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SUPPRESSORS OF *SNF2* MUTATIONS RESTORE INVERTASE DEREPRESSION AND CAUSE TEMPERATURE-SENSITIVE LETHALITY IN YEAST

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ABSTRACT

Mutations in the *SNF2* gene of *Saccharomyces cerevisiae* prevent derepression of the *SUC2* (invertase) gene, and other glucose-repressible genes, in response to glucose deprivation. We have isolated 25 partial phenotypic revertants of a *snf2* mutant that are able to derepress secreted invertase. These revertants all carried suppressor mutations at a single locus, designated *SSN20* (suppressor of *snf2*). Alleles with dominant, partially dominant and recessive suppressor phenotypes were recovered, but all were only partial suppressors of *snf2*, reversing the defect in invertase synthesis but not other defects. All alleles also caused recessive, temperature-sensitive lethality and a recessive defect in galactose utilization, regardless of the *SNF2* genotype. No significant effect on *SUC2* expression was detected in a wild-type (*SNF2*) genetic background. The *ssn20* mutations also suppressed the defects in invertase derepression caused by *snf5* and *snf6* mutations, and selection for invertase-producing revertants of *snf5* mutants yielded only additional *ssn20* alleles. These findings suggest that the roles of the *SNF2*, *SNF5* and *SNF6* genes in regulation of *SUC2* are functionally related and that *SSN20* plays a role in expression of a variety of yeast genes.

EXPRESSION of the *SUC2* (invertase) gene of *Saccharomyces cerevisiae* is regulated by glucose (carbon catabolite) repression. The *SUC2* gene offers a convenient system for studying glucose repression because the gene is not also inducible by sucrose or other substrates of invertase. The regulation of secreted invertase synthesis has been shown to occur at the mRNA level; the 1.9-kb mRNA encoding secreted invertase is produced only when cells are deprived of glucose (derepressing conditions) (CARLSON and BOTSTEIN 1982). In addition to the regulated 1.9-kb mRNA, the *SUC2* gene also produces constitutively a minor, 1.8-kb species of mRNA with a different 5′ end (CARLSON *et al.* 1983); this mRNA encodes an intracellular form of invertase that appears to play no role in sucrose utilization (SAROKIN and CARLSON 1984). An upstream regulatory region that is required for regulated expression of secreted invertase has been identified (SAROKIN and CARLSON 1984) and shown to confer regulated expression to a heterologous gene (SAROKIN and CARLSON 1985a).

To identify genes involved in regulation by glucose repression, we have previously isolated mutants defective in derepression of secreted invertase. Six genes required for normal derepression were identified, *SNF1* through *SNF6* (sucrose nonfermenting) (CARLSON, OSMOND and BOTSTEIN 1981; NEIGEBORN and CARLSON 1984). The *snf1*, *snf2*, *snf4* and *snf5* mutations almost completely prevent secreted invertase synthesis and cause defects in utilization of other carbon sources that are subject to glucose repression. The *snf3* and *snf6* mutations allow some invertase derepression, but in the case of *snf6* we have only one allele, which may be leaky. The effects of these *SNF* genes on *SUC2* expression appear to be mediated by the upstream regulatory region as *snf* mutations were found to affect the expression of a heterologous gene under the control of the *SUC2* upstream region (SAROKIN and CARLSON 1985a).

We previously isolated suppressors of a *snf1* mutation that fell into eight complementation groups, called *ssn1–ssn8* for suppressor of *snf1* (CARLSON *et al.* 1984). The *ssn6* mutations were found to cause high-level constitutive secreted invertase synthesis in a wild-type (*SNF*) background. The interactions between *ssn6* and all of the *snf* mutations were examined, and *ssn6* suppressed the defects in invertase derepression caused by *snf1–snf6*; however, the *snf1 ssn6* and *snf4 ssn6* double mutants displayed the high-level constitutivity of an *ssn6* single mutant, whereas the *snf2 ssn6* and *snf5 ssn6* strains resembled the wild type more closely than either single mutant parent (NEIGEBORN and CARLSON 1984). These findings suggested that *SNF2* and *SNF5* play different roles from *SNF1* and *SNF4* in regulation by glucose repression and that *SNF2* and *SNF5* may act antagonistically to *SSN6*.

