New *SNF* Genes, *GAL11* and *GRR1* Affect *SUC2* Expression in *Saccharomyces cerevisiae*

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ABSTRACT

To identify new genes required for derepression of the *SUC2* (invertase) gene in *Saccharomyces cerevisiae*, we have isolated mutants with defects in raffinose utilization. In addition to mutations in *SUC2* and previously identified *SNF* genes, we recovered recessive mutations that define four new complementation groups, designated *snf7* through *snf10*. These mutations cause defects in the derepression of *SUC2* in response to glucose limitation. We also recovered five alleles of *gal11* and showed that a *gal11* null mutation decreases *SUC2* expression to 30% of the wild-type level. Finally, one of the mutants carries a *grr1* allele that converts *SUC2* from a glucose-repressible gene to a glucose-inducible gene.

MANY genes in *Saccharomyces cerevisiae* are regulated in response to glucose availability. Yeast cells prefer to use glucose as a carbon source and when grown in glucose, repress expression of the genes involved in utilizing alternate carbon sources. To understand the regulatory mechanism(s) responsible for glucose repression, we have focused on understanding the control of *SUC2* (invertase) gene expression. The *SUC2* gene provides a convenient model system because it is regulated solely by glucose repression and is not induced by the substrates sucrose or raffinose. *SUC2* encodes both secreted and intracellular forms of invertase via two mRNAs (PERLMAN and HALVORSON 1981; CARLSON and BOTSTEIN 1982). The secreted enzyme is the physiologically important isozyme, and its expression is regulated by glucose repression at the RNA level. The low level constitutive expression of the intracellular invertase is not relevant to this study.

Genes required for the derepression of *SUC2* in response to glucose starvation have been identified in previous mutant searches (CARLSON, OSMOND and BOTSTEIN 1981; NEIGEBORN and CARLSON 1984). The *SNF* (sucrose nonfermenting) genes fall into three groups of functionally related genes: *SNF1* and *SNF4*; *SNF2*, *SNF5* and *SNF6*; and *SNF3*. These groups are distinguishable on the basis of phenotype and patterns of interaction with extragenic suppressors (NEIGEBORN, RUBIN and CARLSON 1986; ESTRUCH and CARLSON 1990b). Mutations in *SNF1* (also known as *CAT1* and *CCR1*) and *SNF4* (*CAT3*) prevent expression of many glucose-repressible genes (CIRIACY 1977; CARLSON, OSMOND and BOTSTEIN 1981; ENTIAN and ZIMMERMANN 1982; NEIGEBORN and CARLSON 1984; SCHULLER and ENTIAN 1987; SCHULLER and ENTIAN 1988). *SNF1* encodes a protein-serine/threonine kinase (CELENZA and CARLSON 1986). *SNF4* encodes a protein that is physically associated with the SNF1 kinase and is required for maximal SNF1 kinase activity (CELENZA and CARLSON 1989; CELENZA, ENG and CARLSON 1989; FIELDS and SONG 1989).

The *SNF2*, *SNF5* and *SNF6* genes affect not only glucose-repressible genes (NEIGEBORN and CARLSON 1984; ESTRUCH and CARLSON 1990b), but also expression of acid phosphatase (ABRAMS, NEIGEBORN and CARLSON 1986), cell type-specific genes (LAURENT, TREITEL and CARLSON 1990), and Ty elements (HAPPEL, SWANSON and WINSTON 1991). Also *snf2* and *snf5* mutations cause constitutive expression of protease B; a leaky *snf6* allele had no effect (MOEHLE and JONES 1990). Mutations in the three genes were reported to cause singular phenotypes with respect to glucose transport (BISSON 1988). Thus, these genes affect expression of a variety of differently regulated genes, and it seems unlikely that they convey specific regulatory signals. Recent evidence implicates *SNF2* and *SNF5* in transcriptional activation: DNA-bound LexA-SNF2 and LexA-SNF5 fusion proteins activate transcription from a nearby promoter (LAURENT, TREITEL and CARLSON 1990, 1991). The SNF2, SNF5, and SNF6 proteins appear to function interdependently because activation by LexA-SNF2 is dependent on SNF5 and SNF6, and activation by LexA-SNF5 is dependent on SNF2 and SNF6 (LAURENT, TREITEL and CARLSON 1990, 1991). *SNF2* and *SNF5* are the same as *TYE3* and *TYE4*, respectively (CIRIACY and WILLIAMSON 1981) (M. CIRIACY, personal communication).

SNF3 encodes a high-affinity glucose (and fructose) transporter (BISSON *et al.* 1987; CELENZA, MARSHALL-CARLSON and CARLSON 1988) but does not affect invertase expression (NEIGEBORN *et al.* 1986; MARSHALL-CARLSON *et al.* 1990). Mutants were recovered because they are defective in growth on raffinose, which requires ability to transport the low levels of fructose released by the action of secreted invertase.

Genes required for full derepression of *SUC2* have also been identified by other genetic approaches. The *MSN1* gene was isolated as a multicopy suppressor that restored growth on raffinose in a *snf1-ts* mutant. Mutations in *MSN1* cause a few-fold decrease in invertase expression in an otherwise wild-type background (ESTRUCH and CARLSON 1990a).

