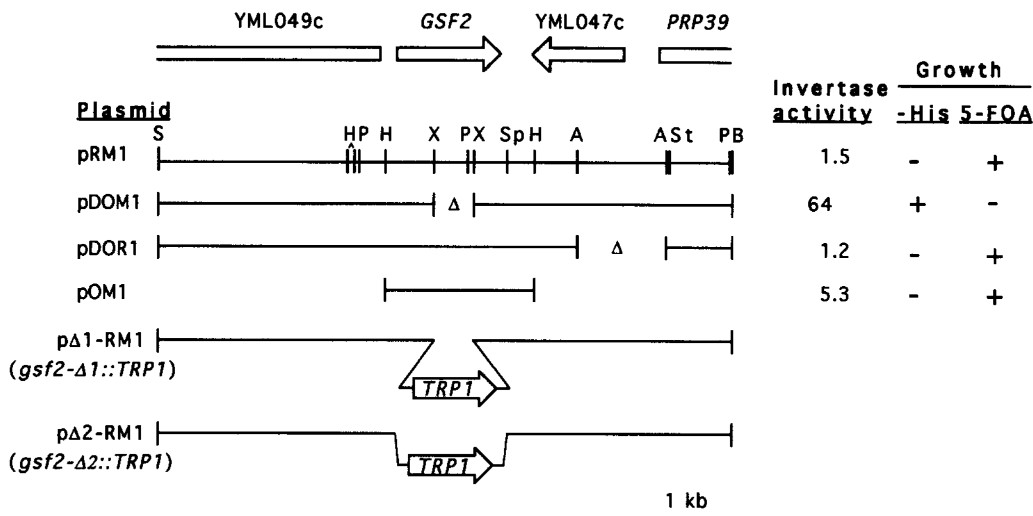

FIGURE 3.—Maps of the *GSF2* locus and plasmids. The map depicts yeast DNA inserts in the vector pRS315 and a corresponding region of chromosome XIII containing YML049c, *GSF2*, YML047c, and *PRP39*. Δ, Sequences deleted from pRM1 to yield pDOM1 and pDOR1. Invertase activity of glucose-grown cells and growth on glucose medium lacking histidine or containing 5-FOA were determined as described in MATERIALS AND METHODS. Restriction sites: A, *Avr*II; B, *Bam*HI; H, *Hin*dIII.

crossed to an *gsf2-1* mutant. The *SUC2* constitutive phenotype of the *gsf2-1* mutation was not complemented in the diploid. Following sporulation, 4:0 segregation of Gsf$^-$:Gsf$^+$ phenotypes was observed in four tetrads that were assayed for invertase activity, confirming that the cloned DNA directed disruption of the *GSF2* locus.

We then constructed an *gsf2-Δ2::TRP1* allele in which the entire *GSF2* coding region was deleted and replaced with the *TRP1* marker. This allele was introduced into an FY250 × FY251 diploid strain (see MATERIALS AND METHODS). The diploid was sporulated, and invertase assays of glucose-grown segregants demonstrated that the *gsf2-Δ2::TRP1* allele confers a *SUC2* constitutive phenotype similar to that observed for the *gsf2-Δ1::TRP1* allele (12 units invertase activity in glucose-grown cells). *gsf2-Δ2* mutants were neither temperature sensitive nor cold sensitive for growth on YPD medium, and the doubling time of an *gsf2-Δ2* mutant in SC glucose medium was indistinguishable from an isogenic wild-type strain (2.5 hr). In addition, the mutants showed normal cell morphology.

Properties of the predicted *GSF2* gene product: The *GSF2* gene encodes a previously uncharacterized protein of 403 residues with a predicted M_r of 46 kD. Despite the absence of a clearly defined signal peptide, Gsf2 contains a hydrophobic region (residues 177–198, LVAQWLFFVMHIFKVGIITLFL) that could serve as a transmembrane domain, dividing the protein into N- and C-terminal domains of roughly equivalent size. Gsf2 also contains a C-terminal dilysine consensus motif for retention of transmembrane proteins in the endoplasmic reticulum (KKXX; NILSSON *et al.* 1989; JACKSON *et al.* 1990; GAYNOR *et al.* 1994) and four potential N-glycosylation sites (Asn89, Asn173, Asn234, and Asn235). No other significant similarity to any other proteins or defined functional motifs is evident in the Gsf2 sequence.

DISCUSSION

In this study, we have isolated mutations in two genes, *GSF1* and *GSF2*, which relieve glucose repression of *SUC2* transcription. Both *gsf1* and *gsf2* mutations have pleiotropic phenotypes: *gsf1* mutations relieve glucose repression of *GAL10* transcription and decrease glucose induction of *HXT1* transcription; *gsf2* mutations also relieve glucose repression of *GAL10* and, in combination with *snf1Δ*, cause an extreme slow growth phenotype.