To explore further the regulatory role of *SNF2*, we have isolated here partial suppressors of *snf2* that relieve the defect in invertase derepression. Both dominant and recessive suppressors were recovered, and all of them were found to be recessive temperature-sensitive lethal mutations defining a single complementation group (*ssn20*). These mutations also suppressed *snf5*, and direct selection for suppressors of *snf5* yielded only more alleles of the same gene.

MATERIALS AND METHODS

Yeast strains: All strains used in this study were isogenic or congenic to strain S288C (*MATα SUC2 gal2*). The origins of all alleles except *snf4-Δ1* and *snf5-5::URA3* have been previously described (CARLSON, OSMOND and BOTSTEIN 1981; CARLSON *et al.* 1984; NEIGEBORN and CARLSON 1984). The *snf4-Δ1* allele is a deletion of part of the *SNF4* gene (F. ENG and M. CARLSON, unpublished results), and *snf5-5::URA3* is an insertion of the *URA3* gene into *SNF5* (E. ABRAMS and M. CARLSON, unpublished results). The *SUC7* gene was introduced into the S288C background from strain FL100 (LACROUTE 1968) through a series of ten backcrosses; *SUC7*, like *SUC2*, is regulated by glucose repression, but produces tenfold lower invertase activity than *SUC2* in this genetic background, which is insufficient to confer a raffinose-fermenting phenotype (SAROKIN and CARLSON 1985b). The presence of *SUC7* in some of these strains, therefore, is not relevant. The strains used to isolate revertants and their genotypes are as follows: MCY637 (*MATα snf2-50 his4-539 lys2-801 ura3-52 SUC2 SUC7*); MCY1947 (*MATα snf5-5::URA3 his4-539 ade2-101 SUC2*); MCY1949 (*MAT***a** *snf5-5::URA3 his4-539 lys2-801 SUC2*).

Genetic methods: Standard genetic procedures of crossing, sporulation and tetrad analysis were followed (SHERMAN, FINK and LAWRENCE 1978). Media and methods for

scoring ability to utilize carbon sources have been described (CARLSON, OSMOND and BOTSTEIN 1981). Scoring for glucose, sucrose, raffinose and galactose utilization was carried out under anaerobic conditions in a Gas Pak disposable system (BBL) or by addition of antimycin A (Sigma) to the medium at a final concentration of 1 μg/ml. 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: Single colonies (10^7 cells) were suspended in water and were spread onto rich medium (YEP) containing 2% raffinose as a carbon source. Cells were then exposed to 100 J/m^2 of ultraviolet radiation; 20% of the cells remained viable. Revertants were selected by incubating the plates anaerobically at 30° for 5 days. Revertants were recovered from seven *snf2* single colonies and five *snf5* single colonies; however, it is likely that all revertants were independent, because mutagenesis stimulated the frequency of reversion by more than 1000-fold. Putative mutants were purified by isolation of single colonies and were tested.

Complementation analysis: To test pairs of mutations for complementation, heterozygous diploids were constructed and isolated by prototrophic selection. The ability of the diploid to utilize raffinose and/or grow at 37° was then determined.

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 carbon source utilization and ability to grow at 37°. The *snf* genotypes of double mutants were confirmed by complementation analysis. For use in these crosses, a *ssn20-6 SNF2 SUC2* segregant lacking the *SUC7* gene [that is, carrying the *suc7°* allele (CARLSON and BOTSTEIN 1983)] was identified by blot hybridization analysis (SOUTHERN 1975); *Eco*RI restriction fragments characteristic of the *SUC7* and *suc7°* loci were detected (CARLSON, CELENZA and ENG 1985).

Assay for invertase: Preparation of glucose-repressed and derepressed cells was as described by CELENZA and CARLSON (1984). Repressed cells were grown to exponential phase (Klett=50) 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). Extracellular invertase activity was quantitatively assayed in whole cells using the method of GOLDSTEIN and LAMPEN (1975), as described by CELENZA and CARLSON (1984).

Assay for β-galactosidase: Cells carrying the *GAL10-lacZ* fusion plasmid pRY123 (WEST, YOCUM and PTASHNE 1984) were grown in supplemented minimal medium (SD) with selection for the plasmid marker *URA3*. Galactose-induced cells were prepared by growth to exponential phase in medium containing 2% galactose and 3% glycerol, and repressed cells were prepared by growth in 2% glucose, 2% galactose and 3% glycerol. Cells were permeabilized by treatment with SDS and chloroform and were assayed for β-galactosidase as described by MILLER (1972).