Genes required for glucose repression of *SUC2* include *HXK2* (ZIMMERMANN and SCHEEL 1977; ENTIAN and MECKE 1982; MA and BOTSTEIN 1986), *REG1* (*HEX2*) (MATSUMOTO, YOSHIMATSU and OSHIMA 1983; NIEDERACHER and ENTIAN 1987), *SSN6* (*CYC8*) (SCHULTZ and CARLSON 1987; TRUMBLY 1988), *TUP1* (WILLIAMS and TRUMBLY 1990 and references therein), *MIG1* (NEHLIN and RONNE 1990), *GRR1* (BAILEY and WOODWORD 1984), *RGR1* (SAKAI *et al.* 1990), and *CID1* (NEIGEBORN and CARLSON 1987). *HXK2* encodes hexokinase PII and may function early in the signaling pathway (ENTIAN *et al.* 1985; MA *et al.* 1989). The *MIG1* product is a zinc-finger protein that binds to *SUC2* DNA (NEHLIN and RONNE 1990). *SSN6* encodes a nuclear protein containing the TPR sequence motif (SCHULTZ, MARSHALL-CARLSON and CARLSON 1990), and *TUP1* encodes a protein with homology to the β-subunit of transducin (WILLIAMS and TRUMBLY 1990). The mechanism of action of these gene products in glucose repression is not understood.

A limitation in our efforts to unravel the regulatory pathway for glucose repression is that, most likely, not all of the relevant genes have yet been identified. For example, the distribution of *snf* alleles obtained in previous studies suggests that some *SNF* genes remain to be identified: only one or two *snf2*, *snf4*, *snf5* and *snf6* alleles were isolated (Table 1). We therefore undertook another mutant search in the hopes of recovering *snf* mutations at new loci.

In this search we wished to optimize the recovery of mutants that were only partially impaired in invertase expression. Such mutants could carry either "leaky" mutations or null mutations that caused only a partial defect; in either case, the genes might be of interest. To detect mild growth defects, we screened mutagenized colonies for defective growth on medium containing raffinose plus antimycin A. Our strains use raffinose less efficiently than sucrose, and antimycin A increases the dependence on raffinose by blocking respiration.

TABLE 1

Distribution of *snf* alleles isolated

Source	Number of alleles							
	suc2	*snf1*	*snf2*	*snf3*	*snf4*	*snf5*	*snf6*	*gal11*
Previous studies[a]	37	10	2	7	1	2	1	ND[b]
This study	21	3	0	10	0	1	0	5

[a] CARLSON, OSMOND and BOTSTEIN (1981); NEIGEBORN and CARLSON (1984).
[b] Not determined.

We also examined the effects of the *GAL11* gene (also known as *SPT13*) on *SUC2* expression. Mutations in *GAL11* cause a partial defect in induction of the *GAL* genes (NOGI and FUKASAWA 1980) and also cause defects in growth on nonfermentable carbon sources, mating of *MATα* strains, expression of cell type-specific genes, and sporulation of homozygous diploids (SUZUKI *et al.* 1988; FASSLER and WINSTON 1989; NISHIZAWA *et al.* 1990). The *spt13* alleles were isolated as suppressors of a Ty insertion mutation (FASSLER and WINSTON 1988). The GAL11 protein appears to have a role in transcriptional activation by GAL4 and RAP1/GRF1/TUF (HIMMELFARB *et al.* 1990; NISHIZAWA *et al.* 1990). Interestingly, GAL11 is the same size as the SSN6 protein and contains similar stretches of polyglutamine and poly(glutamine-alanine) at similar positions (SUZUKI *et al.* 1988). These similarities may be fortuitous or may reflect a functional relationship.

The pleiotropic defects of *gal11* are similar to those of some *snf* mutations. Moreover, *gal11* only partially impairs *GAL* gene expression, reducing expression to 10–30% of the wild-type level (NOGI and FUKASAWA 1980; SUZUKI *et al.* 1988). If *gal11* mutants are only partially defective in *SUC2* expression, we might not have identified them in previous studies. We therefore examined invertase expression in a *gal11* null mutant.

We report here the isolation of mutations that define four new complementation groups, *snf7* through *snf10*. These *SNF* genes are required for derepression of invertase. We show that a *gal11* null mutation partially reduces derepression of invertase, and five new *gal11* alleles were isolated in this search. We also show that one of the newly isolated mutations is an allele of *grr1* and causes glucose-inducible expression of invertase.

MATERIALS AND METHODS

Yeast strains: Strains used in this study are listed in Table 2. Mutant phenotypes were examined in strains with the S288C genetic background. The isolation of a revertant *GAL2* allele was described previously (CARLSON, OSMOND and BOTSTEIN 1981). The plasmid pLS11 contains a *SUC2-LEU2-lacZ* gene fusion; β-galactosidase is expressed from the *LEU2* promoter under control of the *SUC2* upstream regulatory region (SAROKIN and CARLSON 1985).

