GENES AFFECTING THE REGULATION OF *SUC2* GENE EXPRESSION BY GLUCOSE REPRESSION IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Mutants of *Saccharomyces cerevisiae* with defects in sucrose or raffinose fermentation were isolated. In addition to mutations in the *SUC2* structural gene for invertase, we recovered 18 recessive mutations that affected the regulation of invertase synthesis by glucose repression. These mutations included five new *snf1* (sucrose nonfermenting) alleles and also defined five new complementation groups, designated *snf2*, *snf3*, *snf4*, *snf5* and *snf6*. The *snf2*, *snf4* and *snf5* mutants produced little or no secreted invertase under derepressing conditions and were pleiotropically defective in galactose and glycerol utilization, which are both regulated by glucose repression. The *snf6* mutant produced low levels of secreted invertase under derepressing conditions, and no pleiotropy was detected. The *snf3* mutants derepressed secreted invertase to 10–35% the wild-type level but grew less well on sucrose than expected from their invertase activity; in addition, *snf3* mutants synthesized some invertase under glucose-repressing conditions.——We examined the interactions between the different *snf* mutations and *ssn6*, a mutation causing constitutive (glucose-insensitive) high-level invertase synthesis that was previously isolated as a suppressor of *snf1*. The *ssn6* mutation completely suppressed the defects in derepression of invertase conferred by *snf1*, *snf3*, *snf4* and *snf6*, and each double mutant showed the constitutivity for invertase typical of *ssn6* single mutants. In contrast, *snf2 ssn6* and *snf5 ssn6* strains produced only moderate levels of invertase under derepressing conditions and very low levels under repressing conditions. These findings suggest roles for the *SNF1* through *SNF6* and *SSN6* genes in the regulation of *SUC2* gene expression by glucose repression.

GLUCOSE (carbon catabolite) repression is a general regulatory system in *Saccharomyces cerevisiae* that affects the expression of a multitude of genes. The *SUC2* structural gene for invertase provides an attractive system for studying glucose repression because glucose repression appears to be the only regulatory mechanism affecting expression of *SUC2*.

S. cerevisiae is able to utilize sucrose as a carbon source by derepressing synthesis of invertase, which cleaves sucrose to yield glucose and fructose. The *SUC2* gene is the most extensively studied member of the *SUC* gene family (*SUC1-SUC5* and *SUC7*; MORTIMER and HAWTHORNE 1969; CARLSON and BOTSTEIN 1983); an individual haploid strain may contain zero, one or several *SUC*

genes in its genome. Each *SUC* gene encodes two forms of invertase: a glycosylated form that is secreted into the periplasmic space and an intracellular, nonglycosylated form (NEUMANN and LAMPEN 1967; GASCON and LAMPEN 1968; CARLSON and BOTSTEIN 1982; CARLSON *et al.* 1983). The secreted enzyme is responsible for the utilization of sucrose; the *in vivo* function of the intracellular species is unclear. Synthesis of the secreted enzyme is regulated by glucose repression, and the intracellular enzyme is synthesized constitutively at a low level.

The secreted and cytoplasmic invertases are encoded by two differently regulated *SUC2* mRNAs (CARLSON and BOTSTEIN 1982). The secreted invertase is encoded by a 1.9-kb mRNA, the stable level of which is regulated by glucose repression. The cytoplasmic enzyme is translated from a 1.8-kb mRNA which is synthesized constitutively at a low level. These two mRNAs differ at their 5′ ends; the 1.9-kb species includes a signal peptide-coding sequence and, therefore, encodes a secreted form of invertase (CARLSON *et al.* 1983; PERLMAN, HALVORSON and CANNON 1982).

Mutations preventing the expression of the *SUC2* gene have been previously isolated. The *snf1* (sucrose-nonfermenting) mutations abolish derepression of secreted invertase synthesis but do not affect synthesis of the cytoplasmic enzyme (CARLSON, OSMOND and BOTSTEIN 1981). The defect in invertase synthesis probably lies at the transcriptional level; no stable 1.9-kb mRNA is synthesized in *snf1* mutants (CARLSON and BOTSTEIN 1982). The *SNF1* gene is also required to derepress expression of other glucose-repressible genes, and *snf1* mutants are deficient in growth on other carbon sources, the utilization of which is regulated by glucose repression (CARLSON, OSMOND and BOTSTEIN 1981). The *SNF1* gene has been cloned and genetically mapped to a position distal to *rna3* on chromosome *IV* (CELENZA and CARLSON 1984a). Null mutations constructed at the chromosomal *SNF1* locus conferred the expected Snf$^-$ phenotype. The gene encodes a 2.4-kb polyadenylated mRNA that is present in both glucose-repressed and -derepressed cells (CELENZA and CARLSON 1984b).