RESULTS

Isolation of revertants of a *snf2* mutant: Partial phenotypic revertants of a *snf2-50* mutant strain, MCY637, were isolated by subjecting cells to UV mutagenesis and then selecting for ability to form colonies anaerobically on medium containing raffinose as the carbon source. Raffinose-utilizing revertants were obtained at a frequency of approximately 2×10^{-5}.

Twenty-five revertants were colony purified and tested for their ability to utilize a spectrum of carbon sources. The *snf2* parent strain utilizes glucose and sucrose, but not raffinose, galactose or glycerol. All revertants were able to grow on glucose, sucrose and raffinose, as expected, but none was able to utilize galactose or glycerol. Thus, all of the revertants showed only partial

phenotypic reversal of the *snf2* defect. The revertants were also tested for growth on glucose at a range of temperatures; all grew as well as the *snf2* parent strain at 23° and 30°, but were unable to grow at 37°. This temperature-sensitive phenotype was found in subsequent experiments (see below) to be independent of the *SNF2* genotype.

Synthesis of invertase by revertants: The inability of *snf2* mutants to utilize raffinose results from a defect in derepression of secreted invertase. To determine whether the defect in derepression was remedied in the revertants, each was assayed for secreted invertase activity after growth under derepressing conditions. All showed significantly higher levels of enzyme activity than did the parent *snf2* strain, which produces 3 units (wild type produces 200 units); values ranged from 14 to 125 units, and all except three revertants gave values greater than 25 (Table 1). When grown under glucose-repressing conditions, none of the revertants produced more than 10% the derepressed level of invertase activity; repressed values ranged from 1 to 7 units.

Dominance tests: To test for dominance, each revertant was mated to a *snf2-50* strain, and the resulting diploids (homozygous for *snf2* and heterozygous for the suppressor mutation) were tested for both temperature sensitivity and suppression of *snf2*. In each case the diploid was able to grow at 37°, indicating that the temperature-sensitivity defect of the revertants was recessive. The diploids were also assayed for ability to derepress secreted invertase (Table 1). The suppressor mutations fell into three classes. Six proved to be dominant suppressors of the *snf2* defect in invertase derepression; in each case the heterozygous diploid showed ability to derepress secreted invertase comparable to that of the corresponding haploid revertant. Two mutations (subsequently designated *ssn20-5* and *ssn20-6*) displayed a partially dominant phenotype; the diploids produced 40% and 50%, respectively, as much secreted invertase activity as the corresponding haploids. Fifteen mutations were recessive suppressors, and the diploids were unable to synthesize significant secreted invertase. Three of these mutations (*ssn20-19*, *ssn20-21* and *ssn20-28*) appeared to be incompletely recessive; however, we have not crossed these alleles into an unmutagenized background.

Complementation analysis: Because the temperature sensitivity of these revertants was recessive, mutations were analyzed for their ability to complement one another for growth at 37° on YEP-glucose. All mutations failed to complement three representative alleles: *ssn20-1*, *ssn20-6* and *ssn20-7* (*ssn20 snf2* tester strains for complementation were obtained from crosses of the revertants to wild type). These findings suggest that all of the revertants carry mutations at a single locus, which we designate *SSN20*, for suppressor of *snf2*. To confirm these results, we tested the recessive and partially dominant alleles for their ability to complement one another for suppression of the *snf2* defect in invertase derepression. Revertants were crossed to *ssn20-14* and *ssn20-17* mutants, and the resulting diploids were tested for growth on raffinose. As expected, in all cases the mutations failed to complement.

Tight linkage of four of the *ssn20* alleles was demonstrated by tetrad analysis of three heteroallelic diploids (*ssn20-6*/*ssn20-14*, *ssn20-6*/*ssn20-17* and *ssn20-*