TABLE 2

List of *S. cerevisiae* strains

MCY501[a]	*MATα ade2-101 gal2 SUC2*
MCY882	*MATα suc2-Δ9 his4-539 ade2-101 ura3-52 SUC2*
MCY886	*MATα snf4-Δ1 his4-539 ade2-101 SUC2*
MCY947	*MATα snf5-5::URA3 his4-539 ade2-101 SUC2*
MCY1094	*MATα ade2-101 ura3-52 SUC2*
MCY1151	*MATα cyr1-2 ade2-101 ura3-52 SUC2*
MCY1250	*MATα snf2-Δ1::HIS3 lys2-801 ura3-52 his3-Δ200 SUC2*
MCY1389	*MATa ura3-52 leu2::HIS3 SUC2*
MCY1409	*MATα snf3-Δ4::HIS3 lys2-801 ura3-52 his3-Δ200 SUC2*
MCY1594	*MATα snf1-Δ3 ura3-52 lys2-801 leu2::HIS3 SUC2*
MCY1617	*MATa snf3-72 ade2-101 ura3-52 SUC2*
MCY1803	*MATa his4-539 lys2-801 ura3-52::pLS11 SUC2*
MCY1980	*MATa ade6 arg4 met14 &/or met? pet17 trp1 lys2 ura3 spo11? SUC2*
MCY2200	*MATα snf10-68 his4-539 lys2-801 ade2-101 ura3-52::pLS11 SUC2*
MCY2209	*MATa grr1-512 his4-539 ura3-52 SUC2*
MCY2218	*MATα grr1-512 ura3-52 lys2-801 ade2-101 SUC2*
MCY2219	*MATa grr1-512 his4-539 ade2-101 ura3-52::pLS11 SUC2*
MCY2220	*MATα snf7-12 ura3-52 ade2-101 SUC2*
MCY2222	*MATa snf8-210 ura3-52 his4-539 lys2-801 SUC2*
MCY2223	*MATα snf8-210 ura3-52 ade2-101 SUC2*
MCY2224	*MATa snf10-68 ura3-52 his4-539 lys2-801 SUC2*
MCY2236	*MATa snf10-68 his4-539 lys2-801 ura3-52::pLS11 SUC2*
YM3502[c]	*MATα grr1-Δ::URA3 ura3-52 his3-Δ200 ade2-101 lys2-801 met SUC2*
MCY2251	*MATα grr1-512 ura3-52 his4-539 SUC2*
MCY2253	*MATα spt13-101::TnLUK ura3-52 ade2-101 SUC2*
MCY2254	*MATα snf9-612 ura3-52 ade2-101 SUC2*
MCY2255	*MATa ura3-52 his4-539 SUC2* LVM#1-13
MCY2256	*MATα snf6-Δ1 ura3-52 ade2-101 SUC2*
AS14[b]	*MATα snf10 trp1 ura3 ade6 tyr7 arg4*
LS66[b]	*MATa snf10 leu2 ade6 ura3*
YM1871[c]	*MATα grr1-1121 LEU2::GAL1/lacZ ade2-101 his3-Δ200 lys2-801 ura3-52 SUC2*
JF15[d]	*MATα his4-917 lys2-128δ ura3-52 leu2 SUC2*
JF916[d]	*MATα spt13-101::TnLUK his4-917 lys2-128δ ura3-52 leu2 SUC2*

[a] All strains were from this laboratory except as noted.
[b] Obtained from LORRAINE SYMINGTON. Strains AS14 and LS66 contain disruptions of *SNF10* constructed by filling in a *Bam*HI site in the gene and by deleting a *Bam*HI-*Bgl*II fragment, respectively (SYMINGTON *et al.* 1991).
[c] Obtained from JEFF FLICK and MARK JOHNSTON.
[d] Obtained from JAN FASSLER.

General genetic methods and media: Standard genetic procedures were employed for crossing, sporulating and dissecting tetrads (SHERMAN, FINK and LAWRENCE 1978) and transforming yeast (ITO *et al.* 1983). Utilization of glucose and raffinose was scored on solid medium containing 1% yeast extract, 2% bacto-peptone, 1 μg/ml antimycin A (Sigma; prepared as a stock solution of 1 mg/ml in 95% ethanol), and 2% glucose (YPDaa) or 2% raffinose (YPRaa). Utilization of galactose was scored on medium containing 1% yeast extract, 2% bacto-peptone, and 2% galactose, and plates were incubated in GasPak disposable anaerobic systems (BBL). Utilization of glycerol was scored on medium containing 1% yeast extract, 2% bacto-peptone, and 3% glycerol. Except for the original isolation of the mutants, all scoring was performed by spotting cell suspensions onto the appropriate medium and incubating the plates at 30°. Liquid medium was YEP (SHERMAN, FINK and LAWRENCE 1978) containing the indicated sugar.

To assay sporulation, diploid cells were grown in YEP-2% glucose liquid medium overnight, collected by centrifugation, resuspended in a small volume, and spotted on solid sporulation medium (SHERMAN, FINK and LAWRENCE 1978). After 5 days at room temperature, 500 cells were examined for spore formation.

Isolation of mutants: Strain MCY1803 was subjected to mutagenesis with 3% ethyl methanesulfonate for 45 min, and surviving cells (35%) were stored under conditions that were nonpermissive for growth until they were plated for single colonies (CARLSON, OSMOND and BOTSTEIN 1981). Surviving cells were plated onto YPDaa. After 2 days of growth at 30°, 16,000 colonies were replica plated to YPRaa and incubated at 30°. Colonies that showed a growth defect on YPRaa after 1–2 days were retested for their ability to utilize raffinose by spotting cell suspensions onto YPRaa and YPDaa and comparing growth.

Dominance tests and complementation analysis: To test for dominance, each of the mutants was crossed to the wild-type strain MCY501. For complementation analysis with previously identified mutations, mutants were crossed to strains MCY882, MCY886, MCY947, MCY1151, MCY1250, MCY1409, MCY1594, MCY2253 and MCY2256. To test the newly isolated mutations for complementation of one another, segregants derived from backcrosses of mutants to wild type were crossed to the original mutants. Diploids were isolated by prototrophic selection and tested for raffinose utilization.