Mutations causing constitutive synthesis of secreted invertase have also been described. The *ssn6* mutations were isolated as suppressors of a *snf1* mutation which restored capacity for growth on sucrose but not galactose or glycerol (CARLSON *et al.* 1984). Expression of the *SUC2* gene was found to be resistant to glucose repression in either *snf1 ssn6* or *SNF1 ssn6* strains, and secreted invertase was synthesized at levels as high as that of a derepressed wild-type strain. The *ssn6* mutations confer pleiotropic defects and are allelic to *cyc8*, a mutation causing overproduction of iso-2-cytochrome *c* (ROTHSTEIN and SHERMAN 1980).

Mutations in *hxk2*, the structural gene for hexokinase PII (or B) also result in glucose-insensitive synthesis of secreted invertase, maltase, galactokinase and other enzymes, and it has been suggested that this effect is due not merely to a decreased rate of glucose metabolism, but rather to a defect in a regulatory function performed by this hexokinase (ENTIAN 1980; ENTIAN and MECKE 1982; MICHELS, HAHNENBERGER and SYLVESTRE 1983). MATSUMOTO, YOSHI-

MATSU and OSHIMA (1983) have isolated mutations at the *REG1* locus that affect the glucose repressibility of galactokinase synthesis and cause some constitutive production of secreted invertase. The *hex2* and *cat80* mutations described by ENTIAN and ZIMMERMANN (1980) also cause constitutive invertase synthesis; *hex2* and *reg1* both map near the *trp1* gene (ENTIAN and ZIMMERMANN 1982).

We report here the isolation of additional mutations that affect the regulation of *SUC2* gene expression. These mutations define five new complementation groups. We have examined the interactions of these new mutations with each other and with the *snf1* and *ssn6* mutations.

MATERIALS AND METHODS

Yeast strains: All strains used in this study were isogenic or congenic to strain S288C (*MATα SUC2 gal2*), except where noted otherwise. The origins of *snf1*, *suc2*, *ade2*, *his4*, *lys2*, *GAL2* and *MAT***a** alleles have been described previously (CARLSON, OSMOND and BOTSTEIN 1981). The *ura3-52* allele was serially backcrossed into the S288C background as described by CARLSON *et al.* (1984). *SUC7* was introduced into the S288C background from strain FL100 (LACROUTE 1968) through a series of ten backcrosses. The *hxk2-2* mutation was introduced into our strains from strain F445 (*hxk1-1 hxk2-2*) by three serial backcrosses; segregants of genotype *HXK1 hxk2* were identified by their ability to utilize fructose and to secrete invertase constitutively. The genotypes and sources of strains used in this study are listed in Table 1.

Genetic methods: Standard genetic procedures of crossing, sporulation and tetrad analysis were followed (MORTIMER and HAWTHORNE 1969; SHERMAN, FINK and LAWRENCE 1978). Media and methods for scoring ability to utilize carbon sources have been described (CARLSON, OSMOND and BOTSTEIN 1981). As before, scoring for glucose, sucrose, raffinose and galactose utilization was carried out under anaerobic conditions in a GasPak disposable anaerobic system (BBL). Except in the original isolation of mutants, all scoring was determined by spotting cell suspensions onto YEP plates containing the appropriate carbon source.

Isolation of mutants: Yeast cells were mutagenized with 3% ethyl methanesulfonate as described by CARLSON, OSMOND and BOTSTEIN (1981). As before, cells were stored under conditions nonpermissive for growth prior to plating for single colonies. This precaution was taken to ensure the independence of mutants recovered in a single experiment. Surviving cells were plated for single colonies on YEP-glucose and replica plated to YEP-sucrose in the experiments with strains MCY259, MCY517 and MCY527 and YEP-raffinose medium in those with strains MCY520 and DBY782. Raffinose is a poorer substrate than sucrose for invertase, and ability to utilize raffinose proved to be a more sensitive indicator for reduced levels of secreted invertase. Putative mutants were purified and retested for ability to ferment sucrose and raffinose by spotting cell suspensions.

Complementation analysis: To test pairs of mutations for complementation, heterozygous diploids were constructed and isolated, in most cases, by prototrophic selection; when prototrophic selection could not be employed, diploids were identified following single-colony purification by testing ability to sporulate. The ability of the diploid to utilize various carbon sources was then analyzed.