TABLE 1

Phenotypes of revertants of the *snf2* mutant

Relevant genotype	Secreted invertase activity[a] Haploid Repressed	Haploid Derepressed	Diploid from cross to *snf2 SSN20* strain Derepressed
Dominant			
snf2 ssn20-4[b]	1	35	40
snf2 ssn20-12[b]	2	40	50
snf2 ssn20-13	1	20	20
snf2 ssn20-22[b]	5	120	100
snf2 ssn20-24[b]	4	100	90
snf2 ssn20-25[b]	3	85	110
Partially dominant			
snf2 ssn20-5[b]	4	120	65
snf2 ssn20-6[b]	3	100	40
Recessive			
snf2 ssn20-1[b]	6	80	4
snf2 ssn20-2	1	35	6
snf2 ssn20-3	1	65	4
snf2 ssn20-7[b]	6	120	13
snf2 ssn20-9	2	30	5
snf2 ssn20-10	1	45	5
snf2 ssn20-11	4	55	2
snf2 ssn20-14[b]	7	100	3
snf2 ssn20-15[b]	6	65	8
snf2 ssn20-16	1	17	8
snf2 ssn20-17[b]	4	100	10
snf2 ssn20-18	2	60	6
snf2 ssn20-19	2	65	14
snf2 ssn20-21[b]	1	60	11
snf2 ssn20-23	1	14	4
snf2 ssn20-26	4	45	8
snf2 ssn20-28[b]	3	90	12
snf2 SSN20	<1	3	4
SNF2 SSN20	<1	200	200

[a] Units are micromol glucose released/min/100 mg dry weight of cells.
[b] Revertants that were backcrossed to wild type.

6/ssn20-25, all *snf2/SNF2*); in each cross, all four spores of seven tetrads were temperature sensitive for growth and were able to utilize raffinose. Further evidence that these are allelic suppressor mutations which are extragenic to *SNF2* is described below.

***ssn20* mutations are single nuclear mutations unlinked to *snf2*:** Revertants carrying each of 14 *ssn20* alleles (those indicated in Table 1) were crossed to a wild-type (*SSN20 SNF2*) strain, and the resulting diploids were sporulated and subjected to tetrad analysis. In each case, temperature sensitivity segregated 2+:2−, indicating that this defect is due to a mutation in a single nuclear

gene and that the phenotype is independent of the *SNF2* genotype. Segregations of 4+:0−, 3+:1− and 2+:2− for raffinose utilization were observed in ratios approximating 1:4:1 (the ratio for the combined data from all the crosses was 15:63:9). Because *ssn20* suppresses *snf20* and the cross was heterozygous for both markers, these findings indicate that *snf2* and *ssn20* segregate independently. No temperature-sensitive, raffinose-nonfermenting spore clone was recovered, confirming that the temperature-sensitive and suppressor phenotypes segregate together.

Linkage analysis: Analysis of 35 tetrads from crosses including *ssn20* and the centromere-linked marker *trp1* indicated that *ssn20* is not tightly linked to a centromere. Because *ssn20* suppresses only the invertase derepression defect caused by *snf2*, it seemed possible that *ssn20* is a *cis*-acting mutation at the *SUC2* locus, although the recessiveness of many alleles and the temperature-sensitive lethality argue to the contrary. To test this hypothesis, *ssn20 suf2 SUC2* strains were crossed to an *SSN20 SNF2 suc2*am strain, and tetrad analysis was carried out. If *ssn20* and *SUC2* were tightly linked, all *SUC2* segregants would be raffinose fermenters regardless of their *SNF2* genotype, and ability to utilize raffinose would segregate 2:2. This was not the case: only 31 of 50 tetrads showed 2:2 segregation. No linkage to *ura3, lys2, ade2, his4* or *MAT* was detected, and experiments (described below) showed that *ssn20* is not tightly linked to *snf1-snf6* or *ssn6*.

Suppression of *snf2* is not allele-specific: The *ssn20* mutations were isolated as suppressors of *snf2-50*, which was induced by mutagenesis with ethyl methanesulfonate and is probably a missense mutation (NEIGEBORN and CARLSON 1984). The properties of the *ssn20* alleles suggest that they are unlikely to be tRNA suppressors, and, in fact, they do not suppress amber (*his4-539* and *lys2-801*) or ochre mutations (*ade2-101*). To test allele specificity, the ability of the *ssn20-6* mutation to suppress the *snf2-141* ochre allele (NEIGEBORN and CARLSON 1984) was examined. A diploid heterozygous for *ssn20-6* and *snf2-141* was subjected to tetrad analysis. Segregations for raffinose utilization of 4+:0− and 3+:1− were observed (Table 2), suggesting that *ssn20-6* suppresses the raffinose utilization defect caused by *snf2-141*. Five segregants of genotype *ssn20-6 snf2-141* were identified and assayed for secreted invertase after growth under derepressing conditions (Table 3). These results show that *ssn20-6* is an efficient suppressor of *snf2-141* and, therefore, is not an allele-specific suppressor. Further experiments showing that *ssn20* suppresses mutations in other *SNF* genes besides *SNF2* confirm the lack of allele specificity (see below).