Invertase and β-galactosidase assays: Glucose-repressed and derepressed cells were prepared from exponentially

growing cultures as described previously (NEIGEBORN and CARLSON 1984). For assays of *grr1* mutants, cells were grown to mid-log phase (Δ Klett 50) in YEP-2% galactose, washed once in YEP, and then shifted to YEP-2% galactose or YEP-2% galactose plus 2% glucose for three generations (11–12 hr for mutant cells and 9 hr for wild type). Cells were also shifted to YEP-2% raffinose; wild-type cells were allowed to grow two to three generations and *grr1* mutants were assayed after 13 hr, during which they grew less than two generations. Secreted invertase was assayed (GOLDSTEIN and LAMPEN 1975) in whole cells. β-Galactosidase was assayed in permeabilized cells (GUARENTE 1983) and activity is expressed as described by MILLER (1972).

RESULTS

Isolation of mutants: Strain MCY1803 was mutagenized, and 133 mutants that were defective in raffinose utilization were isolated (see MATERIALS AND METHODS). These mutants were able to grow on glucose, but not raffinose, in the presence of antimycin A. To test for dominance, the mutants were crossed to wild type. For 127 mutants, the resulting diploids were able to utilize raffinose, indicating that their mutations are recessive. Five mutants appeared to carry partially dominant mutations; however, none was studied further because two produced high invertase activity, and the other three showed poor spore germination after crossing. One mutant failed to mate.

Complementation analysis with known mutations: To determine if any of the recessive mutations isolated were new alleles of previously identified genes, we carried out complementation analysis with *suc2*, *snf1*, *snf2*, *snf3*, *snf4*, *snf5* and *snf6* null mutations. Complementation of *cyr1* was also tested because mutants carrying a temperature-sensitive *cyr1* allele are defective in invertase production at the permissive temperature (MATSUMOTO, UNO and ISHIKAWA 1984; SCHULTZ and CARLSON 1987). Twenty-one new *suc2* mutations, 3 *snf1* alleles, 10 *snf3* alleles and 1 *snf5* allele were identified (Table 1).

Mutations in *GAL11* affect invertase derepression: The similarity in the spectrum of pleiotropic defects caused by *gal11* and *snf* mutations prompted us to examine the effects of *gal11* on invertase expression. We found that strain JF916, a *gal11* (*spt13*) null mutant in the S288C genetic background (carrying *GAL2*) failed to grow on YPRaa and was partially defective in derepression of invertase (fourfold lower activity than the isogenic *GAL11* strain JF15; data not shown). The mutant was crossed to wild type and the Gal⁻ and Raf⁻ phenotypes cosegregated 2+:2− in six tetrads. Four Gal⁻ Raf⁻ segregants from two tetrads were assayed for invertase activity, and the average value, shown in Table 3, corresponds to about 30% of the wild-type value. The *gal11* mutation affects induction of the *GAL* genes to a similar extent, reducing activity to about 10–30% of the wild-type level (NOGI and FUKASAWA 1980; SUZUKI *et al.* 1988).

Since the *gal11* null mutation causes a Raf⁻ phenotype, we carried out complementation analysis with our new mutations. We identified five new *gal11* alleles. These mutants were all partially impaired in derepression of invertase (data not shown).

Invertase assays: The 87 mutants not yet assigned to complementation groups were assayed for the production of invertase. Cells were grown to mid-log phase in YEP-2% glucose (glucose-repressing conditions) and then shifted to YEP-0.05% glucose for 2.5 hr (derepressing conditions). Derepressed invertase activity was lower than 50 units in 23 mutants (group A) and greater than 50 units in 63 mutants (group B). One mutant (group C) expressed invertase at high levels when grown in glucose, and a shift to low glucose did not cause further derepression.

Surprisingly, many mutants showed impaired growth on YPRaa despite their ability to derepress invertase to high levels. A possible cause of this discrepancy was the presence of antimycin A in the YPRaa plates but not in the liquid medium used to prepare cells for invertase assays. To test this idea, two group B mutants were grown and derepressed in medium containing the antibiotic. Antimycin A did not inhibit derepression of invertase (data not shown).

Complementation analysis: The 23 mutants in group A were backcrossed to wild type (MCY1094), and the resulting diploids were subjected to tetrad analysis. For 17 mutants, 2+:2− segregations for a defect in raffinose utilization were observed in 5–12 tetrads examined. Representative Raf⁻ segregants (two to four) from each of these crosses were assayed for invertase. In 13 cases invertase activity in the segregants was reduced more than twofold relative to wild type and among these were five mutants from which segregants showing low invertase activity (less than 50 units) were recovered. These five mutations define four new complementation groups designated *snf7*, *snf8*, *snf9* and *snf10*. (The name *snf*, for *s*ucrose *n*onfermenting, is used for historical reasons, even though these mutants express sufficient invertase to allow growth on sucrose.) Mutants carrying the alleles *snf7-12*, *snf8-210*, *snf9-612* and *snf10-68* were used for complementation analysis; crosses heterozygous for these mutations yielded 2 Raf⁺:2 Raf⁻ segregations in 7, 15, 9 and 37 tetrads, respectively. Additional alleles in these groups were identified among the 17 mutants showing 2 Raf⁺:2 Raf⁻ segregations. The *snf7* complementation group contains three alleles, the *snf8* and *snf9* groups each have two alleles, and the *snf10* group has a single allele. These mutations all reduce derepression of invertase (Table 3).