Identification of nonsense mutations: Each of the *snf2* through *snf6* mutations was tested for coreversion with the *his4-539* and/or *lys2-801* amber alleles by first plating each mutant on medium selective for reversion to prototrophy and then testing revertants for growth on sucrose and raffinose. The *snf4-319* and *snf5-18* mutations reverted simultaneously with amber markers. Corevertants were crossed to *SNF* strains carrying amber alleles for tetrad analysis; the phenotypic segregations observed were consistent with the segregation of an amber suppressor able to suppress the *snf* mutation.

The *snf2-141* allele was observed to corevert frequently with the *ade2-101* ochre mutation. Such a corevertant was crossed to a *SNF2* strain carrying the *his4-86* and *lys2-802* ochre alleles. Tetrad analysis of this diploid indicated that *snf2-141* is an ochre mutation.

Assays for invertase: Preparation of glucose-repressed and -derepressed cells was as described by

TABLE 1

List of yeast strains

Strain	Genotype	Source
MCY259	*MATα lys2-801 SUC2 SUC7*	This laboratory
MCY517	*MATa lys2-801 his4-539 ura3-52 SUC2 SUC7*	This work
MCY520	*MATa lys2-801 his4-539 ura3-52 ade2-101 SUC2 SUC7*	This work
MCY527	*MATα lys2-801 his4-539 ade2-101 SUC2 SUC7*	This work
DBY782	*MATα ade2-101 SUC2 gal2*	D. BOTSTEIN
F445	*MATα ade1 his2 trp1 met14 suc° hxk1-1 hxk2-2 GLK1*	G. R. FINK

CELENZA and CARLSON (1984a). Repressed cells were grown to exponential phase (Klett 50, measured with a Klett-Summerson colorimeter using a green filter) in YEP medium containing 2% glucose, and derepressed cells were prepared by shifting repressed cells to YEP medium containing 0.05% glucose for 2.5 hr. In the case of clumpy yeast cultures cell density was determined by measuring dry weight as described by CARLSON *et al.* (1984).

For the gel assay, cell extracts were prepared and the two forms of invertase were separated by electrophoresis on a 5.5% polyacrylamide gel as described by CARLSON, OSMOND and BOTSTEIN (1981). Invertase activity was detected *in situ* by the staining procedure of GABRIEL and WANG (1969). Extracellular invertase activity was quantitatively assayed in whole cells using the method of GOLDSTEIN and LAMPEN (1975) as described by CELENZA and CARLSON (1984a).

Assay for galactokinase: Cells were grown in YEP medium containing 2% galactose, or 2% galactose and 2% glucose, and harvested in exponential phase. Crude extracts were prepared by vortexing cells with glass beads (0.45 mm diameter), and assays were then carried out as described by NOGI *et al.* (1977).

Construction of double mutants: Pairwise heterozygous diploids were constructed by selecting for prototrophy. Diploids were sporulated and four-spored asci were dissected. Complete tetrads were tested for genetic markers as well as for sucrose, raffinose, galactose and glycerol utilization. The genotypes of double mutants were confirmed by complementation analysis: complementation of *snf1* through *snf6* was judged by testing diploids for ability to utilize sucrose and/or raffinose, and complementation of *ssn6* and *hxk2* was determined by assaying glucose-repressed diploids for invertase.

RESULTS

Isolation of mutants: Mutants unable to grow anaerobically on sucrose or raffinose (another substrate of invertase), but able to grow on glucose, were isolated from *SUC2* or *SUC2 SUC7* strains as described in MATERIALS AND METHODS. These nonfermenting mutants were expected to lack or have decreased secreted invertase activity. Strains carrying two structural genes for invertase, *SUC2* and *SUC7*, were used to reduce the frequency of recovering mutants in which the defect in fermentation resulted from a lesion in an invertase structural gene.

To test for dominance, each mutant was crossed to a wild-type *SUC2* strain. In each case the resulting diploid was able to ferment raffinose, indicating that the mutations are recessive. To determine whether the fermentation defect resulted from a single nuclear mutation, these diploids were subjected to tetrad analysis. Only those mutants in which ability to ferment sucrose or raffinose segregated 2+:2− were retained for further characterization. Thirty-one such mutants were recovered among approximately 30,000 colonies screened.