Effect of *ssn20* on *SUC2* expression in a *SNF2* background: To determine the effect of an *ssn20* mutation on expression of *SUC2* in a wild-type (*SNF2 SUC2*) genetic background, segregants of genotype *ssn20 SNF2 SUC2* were recovered from crosses. Nine different *ssn20* alleles were tested, including alleles with a variety of suppressor phenotypes: weak and strong, and dominant, partially dominant and recessive. These strains were grown under both glucose-repressing and derepressing conditions at 30° and were assayed for secreted invertase activity (Table 4). These mutants were indistinguishable from the wild type, except that they produced detectable activity (on average, 2 units)

TABLE 2

Suppression of *snf* mutations by *ssn20*

Relevant diploid genotype	Segregation of Raf+:Raf− in tetrads[a] 4+:0−	3+:1−	2+:2−	Suppression of *snf* by *ssn20*
ssn20-6 *SNF1* / *SSN20* *snf1-28*	0	0	7	No
ssn20-1 *SNF1* / *SSN20* *snf1-28*	0	0	4	No
ssn20-6 *SNF2* / *SSN20* *snf2-141*	1	5	0	Yes
ssn20-6 *SNF3* / *SSN20* *snf3-217*	0	0	4	No
ssn20-1 *SNF3* / *SSN20* *snf3-217*	0	0	7	No
ssn20-6 *SNF4* / *SSN20* *snf4-Δ1*	0	0	7	No
ssn20-6 *SNF5* / *SSN20* *snf5-18*	1	4	2	Yes
ssn20-1 *SNF5* / *SSN20* *snf5-18*	2	5	0	Yes
ssn20-6 *SNF6* / *SSN20* *snf6-719*	1	3	0	Yes
ssn20-1 *SNF6* / *SSN20* *snf6-719*	0	6	1	Yes

[a] Four-spored tetrads were tested for growth on raffinose; temperature sensitivity segregated 2:2 in all tetrads.

TABLE 3

Secreted invertase activity in *ssn20 snf* and *ssn20 ssn6* double mutants

	Secreted invertase activity[a]			
	SSN20		*ssn20-6*	
Relevant genotype	Repressed	Derepressed	Repressed	Derepressed
Wild type	<1	200	2	180
snf1-28	<1	<1	1	2
snf2-141	<1	3	2	90
snf3-217	8	22	6	80
snf4-Δ1	<1	2	1	2
snf5-18	<1	5	4	150
snf6-719	<1	22	4	200
ssn6-1	320	430	320	400

[a] Units are micromol glucose released/min/100 mg dry weight of cells; values for *ssn20* strains are the averages of determinations for at least three segregants (ten segregants for *ssn20* in a wild-type background); values for *SSN20* segregants from these crosses are in agreement with previously published values (NEIGEBORN and CARLSON 1984); no strains carried *SUC7*.

TABLE 4

***SUC2* expression in *ssn20 SNF2* strains**

Relevant genotype	Secreted invertase activity[a]	
	Repressed	Derepressed
SSN20	<1	200
ssn20-1	1	200
ssn20-5	1	170
ssn20-6	2	180
ssn20-7	2	210
ssn20-14	1	180
ssn20-15	6	230
ssn20-17	2	200
ssn20-21	5	225
ssn20-22	1	220

[a] Units are micromol glucose released/min/100 mg dry weight of cells; values are the averages of determinations in at least two experiments.

under glucose-repressing conditions. Thus, none of the seven *ssn20* mutations have any significant effect on *SUC2* expression at permissive temperature in the presence of a functional *SNF2* gene.

***ssn20* mutations prevent galactose utilization:** Examination of the original revertants showed that none of the *ssn20* mutations suppressed the defect in galactose utilization caused by *snf2*. We therefore expected to observe 2:2 segregations for galactose utilization in tetrads derived from a cross heterozygous for both *ssn20-6* and *snf2*. Instead, ability to utilize galactose segregated 0+:4−, 1+:3− and 2+:2−, and all temperature-sensitive segregants were galactose nonfermenters. These results suggest that *ssn20-6* in fact prevents galactose utilization. This conclusion was confirmed by analysis of a cross heterozygous for *ssn20-6* and homozygous for *SNF2*; in six tetrads, inability to utilize galactose and temperature sensitivity segregated together 2:2. To determine whether other *ssn20* alleles also prevent galactose utilization, *SNF2* segregants carrying nine other *ssn20* alleles (those listed in Table 4) were tested for growth on galactose. All were negative, suggesting that this phenotype is common to most, if not all, of the *ssn20* mutations. Moreover, in all cases, the galactose-nonfermenting phenotype was recessive, although some of these nine mutations are dominant suppressors of the *snf2* invertase defect.