Many of the mutants recovered in this search derepress invertase to high levels. To determine whether these mutants represent a few or many complemen-

TABLE 3

Expression of invertase and a *SUC2-LEU2-lacZ* fusion in new *snf* mutants

Mutant allele	Invertase activity[a,b]		β-Galactosidase activity[a,c]	
	Repressed	Derepressed	Repressed	Derepressed
snf7-12	1	26 (4)	0.4	13 (1)
snf7-215	1	19 (4)	0.3	10 (2)
snf7-41	1	95 (2)	1.2	35 (1)
snf8-210	<1	32 (6)	0.4	12 (2)
snf8-114	2	88 (2)	ND[d]	ND
snf9-612	<1	50 (6)	0.6	16 (1)
snf9-517	1	55 (2)	0.4	12 (1)
snf10-68	1	55 (5)	0.6	13 (2)
spt13-101::TnLUK (gal11)	1	73 (4)	ND	ND
Wild type	2	260	0.9	36

[a] Values are averages of assays of mutant segregants derived from backcrosses to wild type; the number of segregants assayed is shown in parenthesis. The wild-type value is the average of two assays of MCY1803. Standard errors were <25%, except for *snf7-215* where the values for the segregants were 6, 12, 16 and 43.

[b] Micromoles glucose released/min/100 mg (dry weight) of cells.

[c] Activity is expressed according to MILLER (1972).

[d] ND, no data.

tation groups, we chose 12 such mutants for further complementation analysis. Six were mutants from group B that showed 2+:2− segregation patterns for growth on YPRaa in backcrosses to wild type. Six were chosen from among the group A mutants that showed higher invertase activity after backcrossing, as mentioned above. These 12 mutations were tested for complementation with all of the group A alleles and either a subset or all of the group B alleles. Only one or a few alleles were identified in each complementation group. Thus, mutations in many genes can apparently cause growth defects on YPRaa without impairing derepression of invertase. It is noteworthy that four of these mutations caused an auxotrophy that cosegregated with the Raf⁻ phenotype in tetrads. Three mutations caused a requirement for glutamate and one caused a requirement for aspartate. These mutants were not studied further because derepression of invertase was not significantly defective.

The mutant in group C was backcrossed to wild type, and tetrad analysis showed 2+:2− segregation for utilization of raffinose in 19 tetrads. The mutation was then tested for complementation with all other mutations. It only partially complemented an allele in group B (MCY2255); however, tetrad analysis of the heterozygous diploid yielded Raf⁺ spore clones frequently (1 parental ditype:1 nonparental ditype:5 tetratype asci), indicating that the two mutations are not allelic. Thus, the group C mutation defines a complementation group with a single member, which we show below to be an allele of *grr1*.

Linkage analysis and mapping of *SNF10* on chromosome *III*: Analyses of crosses between the *snf7*, *snf8*, *snf9* and *snf10* mutants and strains containing the centromere-linked marker *ura3* indicated that

TABLE 4

Linkage data

Cross	Gene pair	Number of tetrads PD	NPD	T	Genetic distance (cM)[a]
MCY2236 × MCY1094	*snf10-MAT*	5	0	2	38
MCY2200 × MCY1389		9	1	21	
MCY2236 × MCY1094	*snf10-his4*	4	0	2	23
MCY2200 × MCY1389		15	0	14	
MCY2200 × MCY1389	*snf10-leu2*	29	0	2	3
MCY2200 × MCY1389	*leu2-his4*	18	0	12	20
MCY2223 × MCY1980	*snf8-CEN*	19	33	8	7

[a] Genetic map distances in centimorgans (cM) were calculated according to PERKINS (1949): cM = 100 (T + 6NPD)/2(PD + NPD + T). The distance of *snf8* from its centromere was calculated from the frequency of second-division segregations from the centromere marker *trp1*. PD = parental ditype, NPD = nonparental ditype, T = tetratype.

snf8 and *snf10* are linked to a centromere. Additional analysis showed that *snf8* is located approximately 7 cM from its centromere and *snf10* lies 3 cM from *leu2* on the left arm of chromosome *III* (Table 4). Both tetrads that were tetratype for the *snf10-leu2* marker pair were parental ditype for the *leu2-his4* pair and tetratype for the *snf10-his4* pair, indicating that the gene order is *centromere-snf10-leu2-his4*.

Disruption of a cloned gene located on chromosome *III* near *SNF10* has been shown to cause a sporulation defect (SYMINGTON *et al.* 1991). This gene appears to be identical to *SNF10*, because two different disruptions (AS14 and LS66) fail to complement a *snf10* mutation for growth on raffinose. The physical location of the disrupted gene is consistent with the genetic map position of *SNF10*.

Pleiotropic phenotypes of new *snf* mutants: Pre-

TABLE 5

Regulation of invertase expression in a *grr1* mutant

Relevant genotype	Invertase activity[a]			
	Raf[b]	Gal[b]	Gal + Glu[b]	Glu[c]
grr1-512	4	3	95	153
Wild type	44	58	1	1

[a] Micromoles glucose released/min/100 mgs (dry weight) of cells. Values for the mutant are the average of assays of three *grr1-512* segregants. The wild-type strain was MCY1094.

[b] Cells were grown in YEP-2% galactose to log-phase, washed, and resuspended for growth in YEP-2% raffinose (Raf), YEP-2% galactose (Gal), or YEP-2% galactose plus 2% glucose (Gal + Glu) (see MATERIALS AND METHODS).