TABLE 2

Phenotypes of mutants

Mutant allele[a]	Parent strain	Carbon source utilization					Secreted invertase activity (μmol glucose released/min/100 mg dry weight of cells)	
		Glu	Suc	Raf	Gal[b]	Gly	Repressed	Derepressed
snf1-77	MCY517	+	−	−	−	−	<1	<1
snf1-78	MCY520	+	−	−	−	−	ND	ND
snf1-90	MCY520	+	−	−	−	−	ND	ND
snf1-413	MCY520	+	−	−	−	−	ND	ND
snf1-423	DBY782	+	−	−	−	−	ND	ND
snf2-50	MCY259	+	+/−	−	−	−	<1	3
snf2-141	DBY782	+	+/−	−	−	−	<1	4
snf3-39	MCY527	+	−	−	+	+	40	70
snf3-72	MCY520	+	−/+	−	+	+	14	50
snf3-112	DBY782	+	−/+	−	ND	+	5	70
snf3-121	DBY782	+	+/−	−	ND	+	4	60
snf3-142	DBY782	+	+/−	−	+	+	7	40
snf3-217	DBY782	+	+/−	−	ND	+	5	25
snf3-318	DBY782	+	+/−	−	+	+	2	60
snf4-319	DBY782	+	−/+	−	−	−	<1	1
snf5-18	DBY782	+	+/−	−	−	−	<1	4
snf5-815	DBY782	+	+/−	−	−	−	<1	6
snf6-719	DBY782	+	+	−	+	+	<1	20
Wild type	DBY782	+	+	+	+	+	<1	200

Glu, glucose; Suc, sucrose; Raf, raffinose; Gal, galactose; Gly, glycerol; +, growth in 1 day; +/−, growth in 2 days; −/+, growth in 3 days; −, no growth; ND, not determined.

[a] Mutations were assigned to complementation groups on the basis of complementation tests with the following alleles: *snf1-28*, *snf2-141*, *snf3-39*, *snf3-318*, *snf4-319*, *snf5-18*, and *snf6-719*.

[b] For those mutations isolated in DBY782 (*gal2*), growth on galactose was tested after crossing in a *GAL2* allele.

Complementation analysis: To identify *suc2* mutations, we tested all mutations isolated in DBY782 (*SUC2*) for ability to complement the *suc2-215* amber allele. Thirteen new *suc2* alleles were identified, all of which conferred defects in utilization of both sucrose and raffinose. Because the *SUC7* gene does not confer ability to utilize raffinose, a mutant of genotype *suc2 SUC7* would be able to ferment sucrose but not raffinose. Therefore, mutations isolated in *SUC2 SUC7* strains and conferring this phenotype were also tested for ability to complement *suc2-215*; however, no *suc2* mutations were recovered.

Mutations were also analyzed for their ability to complement a *snf1* mutation for growth on raffinose, and five new *snf1* mutations were identified (Table

2). These new *snf1* mutants were unable to utilize sucrose, raffinose, galactose or glycerol, as was found for the five *snf1* mutants previously isolated by CARLSON, OSMOND and BOTSTEIN (1981).

The remaining 13 mutations were then tested for ability to complement one another. Five additional complementation groups were identified and designated *snf2*, *snf3*, *snf4*, *snf5* and *snf6* (Table 2). The name *snf* (sucrose nonfermenting) is used loosely here; although all these mutants exhibited decreased levels of secreted invertase activity, many of them were capable of slow growth on sucrose. All except the *snf6* mutant showed reduced growth relative to wild type on sucrose (Table 2). None of the mutants were able to utilize raffinose as a carbon source. The *snf2-141* mutation was identified as an ochre mutation, and *snf4-319* and *snf5-18* were identified as amber mutations (see MATERIALS AND METHODS).

Linkage studies of snf2, snf3, snf4, snf5 *and* snf6: Analyses of crosses including the centromere-linked marker *ura3-52* and representative alleles of *snf2*, *snf3*, *snf4*, *snf5* and *snf6* showed that a majority of the tetrads were tetratype, indicating that none of these genes is tightly linked to a centromere. No tight linkage to *lys2*, *his4* or *ade2* was detected. Furthermore, none of the *snf* mutations are tightly linked to each other or to *snf1* or *ssn6* because segregants containing both mutations were frequently recovered from diploids heterozygous for all pairwise combinations.

Secreted invertase activity in new mutants: Secreted invertase was assayed in *snf2*, *snf3*, *snf4*, *snf5* and *snf6* strains grown under glucose-repressing and -derepressing conditions (Table 2). The *snf2*, *snf5* and *snf6* strains contained no secreted invertase when repressed but produced low levels of secreted invertase upon derepression. Because sucrose is a better substrate for invertase than is raffinose, it seemed likely that the limited growth of these mutants on sucrose but not raffinose resulted from the synthesis of low levels of secreted invertase. The *snf4* mutant produced no detectable secreted invertase activity when repressed and only a barely detectable level when derepressed. Strains carrying the *snf3* alleles proved to be more variable in phenotype. All of the *snf3* mutants produced some invertase activity under derepressing conditions, ranging from 10 to 35% that of wild type, and also exhibited significant activity under repressing conditions, ranging from 1 to 20% that of a derepressed wild type (Table 2). The ability of a given *snf3* mutant to utilize sucrose did not, however, reflect its ability to derepress secreted invertase. This discrepancy will be addressed in more detail.