To determine whether this failure to grow on galactose resulted from a defect in expression of the *GAL* genes, we examined the effect of *ssn20* on expression of a *GAL10-lacZ* fusion, in which expression of β-galactosidase is dependent on the *GAL10* promoter and the *GAL1-GAL10* upstream activation site (WEST, YOCUM and PTASHNE 1984). An *ssn20-1 SNF2* mutant and an *SSN20 SNF2* control strain were each transformed (HINNEN, HICKS and FINK 1978) with an episomal plasmid carrying this fusion (pRY123). The transformants were grown under conditions of galactose induction or glucose repression, as described in MATERIALS AND METHODS, and β-galactosidase activity was as-

TABLE 5

Effect of *ssn20* on expression of a *GAL10-lacZ* fusion

Relevant genotype	β-galactosidase activity[a]	
	Repressed	Induced
SSN20 (pRY123)	<1	700
ssn20-1 (pRY123)	<1	90

[a] Units of activity normalized for the OD_{600} of the culture were calculated as described by MILLER (1972); values are the average of two determinations.

sayed. The induced level of β-galactosidase was eightfold lower in the *ssn20* mutant than in the wild-type control strain (Table 5). These results indicate that *ssn20* reduces expression of at least one of the *GAL* genes, *GAL10*.

Interaction of *ssn20* with other mutations affecting regulation of *SUC2* expression: Mutations in the *SNF1* and *SNF3* through *SNF6* genes prevent normal derepression of secreted invertase (CARLSON, OSMOND and BOTSTEIN 1981; NEIGEBORN and CARLSON 1984). To test the ability of *ssn20* to suppress the defects caused by these mutations, diploids heterozygous for *ssn20* and each of the *snf* mutations were constructed and subjected to tetrad analysis (Table 2). If *ssn20* and the *snf* mutation segregated independently and *ssn20* suppressed the *snf* defect in invertase expression, segregations for raffinose utilization of 4+:0−, 3+:1− and 2+:2− would be expected. These patterns were observed for the *snf5* and *snf6* crosses. If *ssn20* did not suppress the raffinose utilization defect or, alternatively, was tightly linked to the *snf* mutation, then 2:2 segregations would be observed. All tetrads from the *snf1*, *snf3* and *snf4* crosses showed 2:2 segregations, and temperature sensitivity segregated independently from the *snf* mutation in each case, thereby showing that *ssn20* is not tightly linked to any *snf* mutation. Therefore, these findings indicate that *ssn20* does not suppress the raffinose utilization defect caused by *snf1*, *snf3* or *snf4*. No differences were observed in the suppression patterns of *ssn20-6* and *ssn20-1*.

To assess the suppression of *snf* mutations by *ssn20* in a quantitative manner, segregants carrying both *ssn20* and the *snf* mutation were recovered from each cross and were assayed for production of secreted invertase (Table 3). These assays confirmed that *ssn20* efficiently suppresses the defect in invertase derepression caused by *snf5* and *snf6*; derepressed values in the double mutants were close to that of wild type. These assays also confirmed that *ssn20* does not suppress *snf1* or *snf4*; the double mutants resembled the single *snf* mutants. The assays of *snf3 ssn20* strains, however, revealed that *ssn20* partially suppresses the defect in invertase derepression, which was not apparent from tests of growth phenotype; the derepressed *ssn20 snf3* strain produced about fourfold more secreted invertase than did the *snf3* strain. This level of activity would be enough to allow a wild-type *SNF3* strain to grow on raffinose. The fact that *snf3 ssn20* mutants do not grow on raffinose and also, like *snf3* single mutants, produce low levels of invertase under glucose-repressing conditions

suggests that *ssn20* does not suppress other defects caused by *snf3* (NEIGEBORN and CARLSON 1984).