[c] Cells were also grown to mid-log phase in YEP-2% glucose (Glu).

viously identified *snf* mutants show pleiotropic defects in their ability to utilize galactose and nonfermentable carbon sources. Tetrad analysis of strains heterozygous for the *snf7*, *snf8* and *snf9* mutations showed no segregation of defects in galactose or glycerol utilization. A strain heterozygous for *snf10* showed cosegregation of a partial defect in glycerol utilization with the Raf$^-$ phenotype in five of six tetrads examined; only one spore clone of the sixth tetrad showed a defect on glycerol. Diploids homozygous for each of these *snf* mutations are defective in sporulation. A low percentage of the cells sporulated (<5% for diploids homozygous for *snf7-12*, *snf8-210*, *snf9-612* or *snf10-68*), and the yield of four-spored asci was low (<1%). In contrast, approximately 70% of wild-type diploid cells sporulated.

New *snf* mutants are defective in derepressing a *SUC2-LEU2-lacZ* fusion: To obtain evidence that the *snf7*, *snf8*, *snf9* and *snf10* mutations affect *SUC2* expression at the transcriptional level, we assayed expression of β-galactosidase from a *SUC2-LEU2-lacZ* fusion, in which the *LEU2* promoter is under control of the *SUC2* upstream region. These mutants carried the fusion integrated on plasmid pLS11 (SAROKIN and CARLSON 1985). Expression of β-galactosidase was reduced relative to wild type in the new *snf* mutants except for the mutant carrying *snf7-41*, which is a weak allele (Table 3).

The group C mutation is a *grr1* allele: The original group C mutant expressed invertase during growth in glucose (Table 5). To test whether this phenotype cosegregates with the Raf$^-$ phenotype, we first assayed invertase activity in all the spore clones from three tetrads. The two phenotypes cosegregated. We then assayed 32 Raf$^-$ segregants from 16 tetrads, and all expressed invertase after growth in glucose. These data strongly suggest that the Raf$^-$ and invertase phenotypes are caused by a single lesion.

No defect in growth on galactose or glycerol was observed in strains carrying the group C mutation. These mutants grow slower than wild type on rich medium containing glucose. Diploids homozygous for the mutation sporulated; however, many cells yielded only two-spored asci.

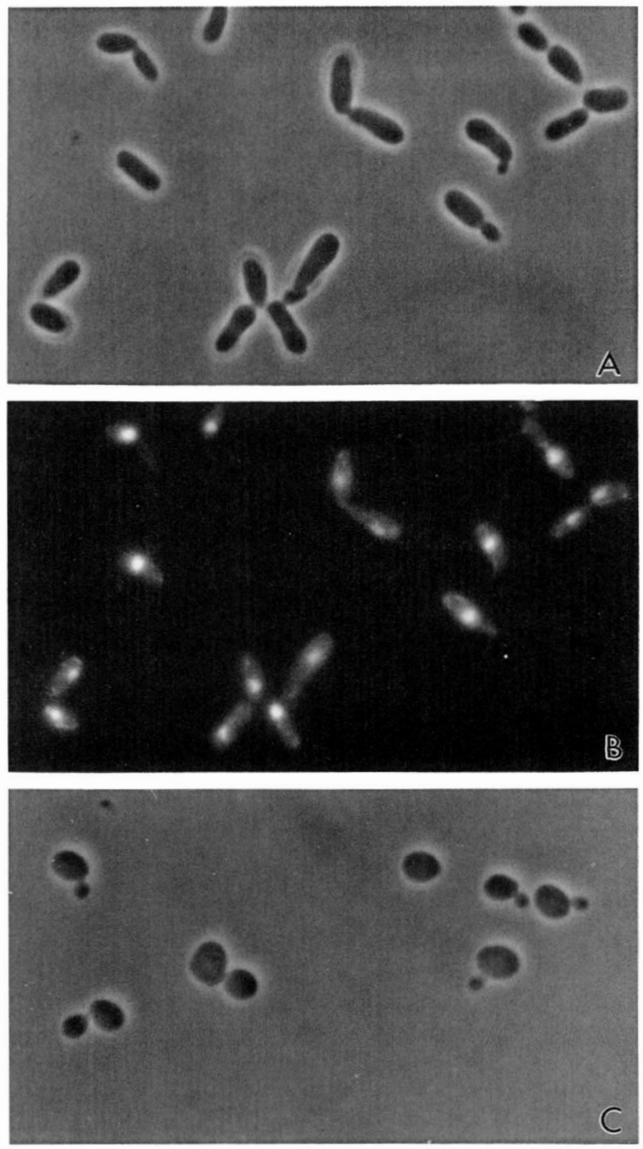

FIGURE 1.—Morphology of *grr1* mutant cells. Cells of strain MCY2218 (*grr1-512*) were grown to mid-log phase in YEP-2% glucose, fixed, stained with 4′,6-diamino-2-phenylindole (DAPI) and examined by fluorescence microscopy as previously described (CELENZA, MARSHALL-CARLSON and CARLSON 1988). The same cells were observed by (A) phase contrast and (B) DAPI staining. A phase contrast micrograph of wild-type cells (MCY1803) is shown for comparison (C).