snf *mutants synthesize cytoplasmic invertase*: Representative mutants from each of the new *snf* complementation groups were assayed for the presence of cytoplasmic invertase following growth in derepressing medium. The secreted, glycosylated invertase and the cytoplasmic, nonglycosylated forms were separated by electrophoresis on a polyacrylamide gel and then detected by staining the gel for activity (GABRIEL and WANG 1969; CARLSON, OSMOND and BOTSTEIN 1981). Although this assay is not quantitative, the nonglycosylated form was present in approximately normal amounts in mutants carrying *snf2-50*, *snf3-39*, *snf3-217*, *snf4-319*, *snf5-18* and *snf6-719* (data not shown).

snf2, snf4 *and* snf5 *mutations are pleiotropic*: Because sucrose utilization in

yeast is regulated by glucose repression, it seemed possible that some of the new *snf* mutations might, like *snf1*, cause general defects in glucose repression. To test this possibility, mutants were assayed for their ability to grow on galactose and glycerol; utilization of these two carbon sources is subject to glucose repression. The *snf2*, *snf4* and *snf5* strains did not grow on either galactose or glycerol, indicating that these mutations are pleiotropic (Table 2). The *snf3* and *snf6* mutants, in contrast, showed normal growth on both carbon sources. The *snf3-217*, *snf3-39* and *snf6-719* mutants were assayed for galactokinase activity (NOGI *et al.* 1977) following growth on galactose, and the enzyme was induced to levels within two-fold that of wild type; no induction was observed when the cells were grown on medium containing glucose in addition to galactose (data not shown).

Genetic properties and growth phenotypes of snf3 *mutants*: A discrepancy was noted between the levels of secreted invertase present in derepressed *snf3* mutants and their ability to utilize sucrose. The most striking example is the *snf3-39* mutant. The invertase produced by derepressed *snf3-39* strains (35% that of wild type) should have been sufficient to sustain growth on sucrose; yet, these mutants were sucrose nonfermenters. Other *snf3* mutants grew much more slowly on sucrose than would be expected from their secreted invertase activity.

To investigate this paradox, the properties of the *snf3-39* mutation were studied further. In three serial backcrosses of the *snf3-39* mutant to wild type, the sucrose- and raffinose-nonfermenting segregants assayed were constitutive producers of secreted invertase. Dominance tests showed that the *snf3-39* allele was recessive to *SNF3*, *snf3-318*, *snf3-217* and *snf3-142* with respect to the level of constitutivity and ability to ferment sucrose. The possibility that sucrose is toxic to a *snf3-39* mutant was tested by comparing the growth of the mutant on medium containing 0.1% glucose with its growth on medium containing 0.1% glucose and 2% sucrose; no difference was observed.

An explanation for the defect in sucrose utilization was suggested by the observation that the mutant with the highest constitutive enzyme level, *snf3-39*, was also the most defective in growth on sucrose (Table 2). Constitutivity could result from a defect in utilization of glucose, and the unexpected severity of the defect in sucrose fermentation could result from a defect in utilization of the low levels of glucose and fructose resulting from limited sucrose hydrolysis. Although all *snf3* mutants appeared to grow normally on plates containing 2% glucose, perhaps they, nonetheless, were inefficient in their use of glucose. To test this hypothesis, we determined the growth rates of *snf3* mutants in medium containing 2 or 0.05% glucose (Table 3). The two strains with the highest levels of constitutive invertase synthesis (*snf3-39* and *snf3-72*) grew markedly slower than the wild type on 0.05% glucose and slightly slower on 2% glucose.

The *snf3-39* and *snf3-217* mutants were also tested for their ability to derepress secreted invertase during growth on rich medium containing 2% galactose as the carbon source. No invertase activity was detected, whereas the wild type produced about 70% as much activity as under our usual derepressing conditions (data not shown).

TABLE 3

Growth rates of snf3 *mutants*

Genotype[a]	Doubling time (hr)	
	0.05% Glucose	2% Glucose
snf3-39	4.4	1.8
snf3-72	3.4	1.6
snf3-142	2.7	1.5
snf3-217	2.8	1.5
snf3-318	2.6	1.5
SNF3$^+$	2.6	1.5

Cells from a freshly growing culture in YEP-2% glucose were diluted into YEP-2% glucose or collected by centrifugation, resuspended in sterile water and then diluted into YEP-0.5% glucose. These cultures were incubated at 30° with rotary shaking. Growth was monitored using a Klett-Summerson photoelectric colorimeter equipped with a green filter, and doubling times were estimated.