The interaction of *ssn20* with *ssn6* was also examined. Mutations at the *SSN6* locus cause constitutive (glucose-insensitive), high-level expression of secreted invertase (CARLSON *et al.* 1984) and suppress the defects in invertase derepression caused by *snf* mutations (NEIGEBORN and CARLSON 1984). Tetrad analysis was carried out on a cross heterozygous at both loci, and ability to utilize raffinose segregated 4+:0− in five tetrads. Double mutants carrying *ssn6* and *ssn20* were recovered and were found to produce invertase constitutively at high levels. The double mutants also resembled the *ssn6* parent in that they exhibited the clumpy phenotype characteristic of *ssn6* mutants; moreover, they were able to grow on galactose, indicating that *ssn6* suppresses the galactose-nonfermenting phenotype of *ssn20*. Scoring temperature sensitivity for growth was not informative, because *ssn6* also causes temperature sensitivity. The frequent recovery of segregants carrying both mutations indicated that *ssn20* and *ssn6* are not tightly linked.

Selection for suppressors of *snf5* yielded only *ssn20* mutations: The *snf2* and *snf5* mutations confer similar phenotypes; both allow very low-level regulated expression of secreted invertase (a few percent of the wild-type level) and cause pleiotropic defects in expression of other glucose-repressible genes (NEIGEBORN and CARLSON 1984). In addition, *snf2* and *snf5* show similar interactions with *ssn6*. Both *snf2 ssn6* and *snf5 ssn6* double mutants resemble the wild type more closely than they resemble either single mutant parent with respect to regulation of *SUC2* expression, suggesting that *SNF2* and *SNF5* act antagonistically to *SSN6*. The finding that *ssn20* suppresses the invertase defect caused by *snf5* provided further evidence that the *SNF2* and *SNF5* genes play similar or related roles in regulation of gene expression. To obtain further information on the relationship of these two genes, we selected for suppressors of the invertase derepression defect of *snf5*, with the intent of examining the effects of these suppressors on *snf2*.

Revertants of two *snf5* mutants, MCY1947 and MCY1949, were isolated by the same procedure used to obtain revertants of the *snf2* mutant (see MATERIALS AND METHODS). These mutants carry a null allele of *snf5*, *snf5-5*: :*URA3* (E. ABRAMS and M. CARLSON, unpublished results). Seventeen revertants were analyzed, as described for the *snf2* revertants. All seventeen carried recessive temperature-sensitive lethal mutations that failed to complement *ssn20*. All displayed a partial reversal of the *snf5* phenotype; although they grew on raffinose, none was able to grow on galactose or glycerol. The suppression of the invertase derepression defect of *snf5* was further characterized by assaying secreted invertase in the revertants. When grown under derepressing conditions, all of the revertants produced significantly higher secreted invertase activity than did the *snf5* parent, which produced only 5 units. Values for the revertants ranged from 15 to 285 units (data not shown). None produced substantial activity when glucose-repressed; values were comparable to those detected in the *snf2* revertants. In each case, the dominance of the suppressor phenotype was assessed by testing diploids homozygous for *snf5* and hetero-

zygous for the *ssn20* mutation for growth on raffinose and/or derepression of secreted invertase activity. Four of these *ssn20* alleles were dominant suppressors, two were partially dominant and the remaining eleven were recessive. Thus, selection for revertants of *snf5* yielded only additional *ssn20* alleles, and the properties of these new alleles were indistinguishable from those of the *ssn20* alleles isolated as suppressors of *snf2*.

DISCUSSION

We have isolated partial suppressors of *snf2* mutations by selecting for raffinose-utilizing revertants of a *snf2* mutant. All 25 revertants recovered carried mutations at a single locus, *SSN20*. The *ssn20* mutations suppressed the defect in invertase derepression caused by *snf2*, but did not remedy the defects resulting in inability to utilize galactose and glycerol. Alleles with dominant, partially dominant and recessive suppressor phenotypes were recovered, suggesting that suppression results from alteration, rather than loss, of the function of the *SSN20* gene product and that the *SSN20* gene product may function as a multimer. It is interesting that these *ssn20* mutations have little effect on expression of *SUC2* in a *SNF2* background, causing only a very low level of constitutivity, but, nonetheless, effectively compensate for the lack of functional *SNF2* gene product and restore nearly wild-type levels of invertase activity.