The *SUC2* constitutive phenotype of *gsf1* mutants is suppressed by mutations in *SNF1*. This result suggests that deregulated Snf1 protein kinase activity may account for the *SUC2* constitutivity observed in *gsf1* mutants and that, in wild type, the *GSF1* gene product may directly or indirectly inhibit Snf1 kinase function in response to a high glucose signal. In contrast, glucose induction of *HXT1* transcription does not require the Snf1 kinase (ÖZCAN and JOHNSTON 1995). Hence, *GSF1* appears to mediate the Snf1-regulated glucose repression of *SUC2* and *GAL10* transcription, as well as the Snf1-independent glucose induction of *HXT1* transcription. Evidence also suggests that the *GSF2* gene product negatively regulates the function of the Snf1 kinase with respect to glucose repression, although interpretation of the data is complicated by the slow growth phenotype of the *gsf2 snf1* double mutant.

The phenotypes of *gsf1* and *reg1Δ* mutants are similar in that both mutations relieve glucose repression of *SUC2* and *GAL10* and curtail glucose induction of *HXT1* to the same degree. The *gsf1* and *reg1Δ* mutations complement, however, and a 2μ-*REG1* plasmid failed to complement *gsf1-1* (data not shown), indicating that *GSF1* and *REG1* are distinct genes. Previous work has implicated Reg1 as a regulatory subunit of the Glc7 type1 protein phosphatase (TU and CARLSON 1994, 1995) and indicated that Reg1-Glc7 affects Snf1 kinase function (JIANG and CARLSON 1996). It is possible that the *GSF1* gene product acts in conjunction with Reg1 to stimulate or direct the activity of the Glc7 phosphatase toward its glucose signaling pathway substrate(s) in response to a high glucose signal. Alternatively, Gsf1 could be a Reg1-Glc7 phosphatase substrate whose func-

tion is stimulated by dephosphorylation in response to high glucose. Because multiple pathways mediate both *SUC2* and *HXT1* regulation, it is also possible that Gsf1 and Reg1 function by distinct mechanisms. This view is supported by the observation that, despite their other phenotypic similarities, *reg1*Δ mutants can use sucrose in the presence of the glucose analog 2-deoxyglucose while *gsf1-1* mutants cannot. We have attempted to clone the *GSF1* gene by complementation of the glucose repression or glucose induction phenotypes of *gsf1* mutants but have thus far been unsuccessful.

Several lines of evidence suggest that multiple pathways convey high glucose signals. ÖZCAN *et al.* (1996) showed that a dominant mutation in *RGT2* (*RGT2-1*) leads to induction of *HXT1* transcription in the absence of glucose, indicating that this mutation constitutively activates a pathway for high glucose induction of *HXT1*. *RGT2* encodes a protein similar to glucose transporters, consistent with a role for Rgt2 as a receptor that generates a signal of high extracellular glucose (ÖZCAN *et al.* 1996). However, the *RGT2-1* mutation does not affect the Snf1-mediated glucose-repression pathway (MARSHALL-CARLSON *et al.* 1991; ÖZCAN *et al.* 1996), indicating the existence of a distinct high glucose signal. WILSON *et al.* (1996) have proposed that glucose signaling via the Snf1 pathway is regulated by a metabolic signal of high intracellular glucose concentration (specifically, a low AMP:ATP ratio resulting from rapid fermentation) that inhibits Snf1 protein kinase activity, based on a correlation between extracellular glucose concentration, the intracellular AMP:ATP ratio, and Snf1 protein kinase activity. These and other studies (BISSON *et al.* 1993; GRANOT and SNYDER 1993; SIERKSTRA *et al.* 1993; H. JIANG and C. A. MICHELS, personal communication) are consistent with the existence of at least two distinct high glucose signals. In this study, we have found that *gsf1* affects both glucose repression and glucose induction, perhaps reflecting crosstalk between different signaling pathways, whereas *gsf2* affects only glucose repression.

We do not yet understand the basis for the extreme slow growth phenotype of *gsf2 snf1* double mutants. However, two previously characterized mutations that also cause a slow growth or lethal phenotype in combination with *snf1*Δ are *grr1* (FLICK and JOHNSTON 1991; VALLIER *et al.* 1994) and *bcy1* (THOMPSON-JAEGER *et al.* 1991). This shared mutant phenotype suggests that the functions of *GSF2*, *GRR1*, and *BCY1* may be related. Indeed, previous studies have established a relationship between *GRR1* and *BCY1* function in the regulation of G1 cyclin expression (BARONI *et al.* 1994; TOKIWA *et al.* 1994; BARRAL *et al.* 1995), and BARRAL *et al.* proposed that *GRR1* acts together with the *BCY1*-regulated RAS/cAMP pathway to couple cell cycle progression to the availability of nutrients. A role for the *GSF2* gene product in such a process is likewise possible. The cloning of the *GSF2* gene enables this and other aspects of Gsf2 function to be addressed.

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