[a] The mutant strains used in this experiment were segregants derived from backcrosses of the original mutant to wild type. The number of backcrosses done in each case was as follows: *snf3-39*, 3; *snf3-72*, 1; *snf3-142*, 1; *snf3-217*, 2; *snf3-318*, 2.

Interactions between ssn6 *and new* snf *mutations:* The *ssn6* mutations were originally isolated as suppressors of *snf1* by CARLSON *et al.* (1984). These mutations not only suppress the sucrose and raffinose utilization defects conferred by *snf1* but also cause constitutive high-level synthesis of secreted invertase in either a *snf1* or *SNF1* background. To test the possibility that *ssn6* would suppress *snf2*, *snf3*, *snf4*, *snf5* or *snf6* mutations, we constructed double mutants and tested their growth properties and synthesis of invertase. The *ssn6* mutation suppressed the defect in raffinose fermentation, but not the defects in galactose and glycerol utilization, conferred by *snf2-50* and *snf2-141*; however, although the secreted invertase activity detected in derepressed double mutants was higher than that of the *snf2* parents, it was still much lower than that of the *ssn6* parent (Table 4). Low levels of activity were found in glucose-repressed cells. In addition, *snf2* suppressed the extreme clumpy phenotype associated with *ssn6* mutants, and the double mutants displayed only a slight tendency to aggregate. The phenotype of the *snf5 ssn6* double mutants was indistinguishable from that of *snf2 ssn6* strains.

The *ssn6* mutation also suppressed the sucrose and raffinose fermentation defects caused by *snf3-39*, *snf3-142*, *snf4-319* and *snf6-719* but not the defects in galactose and glycerol utilization of *snf4*. Moreover, like the *ssn6* parent, the double mutants synthesized secreted invertase constitutively at high levels (Table 4). The *snf3 ssn6* and *snf4 ssn6* strains displayed the clumpy phenotype characteristic of *ssn6* mutants, but the *snf6 ssn6* strains showed a markedly reduced propensity to aggregate.

Interactions between snf *mutations*: Strains carrying pairwise combinations of *snf1*, *snf2*, *snf3*, *snf4*, *snf5* and *snf6* were constructed and tested for growth on different carbon sources and for production of secreted invertase (Table 5).

TABLE 4

Phenotypes of ssn6 snf *double mutants*

Relevant genotype[a]	Growth		Clumpiness	Secreted invertase activity (μmol glucose released/min/100 mg dry weight of cells)	
	Suc	Raf		Repressed	Derepressed
Wild type	+	+	No	<1	200
ssn6-1	+	+	Yes	300	380
snf1-28 ssn6-1	+	+	Yes	200	270
snf2-50 ssn6-1	+	+/−	No[b]	10	80
snf2-141 ssn6-1	+	+/−	No[b]	15	50
snf3-39 ssn6-1	+	+	Yes	360	470
snf3-142 ssn6-1	+	+	Yes	300	380
snf4-319 ssn6-1	+	+	Yes	310	360
snf5-18 ssn6-1	+	+/−	No[b]	15	100
snf6-719 ssn6-1	+	+	No[b]	200	240

+, Growth in 2 days; +/−, growth in 3 days; Suc, sucrose; Raf, raffinose.

[a] Two strains of each genotype were assayed and in all cases nearly identical results were obtained with both strains.

[b] Not clumpy but aggregated more than wild type.

TABLE 5

Secreted invertase activity in strains carrying mutations in two SNF *genes*

Relevant genotype	Secreted invertase activity (μmol glucose released/min/100 mg dry weight of cells)		Epistatic mutation
	Repressed	Derepressed	
Wild type	<1	200	
snf1-28 snf2-50	<1	<1	*snf1*
snf1-28 snf3-39	<1	<1	*snf1*
snf1-28 snf4-319	<1	<1	NC
snf1-28 snf5-18	<1	<1	*snf1*
snf1-28 snf6-719	<1	<1	*snf1*
snf2-50 snf3-142	<1	2	*snf2*
snf2-141 snf3-318	<1	3	*snf2*
snf2-141 snf4-319	<1	<1	*snf4*
snf2-141 snf5-18	<1	10	NC
snf2-141 snf6-719	<1	4	*snf2*
snf3-318 snf4-319	<1	<1	*snf4*
snf3-142 snf5-18	<1	20	NC
snf3-217 snf6-719	2	15	NC
snf4-319 snf5-18	<1	<1	*snf4*
snf4-319 snf6-719	<1	<1	*snf4*
snf5-18 snf6-719	<1	5	*snf5*

NC, No conclusion can be drawn from the data.