Suppression of the *snf2* defect could occur by two possible mechanisms (other than informational suppression). First, if the *SSN20* and *SNF2* gene products interacted physically, an alteration of the *ssn20* gene product could compensate for the structural defect in the mutant *snf2* gene product to restore partial function. This possibility seems unlikely because no allele specificity was observed. The *ssn20-6* mutation efficiently suppressed not only the *snf2* allele for which suppression was selected but also a *snf2* ochre allele. Furthermore, *ssn20-6* and *ssn20-1* suppressed mutations in the *SNF5* and *SNF6* genes. The second possibility, which we favor, is that the *ssn20* mutation allows the cell to bypass its requirement for functional *SNF2*, *SNF5* and *SNF6* gene products to derepress *SUC2* expression. Because previous studies have suggested that *SNF2*, *SNF5* and *SNF6* exert their effects on *SUC2* expression via the upstream regulatory region (SAROKIN and CARLSON 1985a), it is possible that *ssn20* mutations bypass the requirement for one or more functions of the upstream region. Preliminary results indicating that *ssn20* partially suppresses defects in derepression caused by deletions of the upstream region support this notion (L. NEIGEBORN and M. CARLSON, unpublished results).

Studies of the epistasis relationships between *ssn20* and other mutations known to alter regulation of *SUC2* expression revealed that *ssn20* suppresses the invertase derepression defects caused by *snf5* and *snf6*. As is the case for *snf2*, *snf5* is only partially suppressed by *ssn20*, and the double mutants failed to grow on galactose or glycerol; we have only one allele of *snf6*, which appears to be leaky and does not cause pleiotropic growth defects. No suppression of *snf1* or *snf4* was detected. The results with *snf3* were ambiguous: the *ssn20*

snf3 double mutants showed an unimpressive fourfold increase in derepression of invertase relative to *snf3* strains.

These findings on the interactions of the *snf* mutations with *ssn20*, when taken in conjunction with previous studies of their interactions with *ssn6*, suggest that the *SNF* genes include two groups of functionally related genes, one group comprising *SNF2*, *SNF5* and, probably, *SNF6* and the other group comprising *SNF1* and *SNF4*. We previously reported that *snf1 ssn6* and *snf4 ssn6* double mutants synthesize invertase constitutively at high levels and display the clumpy phenotype characteristic of *ssn6* single mutants (NEIGEBORN and CARLSON 1984). In contrast, *snf2 ssn6* and *snf5 ssn6* double mutants synthesize low levels of invertase when glucose-repressed and synthesize reasonably high levels when derepressed, and they are not very clumpy. The *snf6 ssn6* double mutants do not clearly belong in one group or the other; they show high-level constitutivity like that of *snf1 ssn6* and *snf4 ssn6* strains, but are not very clumpy. It is possible that leakiness of the *snf6* allele may contribute to the high level of invertase expression in these strains. Because *ssn20* clearly suppressed *snf6*, we think it likely that the *SNF6* gene is functionally related to *SNF2* and *SNF5*.

All of the *ssn20* mutations were also found to cause recessive, temperature-sensitive lethality regardless of the *SNF2* genotype. One interpretation of this finding is that *SSN20* is an essential gene and that all of our mutations result in a temperature-sensitive *SSN20* gene product. In that case, a true null mutation would be unconditionally lethal. An alternative possibility is that a functional *SSN20* gene is essential for viability at 37°, but is not essential at 30°. To distinguish between these possibilities, we have cloned *SSN20* and are proceeding with construction of a null mutation.

The observation that *ssn20* mutations did not suppress the galactose nonfermenting phenotype of *snf2* led to the surprising finding that, in a *SNF2* background, *ssn20* mutations in fact prevent utilization of galactose at 30°. Studies of the effect of *ssn20* on expression of a *GAL10-lacZ* gene fusion suggested that the galactose nonfermenting phenotype results from a defect in expression of at least one of the genes needed for galactose utilization. This finding that *ssn20* mutants are defective in expression of *GAL10* at a temperature permissive for growth on glucose suggests that the lethality at nonpermissive temperature results from defects in expression of one or more essential genes. It is not clear why *ssn6* suppresses the galactose utilization defect of *ssn20*; *ssn6* does not suppress the galactose-nonfermenting phenotypes caused by *snf* mutations (CARLSON *et al.* 1984; NEIGEBORN and CARLSON 1984).

Taken together, these findings on the pleiotropic effects of the *ssn20* mutations suggest that the *SSN20* gene plays a role in the expression of a variety of genes. Further studies will be required to determine the role of the wild-type *SSN20* gene product and the mechanism by which mutant *ssn20* alleles suppress *snf2*, *snf5* and *snf6*.

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