Microscopic examination revealed that these mutant cells are morphologically aberrant: the buds are elongated, sometimes severely (Figure 1). Previously, BAILEY and WOODWORD (1984) isolated the *grr1* mutation, which causes glucose-insensitive (derepressed) expression of several glucose-repressible enzymes, including invertase, and was associated with a similar morphology. We therefore obtained a *grr1* mutant (YM1871) and found that it was defective in raffinose utilization. Both *grr1* and the Group C mutation are

recessive to wild type with respect to cell morphology, regulation of invertase expression, and raffinose utilization. The two mutations were tested for complementation by crossing MCY2209 and YM1871. The diploid displayed all three mutant phenotypes, indicating that the mutations fail to complement. To test allelism, the diploid was subjected to tetrad analysis. All segregants from three tetrads and four triads were Raf$^-$ and displayed the characteristic aberrant cell morphology. The new allele is designated *grr1-512* after the isolation number of the mutant.

Invertase expression is glucose-induced in *grr1* mutants: The failure of *grr1* mutants to grow on raffinose was initially puzzling in view of the high-level expression of invertase in glucose-grown mutant cells. However, we had not directly assessed the expression of invertase in raffinose-grown cells. Our assay of invertase derepression, performed by shifting glucose-grown cells to low glucose, was not relevant because glucose-grown *grr1* cells already contain invertase, which remains stable during the period of growth in low glucose. Meanwhile, J. FLICK and M. JOHNSTON (personal communication) found that *grr1* mutants express *SUC2* RNA only in the presence of glucose. We therefore grew *grr1* mutant cells to mid-log phase in galactose and then shifted cells to raffinose, galactose or galactose plus glucose. The mutants expressed invertase only in the presence of glucose (Table 5). To examine further this glucose inducibility, we grew both mutant and wild-type cultures to mid-log phase in YEP-2% galactose and then shifted the cells to YEP-2% galactose plus varying concentrations of glucose. Expression of invertase was glucose-inducible in the mutant and glucose-repressible in the wild type (Figure 2). Thus, this confirms the observation of FLICK and JOHNSTON that the *grr1* mutation converts *SUC2* from a glucose-repressible gene to a glucose-inducible gene.

***GRR1* does not affect glucose repression of *SNF3*:** We next tested whether *grr1* affects expression of another glucose repressible gene, *SNF3*, which encodes a high affinity glucose transporter (CELENZA, MARSHALL-CARLSON and CARLSON 1988). Wild-type (MCY1094) and *grr1* mutant (MCY2251) strains were transformed with pSNF3(797)-lacZ (CELENZA, MARSHALL-CARLSON and CARLSON 1988). This plasmid carries a fusion between codon 797 of *SNF3* and *lacZ*. Expression of β-galactosidase was normally regulated in *grr1* transformants: the activity was 0.8 unit in glucose-repressed cultures and 5.2 units in derepressed cultures (values are averages for three transformants). For comparison, average values for repressed and derepressed wild-type transformants were 1.0 and 10 units, respectively. The SNF3-β-galactosidase hybrid protein was localized at the cell surface in *grr1* mutants as it is in wild-type cells, as judged by

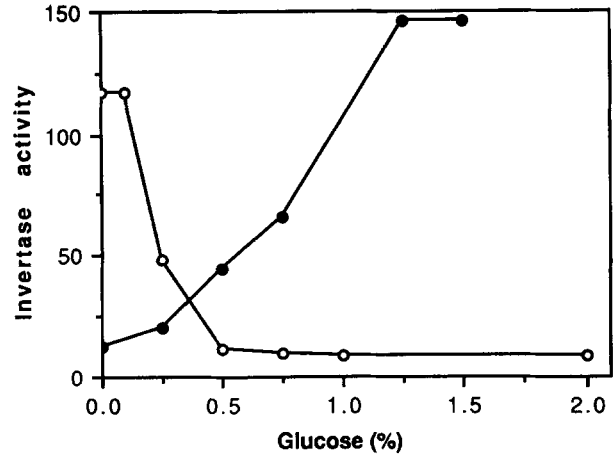

FIGURE 2.—Effect of glucose on expression of invertase in wild-type and *grr1* mutant cells. Wild-type strain MCY1094 (○) and the *grr1-512* mutant MCY2251 (●) were grown to mid-log phase in YEP-2% galactose. The cells were harvested, washed once in YEP and then resuspended in YEP-2% galactose plus glucose at the indicated concentrations. After approximately three generations of growth, cells were assayed for secreted invertase activity. Invertase activity is expressed as μmoles of glucose released/min/100 mg (dry weight) of cells.

indirect immunofluorescence microscopy (data not shown). We also crossed a *grr1* mutant (YM3502) with a *snf3* mutant (MCY1617), and tetrad analysis of the diploid indicated that the double mutant is viable (5 parental ditype: 15 tetratype: 5 nonparental ditype asci).

DISCUSSION

We report here the isolation of mutants defective in raffinose utilization. We identified four new *SNF* genes that are required for derepression of *SUC2* in response to glucose deprivation. As expected, we also recovered additional alleles of *SUC2* and previously identified *SNF* genes. We showed that a *gal11* null mutation partially impairs invertase expression, and included in our collection were five mutants with defects in *gal11*. To our surprise, we also isolated a *grr1* mutant.The *SNF7* through *SNF10* genes appear to affect derepression of invertase at least in part at the transcriptional level. The expression of β-galactosidase from a *SUC2-LEU2-lacZ* fusion was decreased in the mutants relative to wild type. In addition, Northern blot analysis confirmed that the amount of the *SUC2* mRNA encoding secreted invertase was reduced in all of the mutants (data not shown). It was not clear that transcriptional defects could quantitatively account for the reduction in invertase activity; however, that question can be addressed more easily if null mutations in these genes impair invertase expression more severely.