None of the double mutants grew on raffinose. The double mutants carrying either *snf1* or *snf4* in combination with any other *snf* mutation resembled the *snf1* or *snf4* single mutant in their failure to produce any secreted invertase.

TABLE 6

Secreted invertase activity of hxk2 snf *double mutants*

Relevant genotype	Secreted invertase activity (μmol glucose released/min/100 mg dry weight of cells)	
	Repressed	Derepressed
Wild type	<1	200
hxk2-2	80	180
snf1-28 hxk2-2	<1	2
snf2-50 hxk2-2	<1	3
snf3-39 hxk2-2	70	190

With one exception, none of the double mutants carrying a *snf3* allele produced secreted invertase under glucose-repressing conditions; the exceptional case, the *snf3 snf6* strains, carried a *snf6* allele that appeared leaky in single mutants.

One observation worth noting is that, although *snf2-50 snf3-142* strains appeared to grow normally on glucose, the *snf2-141 snf3-318* strains grew very poorly. Moreover, attempts to construct a *snf2-50 snf3-39* double mutant were unsuccessful; the only complete tetrad was parental ditype, and no spores among ten triads and two dyads carried both mutant alleles. For each inviable spore we inferred a genotype of *snf2 snf3* from the genotypes of the viable spores, which were determined by complementation tests. Similar problems with spore inviability were encountered in our attempt to construct a *snf2-141 snf3-39* strain.

Interactions between hxk2 *and* snf1, snf2 *and* snf3: We examined the epistasis relationships between the *hxk2* mutation, which leads to constitutive synthesis of secreted invertase (ENTIAN 1980; ENTIAN and MECKE 1982; MICHELS, HAHNENBERGER and SYLVESTRE 1983) and the *snf1*, *snf2* and *snf3* mutations. Strains of genotype *snf1 hxk2* and *snf2 hxk2* were defective in production of secreted invertase activity under repressing and derepressing conditions (Table 6). The *snf3-39 hxk2* double mutants produced invertase under repressing and derepressing conditions, as did both the *snf3-39* and *hxk2* parents but, like their *snf3* parent, were defective for growth on sucrose and raffinose. The *snf3 hxk2* strains grew on fructose as well as on glucose, suggesting that *snf3* is not allelic to *hxk1*.

DISCUSSION

We have isolated 31 recessive mutations that conferred defects in utilization of sucrose or raffinose. Eighteen mutations affected the regulation of *SUC2* gene expression by glucose repression. These mutations included five new alleles of the previously identified *SNF1* gene (CARLSON, OSMOND and BOTSTEIN 1981) and also defined five new complementation groups: *snf2*, *snf3*, *snf4*, *snf5* and *snf6*. The remaining 13 mutations proved to be lesions in the *SUC2* structural gene for invertase.

The *snf2*, *snf4* and *snf5* mutants produced normal levels of the intracellular invertase but synthesized little or no secreted invertase under conditions that normally allow full derepression. Mutations in these three genes also conferred pleiotropic defects in galactose and glycerol utilization. The recovery of mutations that were phenotypically suppressed by nonsense suppressors indicates that the *SNF2*, *SNF4* and *SNF5* genes encode proteins.

The *snf3* mutants were unable to derepress fully the secreted invertase; derepressed values ranged from 10 to 35% the wild-type value, depending on the *snf3* allele. Two *snf3* mutants were also tested for their ability to derepress invertase during growth in medium containing galactose as the carbon source and showed no derepression; under these conditions wild type produced 70% as much activity as when derepressed in low glucose. The *snf3* mutants also exhibited constitutive (*i.e.*, glucose-insensitive) production of secreted invertase, at levels ranging from 1 to 20% that of the derepressed wild type. These mutants showed decreased ability to utilize sucrose relative to that expected from their enzyme levels. The two mutants with the highest constitutive invertase activity also grew significantly more slowly than wild type in medium containing a low glucose concentration. We suggest that the *snf3* mutations cause not only a defect in derepressing invertase synthesis but also a defect in glucose (and fructose) uptake or metabolism. Such an additional defect would account for the reduced growth rates on low glucose, the constitutive invertase synthesis, and the disproportionate loss of ability to utilize sucrose relative to the defect in derepression of invertase (limited sucrose hydrolysis would result in low levels of glucose and fructose). In bacteria, proteins of the phosphoenolpyruvate-sugar phosphotransferase system are involved both in glucose uptake and in regulating the activity of adenylate cyclase, which produces the cAMP required to derepress expression of glucose-repressible genes (POSTMA 1982).

No pleiotropic defects in utilization of galactose or glycerol or in regulation of galactokinase activity were detected in *snf3* mutants; however, these results do not necessarily indicate that the role of the *SNF3* gene in glucose repression is specific to regulation of the *SUC2* gene. All of the *snf3* alleles allowed partial derepression of invertase synthesis and may be sufficiently leaky that some glucose-repressible genes are unaffected.

The *snf6* mutant derepressed secreted invertase to a level 10% that of wild type. This limited derepression may simply reflect leakiness of the single *snf6* allele that we have isolated. No pleiotropy was detected.

To gain insight into the roles played by the different genes in glucose repression, we studied the interactions between the *snf* mutations and *ssn6*, a mutation that causes constitutive high-level synthesis of secreted invertase and suppresses the sucrose-nonfermenting phenotype of *snf1* (CARLSON *et al.* 1984). The *ssn6* mutation suppressed the defects in derepression of invertase conferred by *snf3*, *snf4* and *snf6* and caused constitutive high-level synthesis of invertase in the double mutants. These epistatic relationships suggest that the *SNF1*, *SNF3*, *SNF4* and *SNF6* gene products function to prevent the repressive effect of *SSN6* in response to conditions of limited glucose availability.

In contrast, the *snf2* and *snf5* mutations suppressed the constitutivity for invertase conferred by *ssn6*, and *ssn6* partially suppressed the defects in derepression of invertase conferred by *snf2* and *snf5*. The *snf2 ssn6* and *snf5 ssn6* double mutants produced only low levels of invertase under repressing conditions and moderate levels of invertase under derepressing conditions. These results indicate that functional *SNF2* and *SNF5* gene products are required for high-level *SUC2* gene expression even when *SSN6* is defective. This finding suggests that the *SNF2* and *SNF5* products act as positive regulators to derepress *SUC2* gene expression in a different manner than do the *SNF1*, *SNF3*, *SNF4* and *SNF6* products. These results also suggest that *SNF2* and *SNF5* play roles antagonistic to that of *SSN6*: the *snf2 ssn6* and *snf5 ssn6* double mutants display a more normal phenotype than the *snf2*, *snf5* or *ssn6* single mutants. It is possible that an as yet unidentified gene product acts directly as a positive regulator of *SUC2* gene expression, and that *SNF2* and *SNF5* only modulate its activity; in the absence of a functional *SSN6* gene product, the requirement for *SNF2* and *SNF5* may be relaxed. Evidence in support of the idea that a positive regulatory factor acts directly to affect *SUC2* gene expression comes from a deletion analysis showing that a region located approximately 400–500 base pairs 5′ to the *SUC2* coding region is required for derepression of *SUC2* gene expression (L. SAROKIN and M. CARLSON 1984).

Another possible explanation for the invertase activity detected in the *snf2*, *snf5*, *snf2 snf5*, *snf2 ssn6* and *snf5 ssn6* strains is simple leakiness of the mutations. This explanation is not compelling in the cases of the *snf2-141 ssn6-1* and *snf5-18 ssn6-1* strains, which produced significant invertase activity under derepressing conditions, because *snf2-141* and *snf5-18* are nonsense mutations. Although the *ssn6-1* allele is not known to be a nonsense mutation, its phenotype is identical with those of our other two alleles, *ssn6-2* and *ssn6-3*, with respect to the invertase activity present in glucose-repressed and -derepressed mutants (L. NEIGEBORN and M. CARLSON, unpublished results).

The *SSN6* product may exert its repressive effect either by preventing the synthesis or activity of positively acting factors or by binding directly to *SUC2* regulatory sequences. We favor an indirect mode of action for *SSN6* because extensive deletion analysis of the noncoding region 5′ to *SUC2* has provided no evidence for a repressor binding site (L. SAROKIN and M. CARLSON 1984). The possibility that the *SSN6* product interacts directly with a positive regulator is analogous to models proposed for regulation of the galactose utilization genes and the phosphatase genes (OSHIMA 1982) and for general amino acid control in yeast (HINNEBUSCH and FINK 1983).

These studies have implications for the general regulation of gene expression by glucose repression in *S. cerevisiae*. The *snf1*, *snf2*, *snf4* and *snf5* mutations confer pleiotropic defects in utilization of galactose and glycerol, which are glucose repressible. The *SNF3* and *SNF6* genes may also be involved in general regulation and the leakiness of our alleles simply prevented detection of pleiotropy. In contrast, *ssn6* does not suppress the defects in growth on galactose and glycerol caused by *snf1* and *snf4*, nor does it cause high-level synthesis of galactokinase under glucose-repressing conditions (L. NEIGEBORN and M.

CARLSON, unpublished results). Thus, the *SSN6* gene appears to affect only a subset of the glucose-repressible genes.

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