None of the *snf7*, *snf8*, *snf9* or *snf10* alleles causes pleiotropic defects in galactose utilization and only *snf10* affects glycerol utilization, although all affect

sporulation. It is possible that these are simply "leaky" alleles (like the original *snf6-719* allele; ESTRUCH and CARLSON 1990b) and that a null mutation would cause more severe, pleiotropic phenotypes. Alternatively, these genes may affect only a subset of glucose-repressible genes. Cloning and disruption of the genes will resolve this question.

The finding that *gal11* mutations partially impair invertase expression is consistent with previous evidence that *gal11* has broad pleiotropic effects (SUZUKI *et al.* 1988; FASSLER and WINSTON 1989). Previously, NOGI and FUKASAWA (1980) reported that *gal11* does not affect invertase activity; however, their strains had different genetic background (A364A). The GAL11 protein appears to function in transcriptional activation by GAL4 and RAP1/GRF1/TUF transcriptional activators (HIMMELFARB *et al.* 1990; NISHIZAWA *et al.* 1990). Perhaps GAL11 similarly affects the transcriptional activation of the *SUC2* gene. Alternatively, GAL11 could affect *SUC2* indirectly, possibly by stimulating expression of an activator of *SUC2*.

We undertook this mutant search to complete the identification of *SNF* genes. Although this search yielded four new *SNF* genes, it is not clear that saturation has been achieved. We recovered additional alleles of some, but not all, previously identified *SNF* genes and only one or a few alleles of the new *SNF* genes. In contrast, many *suc2* and *snf3* mutations were obtained (Table 1). A likely explanation for this disparity is that the *suc2* and *snf3* mutants are healthy on 2% glucose, whereas the other *snf* mutants are distinctly less healthy. Other potential problems in identifying all possible *SNF* genes are that some may be essential for viability and others may have functionally redundant homologs.

In this search we recovered many mutants that grew on glucose, but not raffinose, in the presence of antimycin a and yet expressed invertase at high levels. We expected to recover at least one class of Raf$^-$ mutants that are not defective in invertase expression: the *snf3* mutants, which are defective in high-affinity glucose and fructose transport. Growth on raffinose requires uptake of the low levels of fructose released by the extracellular hydrolysis of the trisaccharide. However, these mutants represent more than a few complementation groups besides *snf3*. It is possible that some of these mutants are defective in other genes required for high-affinity hexose transport or for expression of transporters. For example, KRUCKEBERG and BISSON (1990) found that the *HXT2* gene, which encodes a protein resembling sugar transporters, affects high-affinity glucose transport. Alternatively, a variety of metabolic defects could result in poor growth under conditions of limiting glucose or fructose.

The surprise of this study was the recovery of a *grr1* mutant as a Raf$^-$ mutant. Previously, *grr1* mutants were recovered in a selection for mutations that relieve glucose repression and therefore confer resistance to 2-deoxyglucose (BAILEY and WOODWORD 1984). The mutants were shown to express invertase, maltase and galactokinase when grown in media containing glucose. FLICK and JOHNSTON (1990) showed that *GRR1* acts at the transcriptional level and is required for glucose repression of the *GAL1* promoter. The unexpected phenotype of the *grr1* mutant is that glucose repression of invertase is not simply relieved; rather, invertase expression is glucose-inducible. FLICK and JOHNSTON (personal communication) have shown that *grr1* exerts its effect on invertase expression at the RNA level.

We suggest two possibilities for a mechanism by which a *grr1* mutation converts *SUC2* from a glucose-repressible gene to a glucose-inducible one. First, the *grr1* mutation may somehow switch the polarity of the response of a key regulatory element for *SUC2* expression. Second, *grr1* may effectively eliminate the mechanism responsible for glucose repression and derepression of *SUC2*, thereby unmasking a glucose-inducible response. According to this model, the glucose-inducible activation element would normally be cryptic due to negative regulation by glucose repression.

Certain mutations in the *Escherichia coli lac* repressor gene provide an interesting precedent for the first model. The *lacI^r* (*r* for reverse) mutants are partly constitutive and show increased repression in response to low concentrations of inducer (for review, see MILLER 1978). The mutant repressors bind both nonspecific DNA and the *lac* operator more tightly than the wild-type repressor. As a result, the repressor is trapped on nonspecific DNA, causing partial constitutivity for *lac* expression. Low inducer concentrations increase repression because the inducer-repressor complex retains affinity for the operator. By analogy, a mutation in *GRR1* could effectively increase the binding affinity of a negative regulator for DNA; for example, the GRR1 protein may modify a DNA-binding factor. Because of its increased affinity for nonspecific DNA, the repressor might then show specific binding to the *SUC2* negative regulatory site(s) only when its binding affinity is reduced in response to glucose starvation.

Any acceptable model must account for the finding that *grr1* affects regulation of *SUC2*, *GAL1* and *SNF3* differently. We favor the second model because it is easy to imagine that elimination of a *GRR1*-dependent regulatory mechanism would leave different regulatory components intact at these three loci. Further genetic analysis of the interactions of *grr1* with other mutations affecting glucose repression should prove helpful in elucidating the role of *GRR1* in this regulatory pathway.

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